# Nematode (Phylum Nematoda) community assemblages: A tool to implement environmentally-sound management strategies for root-knot nematodes (*Meloidogyne* spp.) in potato-based cropping systems

**Emil Engelbrecht**

Thesis submitted in fulfilment of the requirements for
the award of the degree Master of Environmental Science
at the North-West University (Potchefstroom Campus)

May 2012

Supervisor: H. Fourie

Co-supervisor: M. Daneel

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIM
NOORDWES-UNIVERSITEIT

**ACKNOWLEDGEMENTS**

There are several people without whom this dissertation and the work it describes would not have been possible. I would like to thank all those people who have contributed towards the successful completion of this work.

God our saviour who held his hand over us with every road trip we made. Without His guidance nothing I have done would have been possible.

My sincere thanks and appreciation to Prof. Driekie Fourie, my supervisor and mentor during this project. Her patience, guidance, constructive criticism, sound advice and encouragement throughout the course of the study are greatly appreciated. It is Prof. who showed me how interesting and dynamic the field of nematology is.

Dr. M. Daneel, co-supervisor, for her contribute and patience with all the questions and assistance.

Dr. Suria Ellis, of the statistical consultation service, which provided assistance with statistical analyses.

I am also grateful to Suria Bekker for her help with laboratory work.

Drs. A. Malan, M, Marias and E. Van den Berg form the ARC-PPRI Pretoria for identification of species.

Mr. G. Posthumus at The Western Free State Seed Potato Growers for funding this research and the potato producers at Christiana, Mrs. N. Coetzee, I. Easby, J. Easby, W. Easby, J. Greyling, C. Kriek, W. Ras, JF. Van der Merwe, and oom Lourens for their dedicated help with the sampling. Also to mr. JC. Olevano for calibrating the planting equipment used in the field trial.

Mr. J. Fourie from Javeline seeds for supplying the *Raphanus* seeds that was used in the field trial.

Finally, I express my appreciation and love to my parents and Jean-maré Truter, for their moral support and motivation during the whole project period, it is much appreciated.

## ABSTRACT

Potato is the number one non-grain food commodity and fourth most important food crop worldwide. Plant-parasitic nematodes (PPN), particularly root-knot nematodes (RKN), are one of the economically most important constraints that adversely affect the quantity and quality of this crop. With the removal of many red-band Class 1 nematicides an increased need exists for producers to improve human, environmental and food safety. Accurate identification of PPN and non-parasitic (NPN) assemblages is, therefore, a prerequisite to investigate and verify alternative, environmentally-friendly management strategies to minimise damage to potato and other rotation crops. The objectives of the study were to assess the status of PPN and NPN in 31 potato fields during two consecutive growing seasons in the Christiana area (North-West Province) of South Africa (SA), with the emphasis on RKN. In addition, the effects of selected Brassicaceae species as cover and/green manure crops on RKN population levels as well as NPN assemblages was investigated in a field experiment.

For both surveys, root and soil samples were obtained from various crops as well as grass cover crops from 31 fields of five producers. PPN and NPN were extracted and prominence values calculated, while the data were also subjected to parametric and non-parametric statistical analyses. RKN were identified, using the molecularly-based SCAR-PCR method, as the predominant PPN associated with potato, other crops and grasses included in potato-based cropping systems in this area. In terms of NPN, soil food web conditions for the majority of the 31 fields were classified as maturing since they were plotted within the "Stable Enriched" quadrant.

In a field trial, conducted during the 2010/2011 cropping season, four Brassicaceae species were evaluated with regard to their host suitability and biofumigation effects in a field where high *M*. *incognita* population levels prevailed. Ethylenedibromide (EDB®) as well as untreated control treatments were also included in a RCBD with five replicates. Relatively high *M. incognita* egg and J2 numbers were maintained by all four cultivars, i.e. Doublet and Terranova, Calienté and Nemat. The follow-up potato crop, planted seven weeks after the incorporation of the Brassicaceae amendments was subsequently parasitized severely in plots planted with all four Brassicaceae cultivars. According to NPN assemblages at termination of the trial, soil food web conditions in plots treated with EDB® as well as untreated control plots were classified as disturbed. In contrast, the status of soil food webs in the four Brassicaceae-amended treatments was classified as maturing.

Results obtained during this study in terms of using NPN assemblages as a tool to classify soil health is the first in SA, particularly for annual crops such as potato. Furthermore, identification of RKN species in fields of these farmers will assist them in choosing cultivars of rotation crops and/or grass cover crops that has been identified as poor hosts of these parasites.

Results emanating from this study are thus applicable in the agricultural sector and could add substantially in terms of our understanding and of both PPN and NPN and their effects in potato-based cropping systems.



**Key words:** biofumigation, Brassicaceae, non-parasitic nematodes, potato, *Raphanus*, root-knot nematodes

## EKSERP

As nie-graan voedselkommoditeit is aartappels wêreldwyd eerste gelys en verteenwoordig dit die vierde belangrikste voedselgewas. Plantparasitiese aalwurms (PPA), veral knopwortelaalwurms (KWA), is een van die ekonomies belangrikste organismes wat die kwaliteit en opbrengs van aartappels nadelig beïnvloed oral waar dit in die wêreld verbou word. Met die onttrekking van al hoe meer Rooiband, Klas 1 aalwurmdoders vanaf wêreldmarkte het 'n toenemende behoefte laat ontstaan by produsente en industrieë om veiligheid ten opsigte van mense, die omgewing en voedselgewasse aan te spreek en te verbeter. Dus is akkurate identifisering van PPA asook nie-parasitiese aalwurm (NPA) groepe 'n voorvereiste om alternatiewe, omgewingsvriendelike bestuurstrategieë ten opsigte van hierdie organismes te ondersoek en te verifieer. Sodoende kan skade aan aartappels en ander opvolggewasse voorkom en/of tot die minimum beperk word.

Die doelwitte van hierdie studie was om die status van PPA en NPA in 31 lande waarin aartappels elke ses tot sewe jaar in wisselbou met ander gewasse en grasdekgewasse geplant word, tydens twee opeenvolgende groeiseisoene in die Christiana gebied (Noord-Wes Provinsie) in Suid-Afrika (SA) te assesseer met die klem op KWA. Die effek van geselekteerde Brassicaceae spesies as dek- en groenbemestinggewasse op KWA bevolkingsvlakke en NPA groepe is ook in 'n veldproef geëvalueer.

Aalwurmwortel- en grondmonsters van verskeie gewasse asook die grasdekgewasse wat as wisselbougewasse op die 31 lande verbou is, is geneem en ontleed vir die teenwoordigheid van beide PPN en NPA. Vervolgens is prominensiewaardes vir die teenwoordige aalwurmgenera/spesies/families bereken. Aalwurmdata is egter ook aan parametriese en nie-parametriese statistiese ontledings onderwerp. KWA is met behulp van die molekulêr-gebaseerde SCAR-PCR metode geïdentifiseer as die dominante PPA groep wat teenwoordig was in hierdie lande waarop aartappels asook ander gewasse en grasse ingesluit word in die aartappelproduserende gebied waar hierdie studie uitgevoer was. In terme van NPA, is die status van die grondgesondheid in die meerderheid van die 31 lande geklassifiseer as ontwikkellend en stabiel, omdat laasgenoemde in die "Stabiele Verrykte" kwadrant van die vereenvoudigde voedselweb geklassifiseer was.

Gedurende die 2010/2011 groeiseisoen is vier Brassicaceae spesies geëvalueer met betrekking tot hul gasheerstatus en bio-berokings effek, in 'n veldproef met hoë *M. incognita* bevolkingsvlakke. Etileendibromied (EDB®) sowel as 'n onbehandelde kontrole is ook in die gerandomiseerde blokontwerp ingesluit met vyf herhalings vir elke behandeling. Relatiewe hoë *M. incognita*-eier en tweede jeugstadium (J2) bevolkingsvlakke was in wortels/knolle van al vier die Brassicaceae kultivars, nl. Doublet, Terranova, Calienté en Nemat teenwoordig tydens die 50% blomperiode van hierdie gewasse. Wortels van die opvolgaartappelgewas (geplant sewe weke na die inwerking van die Brassicaceae kultivars se bogrondse dele) het baie hoë *M. incognita* bevolkingsvlakke getoon tydens knolinisiasie, terwyl eiers en J2 ook reeds in die jong ontwikkelde aartappelknolle teenwoordig was.

Ten opsigte van NPA groeperings is gevind dat die status van die grond ten opsigte van gesondeheid in behandelings met EDB® sowel as die onbehandelde kontrole as versteur geklassifiseer was. In teenstelling daarmee, is die status van grondvoedselwebbe in die vier Brassicaceae-behandelde persele as ontwikkelend en stabiel geklassifiseer. Resultate wat tydens hierdie studie verkry is in terme van grondvoedselwebbe wat deur die teenwoordigheid van NPA groepe gereflekteer word, kan as 'n waardevolle instrument gebruik word om die gesondheid van gronde te klassifiseer en is 'n eerste in SA, veral op eenjarige gewasse soos aartappels. Voorts sal die identifisering van KWA spesies in lande van hierdie produsente in die Christiana gebied hulle alreeds in staat stel om ingeligte keuses te maak ten opsigte van kultivars vir wisselbou- en/of grasdekgewasse wat geïdentifiseer is as swak gashere van hierdie parasiete tydens vorige studies. Resultate wat uit hierdie studie voortspruit is dus onmiddellik van praktiese toepassing in die landbousektor en kan aansienlik waarde toevoeg in terme van die begrip en kennis van beide PPA en NPA en die gevolge van hul teenwoordigheid in aartappelgebaseerde wisselboustelsels.



**Sleutelwoorde:** aartappels, bio-beroking, Brassicaceae, knopwortelaalwurms, nie-parasitiese aalwurms, *Raphanus*

**TABLE OF CONTENTS**

# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

With the progressive increase in the global human population, estimated to reach 9 billion in 2050 (FAO, 2009), it is of vital importance that agricultural and horticultural crops be produced and utilized optimally. Potato is one of the crops that are crucial in terms of food security, taking into account the rapid human population growth and increased hunger rates (IPC, 2011). In 2010, potato was the third largest staple food crop in South Africa (SA) in terms of production (2 071 930 Mt), with a gross value of ZAR 2.4 billion (FAO, 2012). However, seed and table potato crops are exposed to severe damage by various diseases and pests such as plant-parasitic nematodes (PPN) of which root-knot nematodes (RKN; *Meloidogyne* spp.) are economically the most important (Kleynhans, 1991; Kleynhans *et al*., 1996). PPN damage to potato is experienced on a wide scale despite the extensive use of synthetic nematicides on which more severe restrictions are being enforced continuously (Nyczepir & Thomas, 2009). This predicament is true for commercial as well as self-sustaining producers throughout the world, including SA where RKN pose the biggest problem to potato.

Basic, as well as innovative, research to address the RKN problem in optimising sustainable production of this important staple food crop is quintessential for local producers, the industry and the economy. Since RKN has a very wide host range (Kleynhans *et al*., 1996; Moens, 2009), sustainable augmentation in population levels of RKN is also detrimental to production of other crops that are planted in rotation with potato. Management of the Western Free State Seed Potato Growers approached nematologists at the North-West University to assist producers in the Christiana potato-producing area in addressing the RKN problem they experience in their potato-based cropping systems. As a result, this study emanated with the introductory chapter focussing on the potato crop, its origin, classification, anatomy as well as economical and social importance. Furthermore, statistics on potato production in SA and in particular in the Christiana area are also referred to. A concise review of economically important PPN, with special reference to RKN, follows while a synopsis on the use of non-parasitic nematodes (NPN) as indicators of soil health is also provided. Methods used to identify both PPN and NPN are concurrently briefly referred to since accurate identification of genera, species and/or families present in crop fields, in this case potato, is crucial to ensure that management strategies are effective. Ultimately such strategies used to protect the crop against PPN are described briefly, with a final glance to the value of integrated management strategies that are, and can be, used in potato-

based cropping systems. Therefore, this study entailed i) surveys during two consecutive seasons to enable identification of both PPN and NPN in 31 fields of five potato producers in the Christiana area, and, ii) investigation of the effect of four Brassicaceae spp. cultivars crops on a RKN population (*M. incognita*) as well as assemblages of NPN occurring in a field of one of these producers.

### 1.2 Potato (*Solanum tuberosum*)

#### 1.2.1 Origin

*Solanum tuberosum* or the “Irish potato” is generally accepted to have originated in South America in the Peruvian and Bolivian Andes Mountains (Louw, 1982; Scurrah *et al*., 2005). It is not certain how this crop was introduced to SA but it has been suggested that potato tubers were brought from Holland to the Cape in the early 1800’s to be planted as food for seafarers (Louw, 1982; DAFF, 2003).

#### 1.2.2 Classification

The taxonomic classification of potato is as follows (GBIF, 2011):

**Kingdom**: Plantae
**Phylum**: Magnoliophyta
**Class**: Magnoliopsida
**Order**: Solanales
**Family**: Solanaceae
**Genus**: *Solanum*
**Species**: *S. tuberosum* L.

### 1.2.3 Anatomy

#### 1.2.3.1 The potato plant

Potato is a herbaceous, annual plant that grows up to 100cm tall and generally produces several tubers below the ground during one growing season (FAO, 2008). Only the tubers of a potato plant are edible, while the leaves, sprouts and stems contain toxic components known as glycoalkaloids. These natural substances protect the plant against fungi and insects. Potato is vegetatively propagated, with a new plant growing from a tuber or piece of tuber which is referred to as the seed. The crop can be grown from sea level up to 4 700 meters above sea level (IPC, 2011) and shares the genus *Solanum* with at least a 1 000 other species, including tomato (*S. lycopersicum*) and eggplant (*S. melongena*). *Solanum tuberosum* is divided into two subspecies, namely *andigena* (adapted to short day conditions and mainly grown in the Andes) and *tuberosum* (cultivated around the world) (Hawkes, 1990; FAO, 2008).

#### 1.2.3.2 The potato tuber

As the potato plant grows, its compound leaves manufacture starch that is transferred to the ends of its underground stems (or stolons). These stolons thicken/expand to form a few or as many as 20 tubers underneath the soil surface (FAO, 2008), which represent genetic clones of the mother seed (IPC, 2011). Except for the tubers, potato plants also produce flowers and berries that contain between 100-400 botanical seeds. These can be planted to produce new tubers, which will be genetically different from the mother plant (IPC, 2011). The number of tubers that actually reach maturity depends on a range of conditions such as available moisture, temperature and soil nutrients. Tubers may vary in shape and size and normally weigh up to 300g each (FAO, 2008).

At the end of the growing season, the leaves and stems of potato plants naturally die down or are mechanically sprayed with herbicides to terminate their active growth stage. Newly formed tubers detach from the stolons and then serve as a nutrient store that allows the plant to survive the cold, later regrow and reproduce. Each tuber may contain 2-10 buds (or "eyes"), which are arranged in a spiral pattern around the tuber surface. These buds generate shoots that grow into new plants when conditions are favourable again for plant growth (FAO, 2008).

### 1.3 Economic and social importance of potato

Potato forms an integral part of the global food system and is a major food crop in 57 of the more than 100 world countries where it is produced (Scurrah *et al*., 2005; IPC, 2011). It is the world's number one non-grain food commodity and ranks fourth as the world's most important food crop, after maize, wheat and rice. However, unlike the latter crops, potato is not a globally traded commodity and its prices are determined usually by local supply and demand (FAO, 2008). Presently, more than half of the global potato production is provided by developing countries (IPC, 2011). A recent survey by the FAO in more than 70 of the world's developing countries found that increases in potato prices are much lower than that for cereals. Potato is, therefore, highly recommended to support food security and can help low-income countries to alleviate food price increases (FAO, 2008).

World potato production reached up to 321 million tonnes in 2010, with 2 million tonnes being harvested from 53 000ha that were planted with potato in SA (PSA, 2010). Potato is regarded as a universal food crop since it produces more food per unit of water applied during its growth stage than any other major crop and is up to seven times more efficient in using water than cereal crops (IPC, 2011). Except for its general use as a vegetable, potato is used for a variety of purposes. Less than 50% of potato grown worldwide is consumed fresh. The rest are processed as food products and ingredients, feed to cattle, pigs and chickens; processed into starch for industry as well as re-used as seed tubers for growing the next season's crop. However, global consumption of potato as a food commodity is shifting from fresh potato to value-added, processed food products. One of the main items in the latter category is frozen potato, which includes most of the french fries served in restaurants and fast-food chains worldwide (IPC, 2011).

Since the majority of the world's undernourished people live in developing countries (FAO, 2010), potato could fill a substantial demand for food and nutrition. Potato is an excellent, low fat source of carbohydrates, containing only one-fourth the calories that bread contains. When boiled, potato contains more protein and nearly twice the calcium than maize (IPC, 2011). Potato is also a good source of the B vitamins, while their skins are an excellent source of vitamin C. Potato also offers advantages as a subsistence crop because of its high yields and favourable response to intensive gardening techniques. Use of potato as a major food source has grown considerably because of the ease with which it can be manipulated genetically, its versatility in agronomic systems and the expanding number of uses for potato as food and a raw industrial material (Kiple & Ornelas, 2000).

### 1.3.1 Potato production in South Africa (SA)

More than half of the global potato production currently occurs in Africa, Asia and Europe with SA ranking 28th. In Africa, SA currently ranks fourth in terms of potato production with Malawi being first, Egypt second and Algeria third (PSA, 2010). Although potato production only accounts for 53% of vegetable, 12% of horticulture and 3% of the total agricultural crops being produced in SA (PSA, 2010), the local potato industry has grown and established itself as one of the most important food providers in the country (DAFF, 2003). Local potato production has grown substantially during the past 15 years, namely from 1.2 million tonnes in 1990 to a record of 2 million tonnes in 2010 (PSA, 2010). During the same period the area on which the crop has been cultivated, however, actually declined from 66 000ha to 53 000ha, while the average yield and fresh produce have increased steadily (Figure 1.1) (PSA, 2010). Reasons for this could be due to the decrease in potato production under rain-fed conditions together with the increase in production under irrigation (Figure 1.2). The use of higher-yielding cultivars, for example Mondial, and better production practices also contributed to this scenario (F. Niederwieser, pers comm., April 2012).

Locally potato is generally grown under irrigation in 16 regions (Figure 1.3). The top three potato production regions are situated in the Limpopo (18%), Eastern Free Sate (17%) and Western Cape (Sandveld region; 14%) provinces. There are an estimated 654 commercial producers that currently produce potato in SA (PSA, 2010). Yields of approximately 33 tonnes per ha (PSA, 2010) are generally realised and in some regions two potato crops can be produced per year (DAFF, 2011). Since potato is planted at different times of the year due to climatic differences in the various production areas, fresh potato is available for consumption throughout the year (DAFF, 2011).

The most common and popular potato cultivar planted locally is Mondial, followed by Buffelspoort 1 (BP1) and Up-To-Date (UTD) (Figure 1.4) (PSA, 2010). Selection of these potato cultivars, however, depends on the specific production area and purpose (DAFF, 2011). In 2010, 68% of the total potato crop was utilized by local markets, while 17% were processed, 8% used as seed and 7% exported (PSA, 2010). In general SA boasts a sophisticated seed potato industry and an effective and growing processing sector (FAO, 2008). Currently the industry uses 380 000 tonnes of fresh potato, which is processed into french fries, frozen and chilled products and crisps (PSA, 2010). Annual local potato consumption is estimated at ±30kg per person (FAO, 2008).

Although the potato industry is strong, a wide range of pests and diseases as well as other constraints affect local production. Damage inflicted to potato planted locally for example accounts to 10% of tubers being degraded at fresh produce markets. Other factors that adversely affect the crop, resulting in the degrading of tubers are greening, mechanical damage, browning, PPN and others as shown in Figure 1.5 (PSA, 2010).

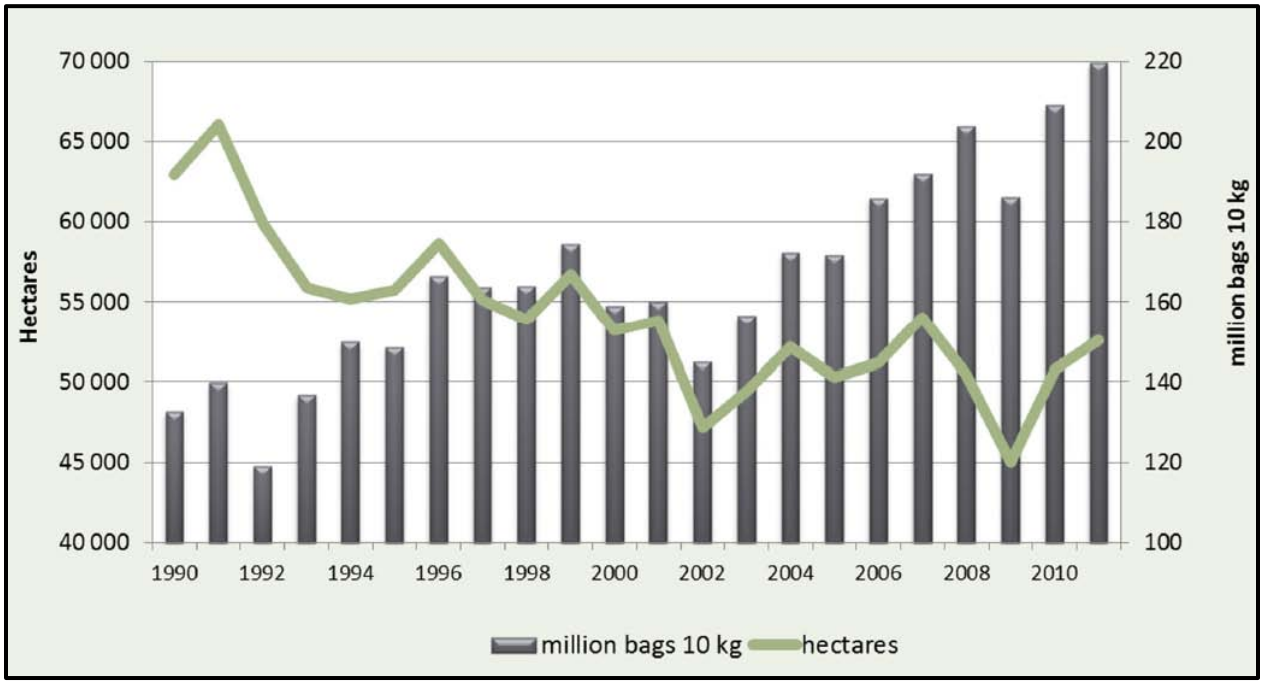


Figure 1.1. Hectares and crop size for potato production in South Africa from 1990-2010 (PSA, 2010).

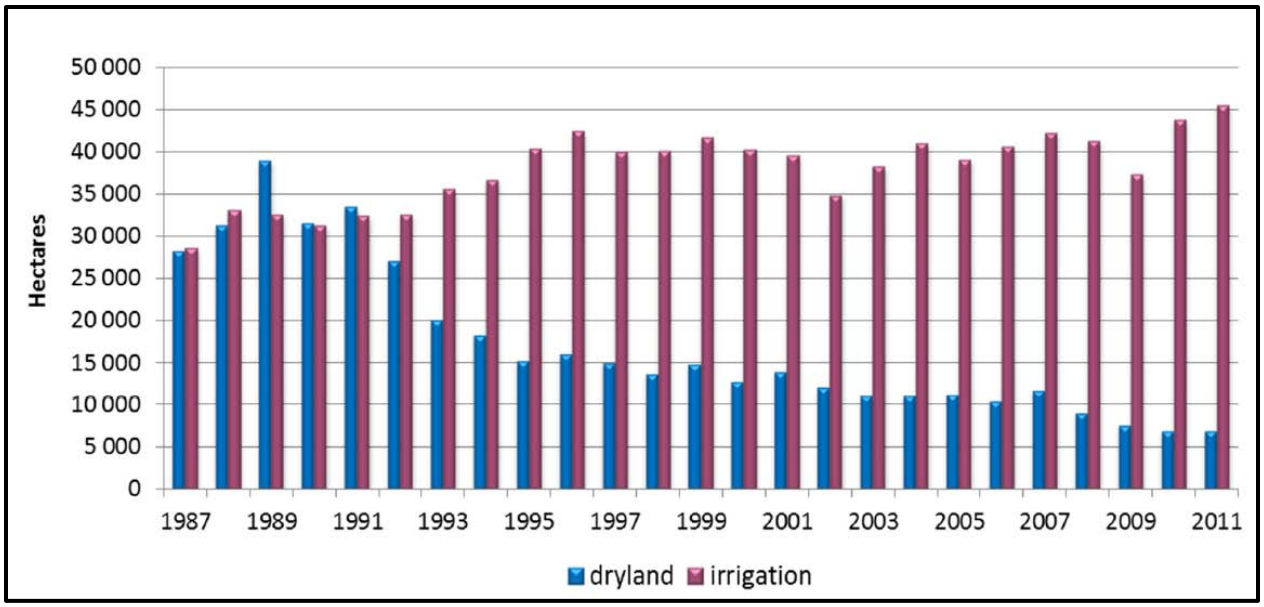


Figure 1.2. Hectares planted to potato in South Africa from 1987 – 2010, indicating rain-fed versus irrigation areas (PSA, 2010).

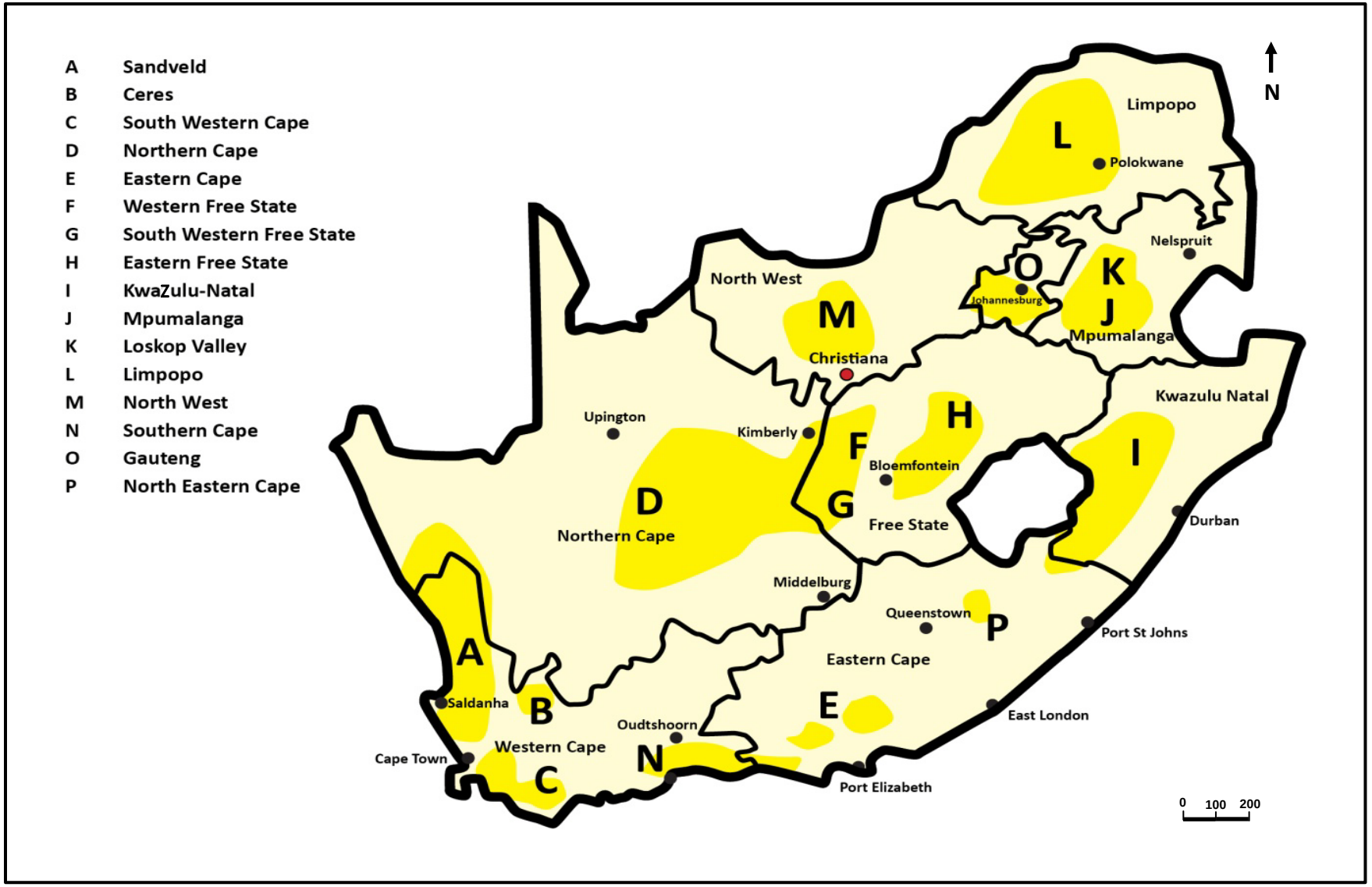


Figure 1.3. The areas where the sixteen potato production regions in South Africa are situated (Adapted from: https://research.cip.cgiar.org/confluence/display/GILBWEB/South+Africa by Thinus du Plessis, Germstyle, Namibia).

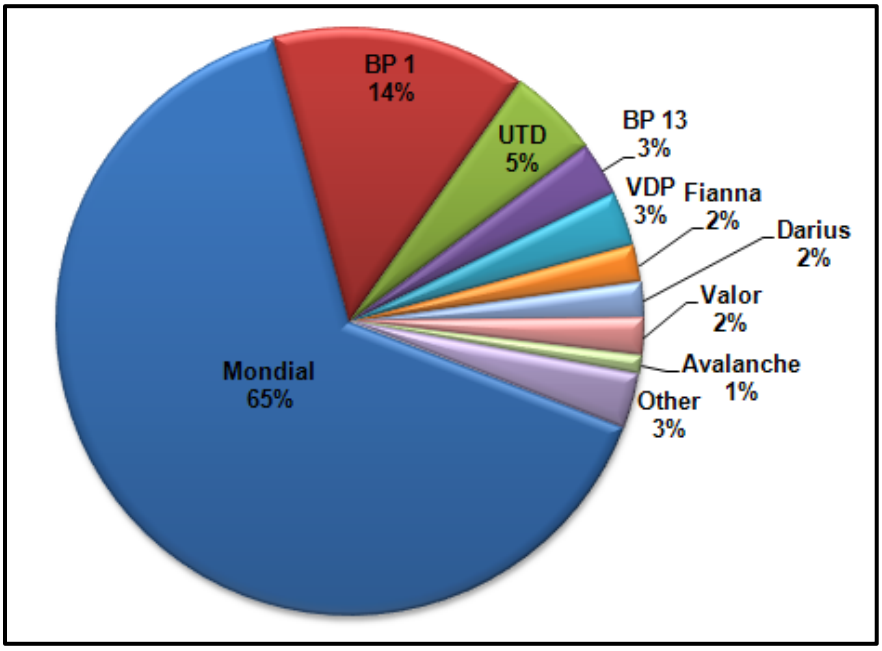


Figure 1.4. Potato cultivars planted in South Africa (PSA, 2010).

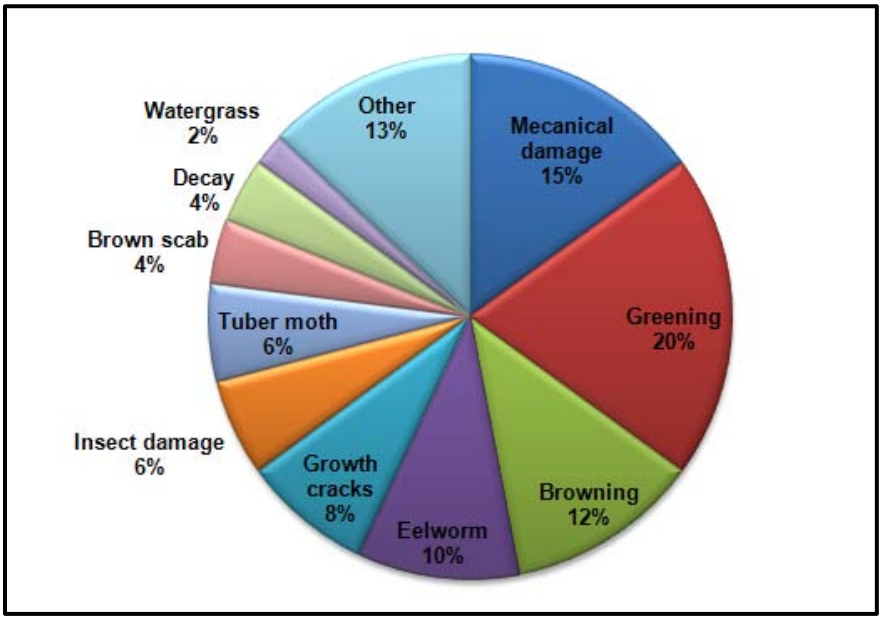


Figure 1.5. Factors related to the degrading of potato tubers in South Africa (PSA, 2010).

### 1.3.2 Potato production in the Christiana area (North-West Province)

Approximately 61 potato producers are actively growing potato on approximately 6 109ha of arable agricultural land that is situated in the region where this study was conducted on the border of the North-West and Free State provinces. Primary cultivars planted for table potato production on a total area of 2 226ha in this area are Mondial (66%), Up-To-Date (UTD) (22%) and BP1 (7%). For seed potato purposes, which is the main objective for producers in this region, cultivars Mondial (80%), BP1 (8%) and Fabula (6%) are planted on a total area of 3 883ha (PSA, 2010).

In this area the average inspections during which tuber infection were recorded, as a result of RKN parasitism, has increased from an average of 4%, with symptoms visible during the 2001/2002 season to nearly 50% during the 2009/2010 season (Table 1.1) (J. van Vuuren, pers comm., May 2010).

Table 1.1. The extent of root-knot nematode damage as recorded during tuber inspections (J. van Vuuren, pers comm., May 2010).

| Growing seasons | Average RKN infection (%) for inspections done | Number of inspections done on tubers | Number of tuber inspections showing tuber infection | Average inspections showing tuber infection (%) |
|---|---|---|---|---|
| 2001/2002 | 0.018 | 736 | 30 | 4.08 |
| 2002/2003 | 0.043 | 372 | 45 | 12.1 |
| 2003/2004 | 0.065 | 407 | 101 | 24.82 |
| 2004/2005 | 0.019 | 371 | 42 | 11.32 |
| 2005/2006 | 0.049 | 513 | 110 | 21.44 |
| 2006/2007 | 0.018 | 489 | 68 | 13.91 |
| 2007/2008 | 0.019 | 458 | 79 | 17.25 |
| 2008/2009 | 0.02 | 528 | 100 | 18.94 |
| 2009/2010 | 0.093 | 697 | 347 | 49.78 |

### 1.4 Plant-parasitic nematodes (PPN) associated with potato

Nematodes are pseudocoelomate, unsegmented worm-like animals that occur in almost every habitat and are either non-parasitic or parasitic. The latter parasitic nematodes infect and parasitize plants, animals and humans (Decraemer & Hunt, 2006). PPN are amongst the most important pest constraints that adversely influence the production of potato worldwide (Scurrah *et al*., 2005). Although occupying many different ecological niches, nematodes are small, essentially aquatic animals (Kleynhans *et al*., 1996; Hunt *et al*., 2005) that require at least a film of water to enable locomotion. Therefore, water content is a primary ecological factor that is important for them to locate and infect plants. Although many PPN species die in dry soils, others may survive in an anhydrobiotic state. Conversely, too much soil water may result in a lethal oxygen deficit for these organisms (Hunt *et al*., 2005). In turn, nematodes are parasitized or preyed upon by viruses, bacteria, fungi, insects, mites and other nematodes (Kleynhans *et al*., 1996).

The life cycle of PPN generally comprises of an egg, four juvenile (three in some longidorids) and an adult stage(s) (Kleynhans *et al*., 1996; Decraemer & Hunt, 2006). Each juvenile moults once and the adult stage appears after the last moult. The second moult, which in Tylenchida occurs within the eggshell gives rise to the second-stage juvenile (J2), which represents the hatching and often infective stage (Kleynhans *et al*., 1996). All PPN species spend at least part of their life cycle in the soil. Their activities are determined by abiotic factors such as soil aeration, temperature, moisture, organic material and other soil properties as well as biotic factors that include availability and suitability of host plants, soil cultivation practices and the presence of pathogens (Kleynhans *et al*., 1996; Bridge & Starr, 2007). Most PPN individuals occur in the upper, cultivated soil layer within the root zones of their host plants (Kleynhans *et al*., 1996).

Most crops are particularly vulnerable to attack by PPN during the seedling stage when the young root system is becoming established (Keetch & Milne, 1982). Feeding of PPN on plant tissue, their interaction with fungi and bacteria and/or their transmission of certain PPN-borne virus diseases can also significantly retard the growth, reduce the yield and/or adversely affect the quality of most crops (Keetch & Milne, 1982). Infection of crops by PPN is often associated with early senescence and proliferation of lateral roots (Scurrah *et al*., 2005) and this usually results in poor crop performance and sometimes crop failure. In fields these symptoms tend to appear in defined patches which often show abundant weed growth and enlarge with time (Kleynhans *et al*., 1996). Globally the potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis* (known as the Golden Cyst Nematode;

GCN) are generally regarded as the economically most important nematode parasites of potato. The latter PPN are followed by species of *Meloidogyne*, *Ditylenchus, Pratylenchus*, *Nacobbus* and *Trichodorus* (Winslow & Willis, 1972; Scurrah *et al*., 2005; Jones *et al*., 2011).

In SA, 39 PPN species that belongs to 19 genera have been identified to infect potato (Keetch & Buckley, 1984; Kleynhans *et al*., 1996), with the most important nematode parasites of this crop being *Meloidogyne* spp. followed by *Pratylenchus* spp. (Kleynhans, 1978; Jones, *et al*., 2011). The golden cyst nematode, *G. rostochiensis*, has only been recorded in certain potato producing areas of SA such as Pretoria (Gauteng Province), surrounding Gauteng areas as well as in the Ceres and Sandveld areas (Western Cape Province) (Louw, 1982; Jones, *et al*., 2011) where they cause significant damage to the crop. Strict quarantine legislation, however, seems to limit the spread of this nematode species in local production areas.

Unfortunately, the conditions that favour successful potato production are also favourable for multiplication and survival of PPN (Jones, 1970). For example, hatch and movement to roots occur most rapidly in sandy soils and this contributes to crops grown on such soils, like potato, being those most likely to suffer the heaviest damage by PPN (Trudgill *et al*., 1998). Key factors that influence the extent of PPN damage to potato tubers are the initial abundance of these parasites as well as the period that the crop is in the soil. If a high RKN population, for example, is present at planting or if infected tubers are planted, potato roots will be invaded by J2 of the particular PPN species present (Jones, *et al*., 2011).

### 1.4.1 Root-knot nematodes (RKN; *Meloidogyne* spp.)

RKN are an economically important polyphagous group of highly adapted obligate endoparasitic organisms that attack potato roots and tubers in local plantings (Jones *et al*., 2011). These parasites are distributed worldwide and infect a wide range of plant species that are cultivated (Castagnone-Sereno, 2006; Moens *et al*., 2009).

*Meloidogyne* species are present in most agricultural soils in southern Africa but especially prefer and cause damage to crops planted in sandy soils (Keetch & Buckley, 1984; Louw, 1982; Kleynhans *et al*., 1996). Six RKN species were reported to be associated with potato in SA (Kleynhans *et al*., 1996; Jones *et al*., 2011) with *M. incognita* and *M. javanica* being the most widespread and damaging and constituting 27% and 41%, respectively, of populations isolated from potato plantings (Coetzee, 1968).

*Meloidogyne acronea, M. arenaria*, *M. chitwoodi* and *M. hapla* have also been recorded to infect potato in SA, but are rare in commercial plantings and little is known about their distribution (Kleynhans *et al*., 1996; Jones *et al*., 2011).

Collectively, *Meloidogyne* species are more damaging to crops such as potato than most other groups of PPN since individuals of this genus are: i) widely distributed throughout agricultural soils worldwide ii) complete several life cycles per growing season, resulting in high fecundity rates and iii) generally have very wide host ranges (Nyczepir & Thomas, 2009). Should these parasites not be managed effectively they can reach population densities that adversely affect vigour, yield (Nyczepir & Thomas, 2009) and quality of crops (Moens *et al*., 2009), such as potato. Due to the combination of factors outlined above and set against the potential financial losses that can occur as a result of RKN infection, growers invest heavily in control strategies to limit the impact of RKN. Currently the input cost for local potato production can amount to R100 000/ha, which usually includes a substantial amount for applying synthetic nematicides (CropLife, 2011; Jones *et al*., 2011).

#### 1.4.1.1 Life cycle of root-knot nematodes (RKN)

RKN typically feed and reproduce on modified, living plant cells within plant tissue such as roots and tubers in the case of potato where they induce specialized feeding cells that are referred to as giant cells (Scurrah *et al*., 2005). Mature females produce eggs in gelatinous masses composed of a glycoprotein matrix, which is excreted by rectal glands in the anal area. The gelatinous substance keeps the eggs together and protects them against environmental extremes and predation by other soil organisms (Moens *et al*., 2009). Depending upon environmental conditions, some J2 may enter diapause and remain in the egg during unfavourable conditions such as during dry seasons or during winter periods when low temperatures are experienced (De Guiran & Ritter, 1979; Hunt *et al*., 2005). In the latter cases, infective J2 may not hatch from eggs for several years after being laid (Hunt *et al*., 2005). Egg masses are usually deposited on the surface of galled potato roots and about 1cm below the surface of developing tubers, but they may also be embedded within the gall tissue. The egg mass initially has a soft, sticky and hyaline structure but becomes firmer and dark brown with age (Moens *et al*., 2009).

Hatching J2 are mobile and can move relatively long distances of between 40-100cm, both horizontally and vertically within the soil profile when soil moisture levels are optimum**.** Mobility allows the J2 to find a suitable host (Eisenback & Hunt, 2009), where it will penetrate just behind the root tip or any place on

a tuber in the case of potato plants. After penetration, the invasive J2 starts to feed on plant cells that are usually located behind the root cap in the differentiated vascular tissue. It then migrates intercellularly through the cortex to the region of cell differentiation where the female becomes sessile and develops a permanent feeding site from which she withdraws nutrients (Karssen & Moens, 2006; Moens *et al*., 2009).

In parthenogenetic RKN species, sex determinism depends strongly on environmental factors. Factors such as high population densities of the particular RKN species, nutritional deficiencies that occur in the host plant, presence of other plant pathogens, the level of resistance exhibited by the host plant as well as the concomitant limitation of food supply influence this phenomenon (Bird, 1971; Triantaphyllou, 1973; Castagnone-Sereno, 2006; Moens *et al*., 2009). When conditions are favourable, juveniles develop into females and into males when adverse conditions (as described above) prevail (Castagnone-Sereno, 2006; Moens *et al*., 2009). However, if such stress is imposed during nematode development, J2 that are developing as females can undergo sex reversal, producing intersexes or males (Triantaphyllou, 1973; Papadopoulou & Triantaphyllou, 1982).

#### 1.4.1.2 Interactions with other organisms

Secondary infection by other pathogens, i.e. fungi and/or bacteria often results in extensive decay of RKN-infected plant tissue (Moens *et al*., 2009). On potato, associations of RKN have been confirmed for bacterial wilt, *Pseudomonas solanacearum* and *Erwinia* spp. and fungi such as *Verticillium* spp., *Fusarium* spp. and *Rhizoctonia solani* (Brodie *et al*., 1993; Manzanilla-Lopéz *et al*., 2004). Resistance of potato to bacterial wilt is known to break down when the plant is simultaneously infected with *M. incognita* (Jatala *et al*., 1975; Jatala & Martin, 1977).

#### 1.4.1.3 Damage and symptoms caused by root-knot nematodes (RKN)

By disrupting the physiology of the host plant, RKN may not only reduce crop yield (quantitative damage), but in the case of potato also the product quality (qualitative damage) and are therefore of great economic and social importance (Karssen & Moens, 2006; Moens *et al*., 2009). RKN damage to potato is usually associated with light (high sand content) soils or peats (Winslow & Willis, 1972; Kleynhans *et al*., 1996). Loamy and sandy soils, usually rich in organic matter with good drainage and aeration, are most suitable for the cultivation of potato (Winslow & Willis, 1972; DAFF, 2011). Soil structure is also an important factor since pore size affects the ease with which nematodes can move

through the soil interstices. In general, sandy soils provide for optimal movement of RKN, while soils with high clay content or those with an excessively open texture inhibit movement of these parasites (Kleynhans *et* al., 1996; Hunt *et al*., 2005). An increase in the severity of RKN infections in local potato plantings as well as the resultant increased dependence on the use of chemical control by local producers, is due to favourable temperatures, soil structure and moisture status that prevail in potato-producing areas (Jones *et al*., 2011).

Potato crops infected by RKN are, however, unlikely to exhibit above-ground symptoms (Scurrah *et al*., 2005). Typical galls or knots are usually only visible on the roots and/or tubers in fields where high RKN population levels occur (Martin, 1972; Jones *et al*., 2011). RKN-infected potato plants may, however, exhibit stunting, yellowing, early senescence and tend to wilt under moisture stress (Manzanilla-Lopéz *et al*., 2004; Scurrah *et al*., 2005). Below-ground symptoms could also result in the root system being reduced or abnormal growth of the roots, excessive branching of secondary roots and overall root galling may occur and be visible (Manzanilla-Lopéz *et al*., 2004; Scurrah *et al*., 2005). Galls are initiated and produced in response to growth regulators, proteins and glycoproteins introduced into the host from subventral oesophageal glands of the feeding J2 which causes hyperplasia and hypertrophy of cortical tissues surrounding the nematode and the giant cells (Jepson, 1987; Bridge & Starr, 2007). While both potato roots and tubers are generally infected by RKN J2, the first generation occurs mainly in the root system while J2 from the second generation usually infect the tubers (Santos, 2001). RKN-infected roots generally have galls or knots of various sizes and shapes depending on the nematode density and the species present (Manzanilla-Lopéz *et al*., 2004; Scurrah *et al*., 2005; Jones *et al*., 2011). It is, however, not recommended to identify RKN species using the size and shapes of galls (Eisenback *et al*., 1981; Manzanilla-Lopéz *et al*., 2004) that are present on potato roots/tubers.

All RKN species that infect potato produce necrotic spots in the region between the tuber surface and the vascular ring. This is the result of tuber tissue reacting to the deposition of eggs and the gelatinous matrix (Scurrah *et al*., 2005). When a RKN infection is severe, tubers may rot in the soil before it is harvested (Martin, 1972) or even be deformed (Jones *et al*., 2011). The depth of J2 penetration into tubers varies but, depending on the tuber size, nematode females are usually found 1-2mm below the skin where it feeds on vascular tissue (Jatala, 1975; Jones *et al*., 2011). Swellings and lesion-like symptoms on the tuber surface as a result of RKN infection (Martin, 1972; Manzanilla-Lopéz *et al*., 2004) are more numerous in the “eye” part of the tubers (Figure 1.6), with lightly infected lesions more likely to be found in the largest tubers (Martin, 1972). At lifting, the lesions on the tubers itself may have a watery appearance and protrude above the surface of the tuber (Martin, 1972; Manzanilla-Lopéz *et*

*al*., 2004). It may, however, collapse later and resembles rough and crinkley scar tissue. Not all lesions are watery and blister-like in appearance and in many cases the presence of RKN results in small swellings only, which are often better detected by touch than by sight (Martin, 1972).

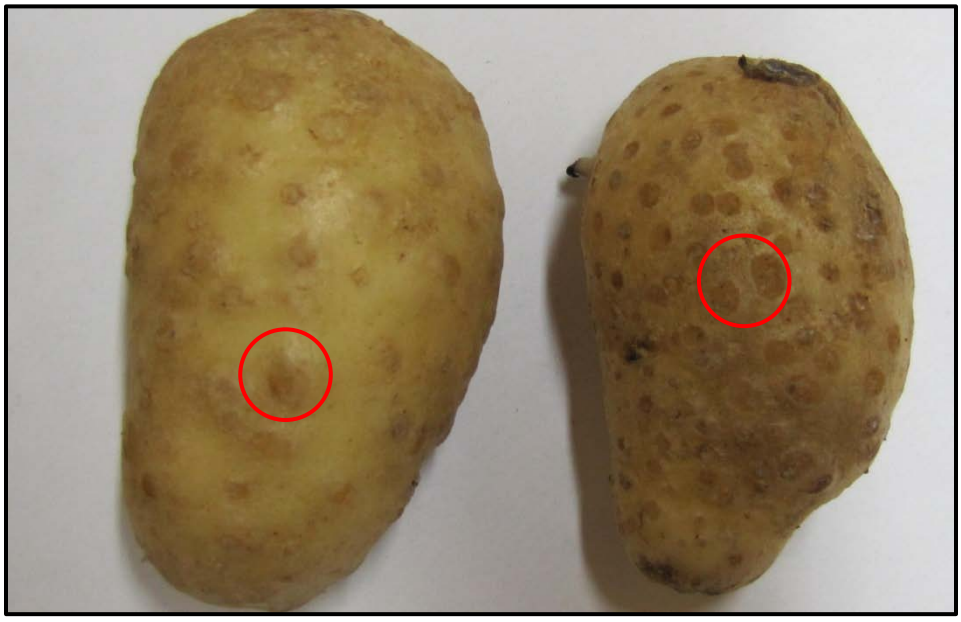

Figure 1.6. Root-knot nematode infected potato tubers, with typical knot-like protuberances visible on the surface of the tubers (Suria Bekker, Unit of Environmental Sciences and Development, NWU, Potchefstroom Campus).

### 1.4.2 Lesion nematodes (*Pratylenchus* spp.)

*Pratylenchus* species are distributed worldwide and are responsible for substantial yield loss in many agricultural and horticulture crops (Duncan & Moens, 2006). Individuals of this species were first found to infect potato in SA during 1953 in the Highveld region (Mpumalanga Province) (Koen, 1960). Seven lesion nematode spp. (Kleynhans *et al*., 1996) have been associated with potato in SA of which *P. brachyurus* is the most damaging (Koen, 1960; Jones *et al*., 2011). According to Van den Berg (1971), Koen (1960) reported *P. brachyurus* to be present in almost all potato-producing areas of SA. Martin (1972) also concluded that it is not uncommon to find lesion and RKN infecting the same tuber.

Lesion nematodes can survive in the soil in the absence of host plants for a long time (Koen, 1960; Hunt *et al*., 2005). When the infected seed potato tuber decomposes in the soil, lesion nematodes are released and can subsequently parasitize a new host (Koen, 1965; Jones *et al*., 2011). Lesion nematodes can, therefore, be spread by the planting of infected tubers in soils not previously infested

with these parasites. Potato are usually not cultivated on the same field for two years in succession and these nematodes can thus parasitize other crops such as maize, wheat, oats and various grasses (i.e. teff) that are rotated with potato and in this way assist in maintaining populations of these nematodes in the soil until the next potato crop is planted (Koen, 1965). A wide range of weeds also serves as hosts for lesion nematodes in which they can be maintained in potato fields (Koen, 1965; Ntidi *et al*., 2012).

#### 1.4.2.1 Life cycle of lesion nematodes

*Pratylenchus* species are polycyclic, polyphagous, migratory root endoparasites (Mc Donald & Nicol, 2005). The life cycle of lesion nematodes generally resembles that of RKN in that it has four moults before the mature male or female stage are reached (Van den Berg, 1982). However, unlike RKN and PCN, lesion nematodes do not induce permanent feeding sites in their host plants. Instead they feed and reproduce while migrating between or through plant cells (Duncan & Moens, 2006). All developmental stages of *Pratylenchus* species are, however, vermiform, including the mature female (Manzanilla-Lopéz *et al*., 2004; Decraemer & Hunt, 2006), and are capable of invading roots and/or tubers of host plants such as potato (Van den Berg, 1982; Bridge & Starr, 2007).

#### 1.4.2.2 Interactions with other organisms

Potato roots/tubers infected with *Pratylenchus* species are, like RKN-infected roots/tubers, more susceptible to secondary invasion by other pathogenic soilborne organisms (Koen, 1960). Since lesion nematodes are migratory endoparasites, they are more prone to interact with other root pathogens and this way enhances the resultant root disease condition (Jones *et al*., 2011). These nematodes interact with a variety of other soilborne pathogens, most notably *Fusarium* and *Phytophthora* spp. They are also associated with the fungus *Verticillium* that can cause *Verticillium*-wilt in potato (Jones *et al*., 2011). Although no such interactions have been recorded locally for lesion nematode interaction with potato, it is likely that these parasites do still influence the incidence of various soil borne pathogens that occur in potato fields (Jones *et al*., 2011).

#### 1.4.2.3 Damage and symptoms caused by lesion nematodes

*Pratylenchus* species typically cause conspicuous, dark brown or black necrotic areas on the root or tuber surface of their host plants (Figure 1.7 A&B) (Van den Berg, 1982; Mc Donald & Nicol, 2005). As they migrate through the plant tissue they cause mechanical destruction of cells (Brodie *et al*., 1993).

Symptoms are also usually associated with lesions on the roots and/or tubers that can turn from dark brown to red (Jones *et al*., 2011). Large numbers of lesion nematodes can, however, damage the roots/tubers to such an extent that the growth of the host plant is adversely affected (Koen, 1967; Manzanilla-Lopéz *et al*., 2004). When the potato plant dies or the above-ground parts are removed, the roots decay and large numbers of lesion nematodes migrate into the soil to attack the tubers (Koen, 1967). Koen and Hogewind (1967) found that lesion nematode-infected tubers are usually firm at lifting but when stored soon wither, loose mass and become hard. Symptoms on tubers can also represent purple-brown lesions that usually occur on the bottom of the tuber at the end of the growing season (Jones *et al*., 2011). The migration of lesion nematodes from the dead and dying roots is mainly responsible for the increased population levels in the soil, as only a few nematodes generally leave the tubers once they invaded and infected it. Another result of the dying-off or removal of the aerial parts of the plant is that soil temperatures may rise by as much as 6°C, which may stimulate the activity and development of PPN, such as lesion and RKN nematodes (Koen, 1967).

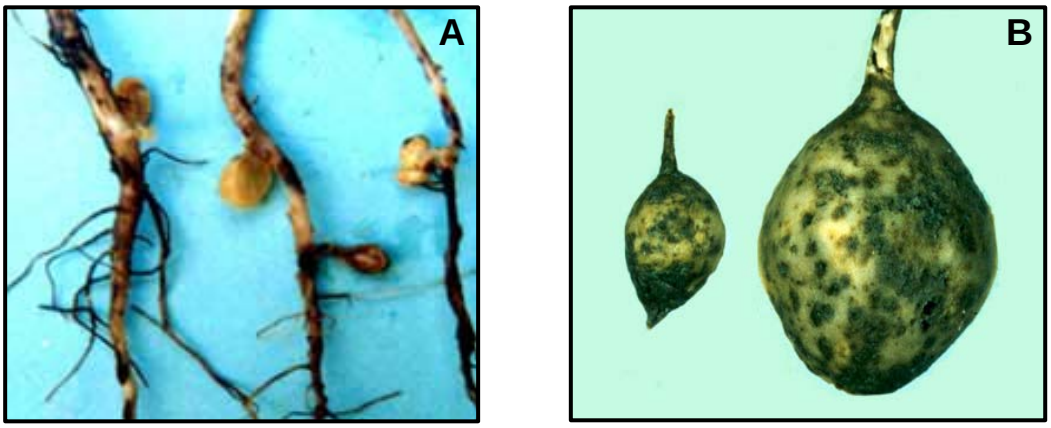


Figure 1.7. (A&B). Brown to black necrotic lesions on potato roots (A) and tubers (B) as a result of root-lesion nematode damage (A: http://jnkvv.nic.in/IPM%20Project/nematode.html) (B: H. Kawagoe & H. Nakasono, APS; http://keys.lucidcentral.org/keys/sweetpotato/key/Sweetpotato%20Diagnotes/Media/Html /TheProblems/Nematodes/LesionNematode/Lesion%20nematode.htm).

### 1.4.3 The golden cyst nematode (GCN; *Globodera rostochiensis*)

Cyst-forming or cyst nematodes, belonging to the family Heteroderidae, are one of the most specialized and successful PPN pests that infect and parasitize agricultural and horticultural crops (Turner & Evans, 1998). The GCN is believed to have evolved along with their principle host, potato, in the highlands of Peru and Bolivia (Scurrah *et al*., 2005). The GCN was reported for the first time in SA in

1971 where it infected potato roots on an irrigated farm North of Pretoria and subsequently on small holdings around Johannesburg and Bon Accord (Gauteng Province) (Knoetze *et al*., 2004). In 1999, it was again recorded in the Hankey (Eastern Cape Province), Randfontein (Gauteng Province), Mitchells Plain, Ceres and the Sandveld areas (Western Cape Province). The biological race of *G. rostochiensis* present in SA appears to be pathotype A (Jones *et al*., 2011).

The GCN, like other cyst nematodes, usually have a very narrow host range and are able to infect potato and some other Solanaceae crops such as tomato, nightshade (*S. nigrum*), bittersweet (*S. dulcamara*) and tobacco (*Nicotiana tabacum*) (Turner & Evans, 1998; Subbotin *et al*., 2010). In heavily GCN-infested fields where no control measures are applied, yields can be less than the mass of the tubers planted (Mai, 1977; Jones *et al*., 2011). Except for directly suppressing yields, GCN can indirectly cause economic losses by interacting with other micro-organisms and this way cause even higher yield losses than those caused by the nematodes alone (Mai, 1977; Turner & Evans, 1998).

The GCN are regarded as one of the most important pests of potato since they cause substantial reductions in yields of susceptible potato cultivars planted in heavily infested fields (Trudgill *et al*., 1998). The lack of inexpensive nematicidal treatments that result in an adequate level of control under such GCN-infested field conditions also contributes to the latter scenario. The relative ease with which GCN can be spread from one area/field to another, their persistence as viable cysts in soil for up to 30 years and their high reproductive capacity in the presence of a growing potato plant contributes to its importance as a major pest of potato worldwide (Winslow & Willis, 1972; Turner & Evans, 1998).

#### 1.4.3.1 Life cycle of the golden cyst nematode (GCN)

The life cycle of GCN, are well adapted towards the host plants they infect and they can survive in various environments (Turner & Evans, 1998; Nicol *et al*., 2011). Eggs inside cysts can remain viable in soil for long periods of time (Scurrah *et al*., 2005) i.e. it can survive for more than 20 years (Oostenbrink, 1966; Subbotin *et al*., 2010) in soils under severe temperature (-15°C) stress or in desiccated soils (Scurrah *et al*., 2005). The eggs contain the infective J2 and are stimulated to hatch as a result of potato root exudates (Scurrah *et al*., 2005; Nicol *et al*., 2011). Hatching of up to 80% of the eggs can be attained under favourable environmental conditions. J2 enters the root near the root tip, migrates intracellulary towards the vascular cylinder and induce a feeding cell or syncytial “transfer cell” (Manzanilla-Lopéz *et al*., 2004; Nicol *et al*., 2011). The J3 and J4 life stages subsequently develop, become sedentary and feed from the syncytia. The 4th moult gives rise to the mature, round and

swollen female, which protrudes from the root/tuber of potato. The males are slender and vermiform, leave the roots, mate and fertilize the females (Nicol *et al*., 2011). After mating, eggs develop within the female. When the female dies the cuticle forms a protective cyst, encapsulating between 200–500 eggs. The life cycle is then completed and may take up to three months to repeat. The small yellow or brown coloured cysts (pinhead size) falls off the roots/tubers, with J2 hatching from the eggs it contains as soon as root exudates of a suitable host plant are excreted (Nicol *et al*., 2011). GCN usually completes one generation during a potato growing season (Morris, 1971).

#### 1.4.3.2 Interactions with other organisms

GCN not only cause wounds in roots/tubers, but also provide entry sites for other micro-organisms as is the case with RKN and lesion nematodes. This is of particular importance to a wide range of fungi and bacteria that are important pathogens of potato (Turner & Evans, 1998; Scurrah *et al*., 2005). A number of PCN species have been found to interact with *Fusarium* wilt species causing wilt disease, for example *G. tabacum* on tobacco. Another interaction is that of *G. pallida* and the bacterium *Ralstonia solanacearum* on potato in which the nematode enhances damage caused by the associated wilt. The economic effect of these interactions varies but their effect can be important with high value crops such as potato. Therefore, the need for research aimed at host-parasite relationships is crucial in order for effective management strategies to be developed (Turner & Rowe, 2006).

#### 1.4.3.3 Damage and symptoms caused by the golden cyst nematode (GCN)

In potato plantings, GCN generally does not cause any specific above-ground symptoms nor any typical symptoms on the roots and/tubers (Kleynhans, 1978; Scurrah *et al*., 2005). However, at flowering of the crop round, yellow or cream cysts are clearly visible on roots (Figure 1.8) when the plant is carefully removed from the soil (Brown, 1969; Kleynhans, 1978; Scurrah *et al*., 2005). Root injury by GCN, causes stress and reduces the uptake of water and nutrients which in turn cause stunting, yellowing and other discolouration as well as wilting of the foliage (Trudgill *et al*., 1998; Scurrah *et al*., 2005). Growth of infected potato plants is retarded and fields heavily infested with GCN usually result in patches of plants showing symptoms such as yellowing of leaves and/or retarded plant growth, especially when low rainfall is experienced (Nicol *et al*., 2011). Root systems can also be reduced and become abnormally branched and brownish in colour. Where low GCN densities occur, tuber sizes are reduced whereas at higher densities both the number and size of tubers can be reduced

(Subbotin *et al*., 2010). Apart from yield reduction of the potato crop, the financial benefits are reduced by the costs of control measures and subsequent reduction of marketable produce (Nicol *et al*., 2011)**.**

Figure 1.8. Golden cyst nematode females visible on roots of potato (Xiaohong Wang, US Department of Agriculture; http://www.sciencephoto.com/media/139385/enlarge).

### 1.4.4 Other plant-parasitic nematodes (PPN) associated with potato in South Africa (SA)

Though PPN other than *Meloidogyne* spp., *Pratylenchus* spp. and *G. rostochiensis* have been recorded to parasitize potato in SA (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Jones *et al*., 2011), they have not been associated with crop and/or quality losses. These include various species of *Anguina*, *Aphelenchus*, *Aphelenchoides*, *Ceocenamus*, *Criconema*, *Helicotylenchus*, *Mesocriconema*, *Nanidorus*, *Paratrichodorus*, *Rotylenchus*, *Rotylenchulus*, *Tylenchus*, *Tylenchorhynchus*, *Xiphinema*, as well as *Ditylenchus africanus* (peanut pod nematode) and *Radopholus similis* (Keetch & Buckley, 1984; Kleynhans *et al*., 1996).

Internationally, however, the root tip feeders belonging to the *Paratrichodorus*, *Trichodorus* and *Nanidorus* genera are considered as important pests of potato (Scurrah *et al*., 2005; Bridge & Starr, 2007). They have the potential to cause severe damage to the root system of young developing potato plants, while they also have the ability to transmit viral diseases (Scurrah *et al*., 2005; Bridge & Starr, 2007) like the tobacco rattle virus that cause tuber spraing or corkey ringspot in potato (Louw, 1982; Manzanilla-López *et al*., 2004). This virus together with its vector *N. minor* does occur in SA and were reported from soil samples of seven of the nine provinces where potato has been planted in potato-

producing regions. *Trichodorus* spp., however, are all endemic to the natural veld and indigenous forests in SA and have not been associated with any agricultural and/or horticultural crop to date (M. Marias, pers comm., January 2011). Although *Paratrichodorus* species occurs widely in sandy soils planted to potato, their concomitant occurrence with tobacco rattle virus have not yet been reported in SA (M. Marias, pers comm., January 2011).

### 1.5 Identification of plant-parasitic (PPN) and non-parasitic nematodes (NPN)

Accurate identification of nematodes is the cornerstone upon which all aspects of research, advisory work, implementation of quarantine legislation and selection of control strategies is based. At present classical taxonomy, using morphological characteristics to describe and identify nematode genera and species, is being replaced to a large extent by the use of more sophisticated molecular methods (Perry & Moens, 2006). In some cases, molecular and bar-coding techniques used to identify nematodes may even replace traditional morphological identification of nematodes (Perry & Moens, 2006). This is mainly due to a progressive decline in the numbers of expert taxonomists that were trained to use the traditional morphological approach to identify these parasites. However, from a worldwide perspective, both classical and molecular techniques are required at present to ensure the accurate identification of nematodes (Perry & Moens, 2006).

However, to differentiate between PPN species of the same genus, for example some *Meloidogyne* spp., using morphological characteristics of their perineal patterns alone often prove to be inconclusive (Zijlstra *et al*., 2000; Hu *et al*., 2011). Reasons for this are that a morphological approach requires much skill and experience (Jepson, 1987) since considerable similarity exists between some species with regard to the formation of striae of the cuticle that represent the perineal pattern (Zijlstra *et al*., 2000; Hu *et al*., 2011). The occurrence of high intra-species variation also contributes to accurate identification of RKN being elusive (Hartman & Sasser, 1985; Zijlstra *et al*., 2000; Hu *et al*., 2011). DNA-based diagnostics, however, provide attractive solutions for reliable identification of PPN that are considered to be economically important, such as RKN (Ziljstra *et al*., 2000), and have been applied with success by a number of researchers (Powers *et al*., 1986; Piotte *et al*., 1992; Xue *et al*., 1992; Castagnone-Sereno *et al*., 1993; Fargette *et al.,* 1996, Ziljstra *et al*., 2000; Fourie *et al*., 2001; Berry *et al*., 2008).

Before research programmes or proper management strategies can be implemented, particularly where quarantine organisms are concerned, accurate and reliable identification of RKN are absolute prerequisites (Piotte *et al*., 1992; Zijlstra *et al*., 2000). Accurate identification of *Meloidogyne* spp. is

also important for proper selection of non-host crops for rotation purposes or for the use of a resistant cultivar, when/if available (Thomason & Caswell, 1987).

DNA-based methodology has been used extensively during the past few decades for the identification of the economically most important RKN species (Hu *et al*., 2011). According to literature, *M. incognita*, *M. javanica*, *M. hapla*, *M. arenaria*, and *M. chitwoodi* are discriminated from each other mainly by using COII polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) (Powers & Harris, 1993). *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are, however, distinguished from each other by means of PCR using sequence-characterized amplified region (SCAR) primers (Zijlstra *et al*., 2000; Meng *et al*., 2004). In addition, *M. incognita* or *M. javanica* could also be discriminated by using real-time PCR (Berry *et al*., 2008; Toyota *et al*., 2008). More recently, various molecular techniques have also been developed to identify an emerging RKN species, *M. enterolobii*, that is causing problems in a variety of crops and include analyses of mitochondrial DNA (mtDNA) (Blok *et al*., 2002; Brito *et al*., 2004; Xu *et al*., 2004; Tigano *et al*., 2005; Zhuo *et al*., 2010) and ribosomal DNA (rDNA) intergenic regions (IGS) (Blok *et al*., 1997; Adam *et al*., 2007),

Research-to-date has demonstrated the effectiveness of identifying and differentiating *Meloidogyne* spp. using molecular techniques. This approach together with the use of morphological methods is fundamental for research programmes and proper management strategies to be implemented, since it could improve progress and crop management decisions (Powers & Harris, 1993; Zijlstra *et al*., 2000).

On the other hand, morphological identification of nematodes (both PPN and NPN) to family and/or genus level generally poses no problems and is usually done as a standard method. Since nematodes feed on a wide variety of soil organisms their greatest apparent morphological diversity can be seen in the head and mouth structures, which are closely related to their feeding habits. According to Yeates *et al.* (1993) the following nematode feeding or trophic groups are recognised:

1. Herbivores or plant-feeders (PPN) - feeds on host plants using a stomatostylet (Tylenchida, Aphelenchida), onchiostylet (Triplonchida) or odontostylet (Dorylaimida).

2. Fungivores or hyphal feeders - NPN that penetrate fungal hyphae using a stomato- or odontostylet. In addition to obligate hyphal feeders, this group also includes the alternative life cycle of some invertebrate parasites (e.g. *Deladenus* spp.).

3. Bacterivores or bacterial feeders - NPN that feed on any prokaryote food source. These organisms (e.g. *Rhabditis* spp., *Alaimus* spp.) ingest their prey through a narrow or broad mouth (e.g. *Diplogaster* spp.), followed by an oesophagus with strong muscles.

4. Substrate feeders - ingestion of substrate material may be incidental to bacterial feeding, predation and unicellular, eukaryote feeding in many NPN. Mouths of these NPN ranges from short and broad too long and narrow and teeth may be present, suggesting a more predatory life style. The expression “non-selective deposit feeding” used for aquatic nematodes relates to substrate feeding NPN.

5. Predators or animal feeders - NPN that ingest invertebrates such as protozoa, nematodes and rotifers either as “ingesters” (e.g. *Diplogaster* spp., *Mononchus* spp., *Nygolaimus* spp.) or as “piercers” (e.g. *Seinura* spp., *Labronema* spp., *Laimaphelenchus* spp.) by sucking body fluids of their prey through a narrow stylet.

6. Feeders on eukaryotes - NPN that feed on diatoms or other algae, as well as fungal spores and yeast cells. Examples of this trophic group are *Achromadora* spp., *Diplogaster* spp. and *Fictor* spp.

7. Omnivores - NPN that feed on a wide range of foods occurring in more than one trophic level (Yeates *et al*., 2009) (particularly combining feeding types 2-6). These species are restricted to a few members of the Dorylaimida. Examples are *Actinolaimus* spp., *Aporcelaimellus* spp. and *Kochinema* spp.

8. Dispersal or infective stages of animal parasites - includes life stages of animal parasitic nematodes that occur in the soil, for example invertebrate- (e.g. *Deladenus* spp., *Heterorhabditis* spp.) or vertebrate parasites (e.g. *Strongiloides* spp.). When these stages feed and contribute to soil processes, they should be included in other appropriate categories. If they die in the soil they contribute to the nutrient pool. Families (e.g. Rhabditidae and Diplogasteridae) that use animals as phoretic (transport) hosts are not included in this group.

The relationship between nematode community structure and various agricultural practices (e.g. Wang *et al.*, 2004), however, generally refer to only five main trophic groups of NPN *viz*. bacterivores, fungivores, herbivores, omnivores and predators which are the groups that are mainly focused upon in research of this nature.

Figure 1.9 below represents a graphical illustration and explanation of the proposed faunal profile into which soil can be categorised according to the presence, abundance and diversity of NPN, soil nematodes (Ferris *et al*., 2001).

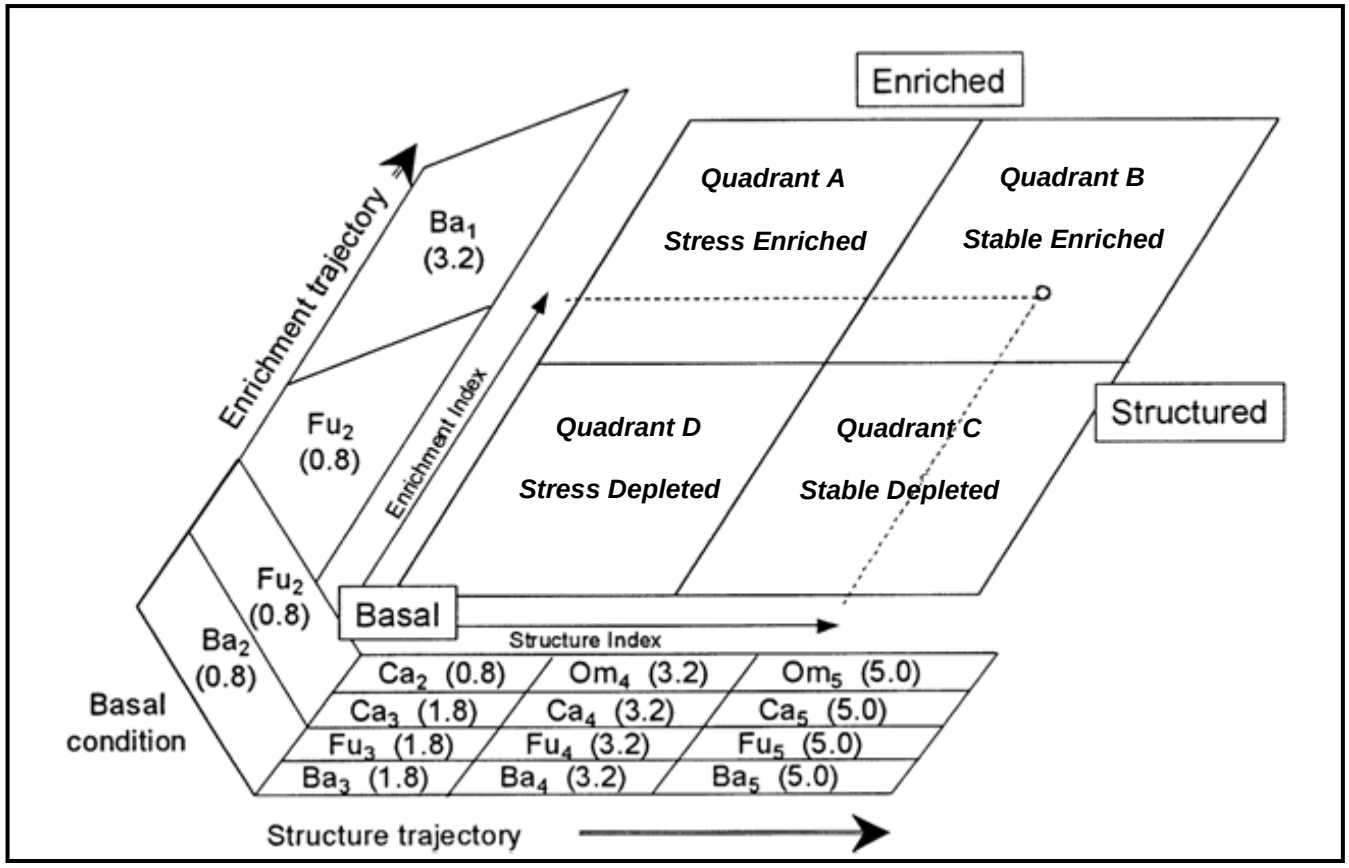


Figure 1.9. Functional guilds of non-parasitic, soil nematodes characterized by their respective feeding habit (trophic group) and by life history characteristics as expressed along a colonizer–persister (cp) scale (after Bongers & Bongers, 1998) (Graphical illustration from Ferris *et al*., 2001).

The Enrichment Trajectory (representing the Enrichment Index; EI) and the Structure Trajectory (representing the Structural Index; SI) are both based on the indicator importance of functional guilds of nematodes and are thus descriptors of food web conditions (Neher *et al*., 2004). This way quantification of the state of the soil food web through the EI (a measure of opportunistic bacterivore and fungivore nematodes), the Channel Index (CI; indicator of predominant decomposition pathways) and the SI (indicator of food web state affected by stressor disturbance) can be determined (Neher *et al*., 2004).

Functional guilds of nematodes are defined as a matrix of their feeding habits, their biological, ecological as well as life history characteristics which are all incorporated in the cp classification and ultimately graphically illustrated in the simplified food web (Neher et al., 2004). Thus, the Ba1 functional guild comprises cp1 bacterivores such as those in the Rhabditidae and Panagrolaimidae families; Ba2 those grouped in the Cephalobidae, Monhysteridae and Plectidae families and Ba3 those belonging to the Prismatolaimidae family (Ferris & Matute, 2003; Neher *et al*., 2004).

NPN representing bacterial-feeding in cp1 and fungivores in cp2 are indicators of enrichment (*e*), while nematodes of all feeding habits classified as cp2 are considered basal (*b*) to both enrichment and structure trajectories. Ultimately, nematodes of all feeding habits in cp3-5 are indicators of structure (*s*). Functional guilds of NPN, soil nematodes are characterized by feeding habit (trophic group) and by life history characteristics (Bongers & Bongers, 1998). Indicator guilds of soil food web conditions (basal, structured, enriched) are then designated and weightings of the guilds along the structure and enrichment trajectories are provided, for determination of the Enrichment Index and Structure Index of the food web (Neher *et al*., 2004). Although the enrichment trajectory can de dissected further to determine flow down fungal and bacterial decomposition channels according to the Channel Index, soil food webs in fields in the Christiana area sampled during this study were only categorised according to the Enrichment and Structure Trajectories to indicate the basic status of these fields in terms of soil health. An explanation of terminology (Ferris *et al*., 2001) important to understand and interpret faunal graphs are given below:

- Colonizer-persister (cp) scale: Assignment of taxa of soil and freshwater nematodes to a 1-5 linear scale according to their *r* and *K* characteristics.

- cp1: Short generation time, small eggs, high fecundity, mainly bacterivores, feed continuously in enriched media, form dauer larvae as microbial blooms subside.

- cp2: Longer generation time and lower fecundity than the cp-1 group, very tolerant of adverse conditions and may become cryptobiotic. Feed more deliberately and continue feeding as resources decline. Mainly, bacterivores and fungivores.

- cp3: Longer generation time, greater sensitivity to adverse conditions. Fungivores, bacterivores and carnivores.

- cp4: Longer generation time, lower fecundity, greater sensitivity to disturbance. Besides the other trophic roles, smaller omnivore species.

- cp5: Longest generation time, largest body sizes, lowest fecundity, greatest sensitivity to disturbance. Predominantly carnivores and omnivores.

### 1.6 Management practices to reduce plant-parasitic nematodes (PPN)

Producers in intensive crop production areas worldwide usually have to choose the practice(s) that is best suited to reduce PPN populations that damage their crops. Such decisions are mainly influenced by crop history, characteristics of a particular crop as well as PPN species that are present in a given field (Nyczepir & Thomas, 2009)**.** Traditional knowledge and agricultural practices are being recalled and reviewed on a frequent basis to assess its potential to address damage caused by PPN in current potato-based cropping systems (Manzanilla-López *et al*., 2004).

The control of PPN, in this case RKN, is basically aimed at maintaining their population levels below economic threshold densities since total eradication of these parasites is virtually impossible (Keetch & Milne, 1982; Kleynhans *et al*., 1996). Therefore, management approaches currently used to manage PPN are more holistic, including a combination of tools based on producers' needs rather than scientific ideology (Sikora *et al*., 2005). Crop protectors and scientists should also acquire knowledge on the ecological nature of plant diseases (Manzanilla-López *et al*., 2004) as well as other important pests that attack crops. While the main focus previously was on the eradication of PPN in the soil and crop tissue by means of synthetically-derived chemical substances, a wider and collective view emerged during the last decade which is aimed at applying sustainable nematode management strategies with some yield loss being accepted (Sikora *et al*., 2005). Increasingly important is the need to take into consideration the impact of the pest management strategies on biodiversity (both fauna and flora) and the ecological balance in soils (Moens *et al*., 2009). Even though there are differences in the various approaches to control PPN, the ultimate goals remain the same, namely to reduce PPN populations and enable sustainable crop production by increasing yields at cost-effective levels (Sikora *et al*., 2005).

Although multiple nematode management strategies are available to reduce levels of RKN in potato-based cropping systems, only a few will be elaborated on.

### 1.6.1 Chemical control

Chemical control is the most expensive but also the quickest and usually the only practical way to control PPN that infect high-value cash crops such as potato (Kleynhans, 1978). The main advantage of chemical control compared to other methods is that target PPN populations such as RKN, are usually reduced to very low levels within a few days after application of a nematicide. The producer can thus grow a crop during or soon after nematicide application (Keetch & Milne, 1982).

At present the use of synthetically-derived nematicides is the predominant and most popular strategy applied by local potato producers to minimise damage to their produce (Jones *et al*., 2011). A wide range of nematicides, fumigants and non-fumigants are registered in SA for use on a range of agricultural and horticultural crops and in particular on potato (Nel *et al*., 2007; CropLife, 2011; Table 1.2). These nematicides are generally classified into two main groups, namely fumigant and non-fumigant (contact and systemic) products with a third group being added recently and which include the seed treatment nematicidal applications (Nyczepir & Thomas, 2009). Generally the most effective nematicidal treatment, however, is fumigation of soils prior to planting using various products such as methyl bromide or EDB® (ethylenedibromide), Telone®, Telopic® or Herbifume® (Sikora & Fernández, 2005; CropLife, 2011). Methyl bromide is, however, not available for commercial use in SA anymore (Nel *et al*., 2007; CropLife, 2011), with potato producers still being heavily reliant on the use of EDB® and other fumigants mentioned earlier.

Another consideration is that chemical control of PPN on potato in SA is very expensive and is only a short term solution because populations of these parasites can increase to reach damaging thresholds within a couple of months after application. This could lead to more applications as well as higher dosage rates of the product being applied. Therefore, chemical control should only be used on a preventative rather than curative basis (Jones *et al*., 2011).

Although the use of nematicides is at present still an important tool for controlling PPN, its dominance as a control strategy is progressively decreasing (Nyczepir & Thomas, 2009). Many of the nematicides that were previously available have been or are being removed from agricultural markets due to worldwide environmental and human health awareness issues. Therefore, effective control practices that employ alternatives to conventional chemical control should increasingly be investigated and implemented (Batchelor, 2002).

Also, due in part to the small market share of nematicides relative to herbicides, insecticides and other pesticides, there is little prospect for the development of novel chemistry in terms of nematicides in the near future (Moens *et al*., 2009). This increases the risk that PPN could adapt to the few chemicals that are still registered for use on crops such as potato. The latter could result in the selection of PPN populations developing resistance against a particular nematicide (Kleynhans *et al*., 1996) that is used continuously on potato crops.

Table 1.2. Synthetic nematicides currently registered on potato in South Africa to reduce population levels of plant-parasitic nematodes (PPN) (CropLife, 2011).

| Mode of action | Active(s) | F/T | Concentration | Product name | Reg no. | Company name |
|---|---|---|---|---|---|---|
| Fumigant | 1,3 Dichloropropene | FU-O | 1 110 g $l^{-1}$ | Telone II® | 5223 | Dow AgroSciences SA (Pty) Ltd |
| Fumigant | 1,3 Dichloropropene | GE | 1 110 g $l^{-1}$ | DD92® | 4988 | BASF South Africa (Pty) Ltd |
| Systemic | Aldicarb | GR | 150 g $kg^{-1}$ | Sanacarb® | 4717 | Bayer (Pty) Ltd |
| Systemic | Aldicarb | GR | 150 g $kg^{-1}$ | Temik® | 590 | Bayer (Pty) Ltd |
| Contact | Cadusafos | EW | 100 g $l^{-1}$ | Rugby 10 ME® | 6368 | FMC SA (Pty) Ltd |
| Contact | Cadusafos | GR | 100 g $kg^{-1}$ | Rugby 10 G® | 4110 | FMC SA (Pty) Ltd |
| Contact & systemic | Ethoprophos | GR | 150 g $kg^{-1}$ | Mocap GR® | 6985 | Bayer (Pty) Ltd |
| Systemic | Fenamiphos | CS | 240 g $l^{-1}$ | Nemacur 240 CS® | 7247 | Bayer (Pty) Ltd |
| Systemic | Fenamiphos | EC | 400 g $l^{-1}$ | Fenamiphos 400 EC® | 7167 | Erintrade cc t/a RT Chemicals |
| Systemic | Fenamiphos | EC | 400 g $l^{-1}$ | Nemafen® | 7310 | Novon Crop Protection |
| Systemic | Fosthiazate | EC | 900g $l^{-1}$ | Nemathorin 900 EC® | 5612 | ISK Biosciences |
| Systemic | Fosthiazate | GR | 100 g $kg^{-1}$ | Nemathorin 100 GR® | 5613 | ISK Biosciences |
| Fumigant | Furfural | EC | 900 g $l^{-1}$ | Crop Guard® | 6864 | Illovo Sugar Ltd |
| Fumigant | Ethylenedibromide | VB | 1 800 g $l^{-1}$ | EDB® | 4509 | Cropchem |
| Contact & systemic | Oxamyl (root-knot nematodes) | GR | 100 g $kg^{-1}$ | Blockade G® | 7552 | Klub M5 |
| Contact & systemic | Oxamyl (root-knot nematodes) | GR | 100 g $kg^{-1}$ | Oxagran 100 GR® | 6406 | Nialcor |
| Contact & systemic | Oxamyl (root-knot nematodes) | GR | 100 g $kg^{-1}$ | Vydate 100 GR® | 3945 | Du Pont de Nemours Int SA |
| Contact & systemic | Oxamyl | SL | 250 g $l^{-1}$ | Oxatak SL® | 7589 | Meridian Agrochemical Co.(Pty) Ltd |
| Contact & systemic | Oxamyl | SL | 250 g $l^{-1}$ | Oxadate® | 7588 | Tsunami Plant Protection (Pty) Ltd |
| Contact & systemic | Oxamyl (root-knot nematodes) | SL | 310 g $l^{-1}$ | Blockade SL® | 7345 | Klub M5 |
| Contact & systemic | Oxamyl (root-knot nematodes) | SL | 310 g $l^{-1}$ | Platoon SL® | 7370 | Villa Crop Protection (Pty) Ltd |
| Contact & systemic | Oxamyl (root-knot nematodes) | SL | 310 g $l^{-1}$ | Vydate SL® | 5057 | Du Pont de Nemours Int SA |
| Contact & systemic | Oxamyl (root-knot nematodes) | WSC | 310 g $l^{-1}$ | Vigour 310 SL® | 8088 | Agchem |

### 1.6.2 Alternative strategies to manage plant-parasitic nematodes (PPN)

#### 1.6.2.1 Host plant resistance

Breeding of plants resistant to specific species of for example RKN or cyst nematodes is probably one of the most economical and effective methods of control since it affords the grower at least the following advantages, namely:

- should the growth and yield of the resistant plant be comparable to those of its susceptible counterpart, its use to control nematodes will involve little additional expense (Keetch & Milne, 1982; Cook & Starr, 2005; Nyzepir & Thomas, 2009) and

- soil and climatic conditions required for the cultivation of resistant plants are not as critical as those necessary for the efficient use of nematicides (Keetch & Milne, 1982).

Host plant resistance is generally defined as the ability of the host to minimise penetration, development, reproduction and fecundity of PPN and ultimately result in minimal damage to the host plant (Bos & Parlevliet, 1995; Hussey & Jansen, 2002; Roberts, 2002). On the other hand, crops highly susceptible to attack by PPN, such as RKN, allow a rapid build-up of population levels of these parasites since they support optimal development and reproduction (Bos & Parlevliet, 1995; Roberts, 2002) of the parasites even when low initial population densities are present at planting (Keetch & Milne, 1982; Whitehead & Turner, 1998). Resistance/susceptibility on the one hand and tolerance/sensitivity on the other hand are defined as independent, relative qualities of a host plant's reaction to nematode infection based on comparison between a susceptible and resistant genotype (Bos & Parlevliet, 1995).

Various mechanisms exist in terms of host plant resistance, including non-preference (antixenosis), antibiosis and tolerance (Bos & Parlevliet, 1995). Antixenosis refers to the response of a pathogen to a host plant that lacks characteristics to serve as a host, resulting from negative reactions or total avoidance during the search for food, oviposition sites or shelter. Antibiosis, on the other hand, includes all adverse effects exerted by the host plant on the biology, i.e. survival, development and reproduction of the pathogen (Bos & Parlevliet, 1995). Host plants that suffer little damage, even when heavily infected with nematodes that would severely damage susceptible host plants are, on the other hand, classified as tolerant (Bos & Parlevliet, 1995). Furthermore, a host plant is hypersensitive when high levels of damage are suffered, even when relatively lightly infected with PPN (Bos & Parlevliet, 1995).

However, only a few plants have been classified as being immune to PPN (Keetch & Milne, 1982) which entails total exclusion of the pathogen (Bos & Parlevliet, 1995).

Most resistant plant cultivars that are used in cropping systems are usually resistant to only one species of RKN (vertical/qualitative resistance) (Roberts, 2002). Such resistant hosts may also be susceptible to other PPN genera or even to other populations or biotypes of the same species (Roberts, 2002; Starr & Mercer, 2009). The lack of resistance to multiple nematode genera is one of the main limitations of nematode-resistant plants. A further complication may be the occurrence of biotypes within a given RKN species that are able to overcome the resistance of a plant (Roberts, 2002). Quantitative/horizontal resistance on the other hand refers to a host that is resistant against more than one species/race/population of a specific PPN (Roberts, 2002).

In terms of RKN in particular, inclusion of host plant resistance in potato cropping sequences together with non-host crops is suggested to be one of the most economical ways to reduce population levels of these parasites (Scurrah *et al.*, 2005). None of the potato cultivars available in SA are, however, resistant against lesion nematodes, PCN or RKN that occur in local potato-producing soils (Jones *et al*., 2011). In fact, no potato breeding material adapted to warmer climates of the world that confer resistance to thermophyilic RKN species (*M. incognita* and *M. javanica*) had been developed by the mid 2000's (Scurrah *et al*., 2005). However, the dominant *Rmc1* gene in *S. bulbocastanum* (wild potato) has been reported to confer resistance to *M. chitwoodi, M. fallax and M. hapla,* which represents cryophilic RKN species that prefers colder climates (Brown *et al*., 1996; Cook & Starr, 2006; Evans & Perry, 2009). On the other hand, potato cultivars resistant to PCN are available in Australia, Europe, New Zealand and the USA (Finlay *et al*., 1998; Cook & Starr, 2006) but not in SA (Jones *et al*., 2011).

The only way to use available cultivars to address RKN problems in local potato plantings is to plant short growing cultivars that could be lifted before too many life cycles of RKN developed in their roots and tubers. This way population levels of these parasites in soils could be limited, while damage to tubers could also be reduced substantially or even prevented (Jones *et al*., 2011).

#### 1.6.2.2 Biological control

Biological control is defined as the management of plant diseases and pests by using living organisms or antagonists such as predators and parasites of organisms that kill or damage their hosts (Moens *et*

*al*., 2009). Furthermore, the use of microbes that indirectly influence the establishment, function and survival of pathogens and pests adversely is included in this definition (Moens *et al*., 2009).

A wide range of bacterial and fungal pathogens and antagonists have been evaluated for use as biological agents to reduce population levels of economically important PPN, such as RKN, PCN and *Pratylenchus* spp., of potato. These include active antagonists such as *Paecilomyces lilacinus*, *Bacillus firmus*, *B. chitinosporus*, *B. laterosporus*, *B. licheniformis*, *Pochonia chlamydospona*, *Pasteuria penetrans*, *Burkholderia cepacia*, *Rhizobium* spp., *Pseudomonas* spp., *Glomus* spp., *Fusarium* spp. and *Trichoderma* spp. (Hallman *et al*., 2009). Studies with *P. lilacinus* showed that the fungus consistently infected eggs (80-90%) and occasionally females of *M. incognita* that parasitized potato roots in Peru. The same fungus also penetrated 70-90% of eggs inside *G. pallida* cysts (Jatala *et al*., 1979).

Except for fungi and bacteria that act as biological antagonists of PPN, other natural-occurring predatory nematodes as well as protozoa that live in soil have also been documented to feed on PPN in local agricultural soils (Keetch & Milne, 1982; Sikora, 1992). It is, however, important that population levels of such micro-organisms are stimulated by implementing soil-friendly approaches to optimize the impact thereof. Such strategies include the incorporation of organic material, i.e. manures, compost and above-ground parts of cover crops, which is seldom used by local potato producers. However, although notable effects on PPN population levels have been obtained with the application of biological agents itself under experimental conditions, their use in local, annual cropping systems such as potato-based sequences at present is very rare (Jones *et al*., 2011).

#### 1.6.2.3 Quarantine

Quarantine aims at excluding or intercepting exotic PPN, that cause major yield and quality problems of crops in certain regions of the world, or isolating and eradicating them once they have been detected and/or become established (Keetch & Milne, 1982). Such strategies are considered a preventive and not a curative approach that are applied to prevent and/or stop the introduction and/or increased dissemination of economically important PPN into a country, local region or planting site (Nyczepir & Thomas, 2009). The prevention of an alien, PPN pest from becoming established in a country, area or field, however, is seldom achieved because of the efficient natural spread of these parasites, the large scale movement of infected plant and planting material, the ability of PPN to establish themselves rapidly on host plants and the difficulty in completely eradicating them from infected plants or infested

soil (Nyczepir & Thomas, 2009). Furthermore, the information on which the implementation of control measures is based is derived almost exclusively from the sampling of bulk consignments of soil or plant material (Nyczepir & Thomas, 2009). Since only a few small samples can, however, be examined the chances of detecting one or two live PPN in a consignment of potato for example are minimal (Keetch & Milne, 1982). The long-distance movement of plant material supported by the global markets for agricultural products has also supported the spread of important PPN around the world and between countries on the same continent (Sikora *et al*., 2005). Therefore, quarantine services cannot indefinitely prevent the introduction into and establishment of alien PPN species in an area. The best that can be done is to delay and monitor the latter scenario (Swart, 2011).

Currently, the PPN classified as quarantine organisms for local purposes are *G. rostochiensis*, *Ditylenchus destructor*, *M. chitwoodi* and *M. fallax* (Swart, 2011). Control of GCN for example in SA is achieved mainly by quarantine measures since no potato plantings are permitted on GCN-infested fields (Jones *et al*., 2011). The nematode *Ditylenchus africanus* was first isolated in 1987 in SA from the pods of groundnuts and was initially identified as *D. destructor* (De Waele *et al*., 1989). The latter PPN is regarded as a serious pest of potato in parts of Europe and the Soviet Union (Hooper, 1973). Experiments, however, later showed that all local potato cultivars tested were identified as poor hosts to the South African population of this PPN species and that no damage was caused to the tubers (De Waele *et al*., 1991). The South African population was therefore, later considered to be a new species and described as *D. africanus*, which does not pose a risk to potato (Wendt *et al*., 1995).

The RKN *M. chitwoodi* and *M. fallax* are also quarantine organisms and important pests of potato in Europe, North America, New Zealand and Australia where it causes serious economic losses due to direct damage to the plant and tuber (Nicol *et al*., 2011). *M. chitwoodi* was reported for the first time in SA on potato in the Maclear area (Eastern Cape Province). Since then it has been reported on potato in the Clocolan (Free State Province), Boston and Mooiriver areas (KwaZulu-Natal Province) (Jones *et al*., 2011). Although Fourie *et al*. (2001) reported the first record of *M. fallax* from groundnut in the Vaalharts area (Northern Cape Province), the distribution of this species as well as *M. chitwoodi* in potato production areas remain unknown.

#### 1.6.2.4 Crop and fallow rotations

The basic concept of nematode control using crop rotation is to only grow crops susceptible to PPN sporadically to reduce populations of these parasites and prevent them from damaging crops (Keetch &

Milne, 1982; Coyne *et al*., 2009). This way it may also prevent low PPN population densities from increasing to levels at which these parasites would cause economic losses to the potato and other rotation crops (Keetch & Milne, 1982; Coyne *et al*., 2009; Jones *et al*., 2011).

Crops that are good hosts to RKN and should not be included in local rotation cycles with potato include Fabaceae crops (beans and peas), maize (*Zea mays*), Solanaceae crops (tomato, paprika, tobacco) and lucern (*Medicago sativa*) in particular (Jones *et al*., 2011). Inclusion of maize in potato-based systems could for example lead to high RKN populations since *M. incognita* and *M. javanica* are the predominant economically important nematode pests in maize-production areas where potato are also cultivated (Riekert, 1996; Riekert & Henshaw, 1998). Ngobeni *et al*. (2010), however, reported various local maize hybrids that are resistant to both *M. incognita* and *M. javanica*, which can be used by local potato producers to reduce RKN population in such areas.

As part of crop rotation systems, cover crops that are non-hosts to PPN have already been used successfully in various crop rotation systems worldwide (Sikora *et al*., 2005; Coyne *et al*., 2009). A variety of cover crops/grasses are listed as poor hosts of the predominant *M. incognita* and *M. javanica* that occur in local potato-producing areas (Taylor & Martin, 1960; Fourie *et al*., 2007; Jones *et al*., 2011) and could be used in such rotation systems. These include grasses like Bloubuffel grass (*Cenchrus ciliaris*), Borseltjie grass (*Anthephora pubescens*), *Crotalaria* spp., Kleinbuffels grass (*Panicum coloratum*), mustards (*Brassica* spp.), Oulands grass (*Eragrostis curvula* cv. Ermelo), Rhodes grass (*Chloris gayana*), Smutsvinger grass (*Digitaria eriantha*) and Vetiver grass (*Vetivera zizanioides*). Van Biljon (2004) also reported that several *Sorghum* spp., wheat (*Triticum* spp.), oats (*Avena sativa*) and alfalfa (*Medicago sativa*) cultivars as well as a variety of grass species are resistant to *M. incognita* and *M. javanica,* while Fourie *et al*. (2006) listed the soybean (*Glycine max*) cultivar Egret as resistant to both *M. incognita* and *M. javanica*. The current situation in SA is that potato producers generally include the crop in a 3-4 year rotation system with maize and wheat (Sellschop & Du Toit, 1948; Black, 2008), while producers in the Christiana area only plant potato every 7-8 years. Wheat, maize and grasses are used as the main rotation crops and/or cover crops in these cropping systems while some fields are also often left fallow for a year.

The biggest advantage of a crop rotation strategy compared to most other cultural control methods or the use of a nematicide is that it generally produces an economic return on investment (Nyczepir & Thomas, 2009). If sufficient markets are available to support profitable production of crops that are poor

or non-hosts of RKN, studies can be done to exploit host range limitations of many PPN species. Key factors in determining economic viability of such an approach are (Nyczepir & Thomas, 2009):

- accurate identification of PPN species, RKN in particular in the case of potato in SA, and knowledge of the host range for these target species,
- knowledge about crops that are non-hosts to these PPN species and that are agronomically suited to the geographic region, and
- ensuring that populations of other pathogens or pests are not significantly enhanced by the rotation crop.

However, the implementation of crop rotation is not as simple as a nematicide application because rotation crops often require specialized farm equipment and production practices (Halbrendt & LaMondia, 2004; Nyczepir & Thomas, 2009). Crop rotation *per se* also frequently results in less profit per unit of land, while use of varieties resistant to RKN in this case may carry yield penalties when compared with their susceptible counterparts (Nyczepir & Thomas, 2009). Moreover RKN and lesion nematodes have very wide host-ranges which is the main factor that limits the use of crop rotation as a management strategy. The latter limitation is aggravated by the occurrence of physiological races of *Meloidogyne* species since crops that are tolerant to some races may be susceptible to others (Keetch & Milne, 1982).

Except for rotating crops, a period(s) of fallowing is usually included for a specific field(s) within cropping cycles (Nyczepir & Thomas, 2009). Keeping a land free of all vegetation for a specific time is known as clean fallowing. It can be done by frequently tilling or ploughing the soil or by eliminating all plant growth by means of herbicide application (Keetch & Milne, 1982). Some crop rotations incorporate an extended period of time where the land is left fallow before replanting of another agricultural crop (Nyczepir & Thomas, 2009). The general objective in using a fallow rotation is to reduce the population of PPN prior to replanting the site through starvation, desiccation and increase in soil temperature (Keetch & Milne, 1982; Nyczepir & Thomas, 2009). The apparent benefits of applying fallowing to crop fields to reduce PPN populations are clear. Nearly all PPN are suppressed simultaneously, with minimal expenses being associated with this strategy compared to planting and maintaining a crop (Nyczepir & Thomas, 2009). Many potato growers involved in intensive crop production systems do, however, not rely on fallowing as a primary tactic to suppress RKN. Lack of income from the fallow land, the expenses associated with regular weed control which is preferably every three weeks to

prevent RKN reproduction and potentially deleterious effects from soil erosion are all factors that contribute towards the limited acceptance of fallowing (Halbrendt & LaMondia, 2004; Sikora *et al*., 2005; Nyczepir & Thomas, 2009).

However, essential to the success of any rotation cycle is effective weed control because the presence of a variety of weed species that grow in potato fields can result in the build-up of high RKN and other PPN populations despite the poor-host status of for example a resistant and/or non-host crop (McSorley *et al*., 1994; Bélair & Parent, 1996). Various weed species have been associated with economically important RKN as well as other PPN that occur in local agricultural soils (Keetch & Buckley, 1984; Ntidi *et al*., 2012). Occurrence of such weeds in potato fields may have serious effects for local producers in terms of RKN infection of the crop.

#### 1.6.2.5 Soil amendments and cover- and/or green manure crops

Soil amendments, such as livestock or poultry manure and organic compost are often applied to soils used for intensive annual crop production (Nyczepir & Thomas, 2009). Suppression of PPN due to application of such soil amendments has been recorded and is suggested to result from the enhanced activity of antagonists of PPN in response to elevated levels of organic matter decomposition (Widmer *et al*., 2002). The control of PPN is however, seldom the primary reason for such applications. Amendments are rather applied as low cost sources of plant nutrients, acceptable methods for the disposal of animal waste or other agricultural by-products or to improve soil properties (Nyczepir & Thomas, 2009)**.** As organic waste production increases in the future and disposal options become more restricted, the addition of soil amendments to intensive cropping systems is, however, likely to increase (Zasada *et al*., 2008). Application of such amendments, however, poses problems for local commercial potato producers whose crop fields are big, usually in excess of 20ha. Huge amounts of manures or compost are needed in such cases to obtain the desired effect in terms of increasing the organic content of soils and concurrently the numbers of soilborne antagonists of PPN (Coyne *et al*., 2009).

A strategy that promises potential to support/supplement the incorporation of organic substances is, however the use of green manure crops. A more effective and viable method to manage PPN is to use plant species of which the aerial parts can be cut off, slashed and ploughed into the soil as a green manure (Keetch & Milne, 1982). Cover and green manure crops that are intended to suppress PPN populations are increasingly used (Nyczepir & Thomas, 2009). In addition such crops prevent/reduce

soil erosion and increase soil quality. Crops that are poor or non-hosts for RKN and prevent the build-up of population levels of these parasites are the best to use for this purpose (Timper *et al*., 2006).

Brassicaceae crops, also referred to as cruciferous crops, offer a potential alternative to the use of synthetically-derived nematicides in intensive cropping systems to reduce PPN populations (Akhtar & Alam, 1991; Sarwar *et al*., 1998; Bending & Lincoln, 2000; Zasada & Ferris, 2004). These crops produce glucosinolates (GL), that when hydrolysed, produce isothiocyanates that are biocidal to a range of soil organisms (Akhtar & Alam, 1991; Sarwar *et al*., 1998; Bending & Lincoln, 2000; Zasada & Ferris, 2004). The term biofumigation is used to indicate that volatile substances are produced through microbial degradation of organic amendments that result in significant toxic activity towards PPN and/or other soilborne pests or diseases (Bello, 1998).

The efficacy of a range of Brassicaceae species is used to reduce population levels of PPN (Mojtahedi *et al*., 1991; Lazzeri *et al*., 2004), other soilborne pathogens (Ramirez-Villapudua & Munnecke, 1988; Subbarao *et al*., 1999), insects (Williams *et al*., 1993) and weeds (Brown & Morra, 1995) in agricultural systems. The modes-of-action of these plant species in terms of reducing PPN is suggested to be the production of glucosinolate-degradation products (i.e. isothiocyanates, thiocyanates and nitrogenous substances) that are toxic to nematodes (Lazzeri *et al*., 1993; Zasada *et al*., 2003), but beneficial towards stimulation of communities of microbial communities present in the amended soil that are antagonistic to nematodes (Cohen *et al*., 2005). Various members of the Brassicaceae family have been used as green manures to manage RKN in particular (Mojtahedi *et al*., 1991; Lazzeri *et al*., 2004).

To achieve the maximum benefit from utilizing Brassicaceae species as cover and/or green manure crops for the control of soilborne pathogens, a Brassicaceae species with a high potential of GL production and also one that produces large quantities of biomass in the selected geographic location or environment should be selected (Kirkegaard & Sarwar, 1998; Morra & Kirkegaard, 2002; Zasada *et al*., 2003). Research by Zasada *et al*. (2003) demonstrated that amended materials of Brassicaceae species differed substantially in terms of their effect on PPN and other soilborne organisms. For example, population levels of the citrus nematode *Tylenchulus semipenetrans* was consistently suppressed by brassicaceous amendments (Zasada *et al*., 2003), while its effect on *Fusarium* spp. (Ramirez-Villapudua & Munnecke, 1988) and weeds varied (Brown & Morra, 1995). The latter authors suggested that for consistent and reliable suppression of pests and diseases in crop management systems amended with Brassicaceae species, it is crucial to investigate and understand all component mechanisms that have an impact against the target soilborne organisms present.

Another important factor to keep in mind when chemical biofumigant properties of Brassicaceae crops is exploited for use in a cropping system(s) is that such species should not be good hosts of the target PPN (Stirling & Stirling, 2003). Such species of Brassicaceae exists and according to Pattison *et al*. (2006) *Raphanus sativus* represents an example of such a species that generally had the most consistent resistance of the 43 Brassicaceae cultivars screened for host suitability against *Meloidogyne* species during their study. It also proved to be a robust performer under tropical conditions where it was less sensitive to hot conditions as well as attack by insect pests. Other authors, however, argue that the resistance of Brassicaceae cultivars to RKN appear to be more related to genotype than its GL content (Potter *et al.*, 1998). However, other studies indicated that the suppression of *M. javanica* and *M. arenaria* after soil incorporation of Brassicaceae tissues is not related to the GL content, but that mechanisms other than biofumigation may have caused reduction in numbers of these parasites (McLeod & Steel, 1999). The latter results, however, contradict those by Kirkegaard and Sarwar (1998); Morra and Kirkegaard (2002); Zasada *et al*. (2003) and Curto *et al.* (2005). The latter authors evaluated and selected different Brassicaceae species, namely *E. sativa*, *B. juncea* and *R. sativus* as well as Capparaceae species for their high active GL content and biocidal activity against *M. incognita* J2. Accordingly all varieties screened could be used in pest management programmes based on their ability to reduce *M. incognita* infestations in the soil due to their high GL content and subsequent good nematicidal activity.

However, although used widely, controversy still exists about the ability of Brassicaceae crops to reduce populations of PPN through biofumigation. The potential reduction of PPN populations in the soil following incorporation of the green manures may be offset by the reproduction of the PPN in the roots of Brassicaceae plants susceptible to these parasites during their growth period (McLeod *et al*., 2001). It will thus be necessary to define the best strategy to obtain significant PPN control by careful selection and optimal use of cover and green manure crops, such as Brassicaceae, with biofumigation properties in rotation systems (Curto *et al*., 2005). At present the use of such crops in local cropping systems, including potato, is limited. There is, however, a movement in this direction since producers realise that alternatives must be investigated and found due to the withdrawal of various effective, synthetically-derived nematicides that were traditionally used to address the RKN problem in local potato plantings.

### 1.7 Integrated nematode management and future strategies

At present, substantial gaps exist in our knowledge about multiple interactions between the soil ecosystem, weeds and other pests (including PPN) and diseases (Nyczepir & Thomas, 2009). Development of nematode-integrated management programmes thus necessitates analysis of all biotic and abiotic factors, on target PPN populations (Sikora *et al*., 2005). Furthermore, cost-benefit ratios for grower acceptance should be calculated to estimate the maximum impact a particular management strategy can have in reducing PPN population levels in cropping systems (Sikora *et al*., 2005). Such integrated approaches are designed to attain high crop yields, while simultaneously reducing PPN as well as other pest, disease and weed problems. Ultimately, the reduction of erosion and improvement of soil fertility should be selection dependant on the main crop, in this case potato (Sikora *et al*., 2005).

Scurrah *et al*. (2005) listed the use of RKN-resistant cultivars and rotation with non-host crops as the economical most important tools to reduce RKN numbers in potato-based cropping systems effectively. Although RKN problems in local potato producing areas are at present still mainly dealt with by means of chemical control (Jones *et al*., 2011), planting of RKN-free tubers, crop rotation as well as the inclusion of poor-host rotation crops (Sikora *et al*., 2005) are all strategies currently used by local potato producers to protect their produce. The wide host range of the predominant PPN parasite of potato, i.e. RKN (Jones *et al*., 2011), however, continue to pose challenges to producers in terms of aligning available management strategies optimally to address this problem.

Local producers realise that the management of PPN in the future will not be dependent on only one of these strategies, but that logical and effective integration thereof will be crucial to enable them to farm sustainably. Most important is that such an integrated approach should be economically acceptable to producers (Sikora *et al*., 2005). However, an integrated approach should be dynamic and flexible and may require frequent adjustment in response to changes related to the production system (e.g. new cultivars that enter the market, economic factors, environmental conditions or availability of registered nematicides and/or biological products) (Nyczepir & Thomas, 2009). Fortunately, the challenges that the agricultural sector face these days provide opportunities to adapt recent technological, molecular as well as biological, advances together with novel emerging chemical and biological agents for the management of PPN such as RKN (Nyczepir & Thomas, 2009). Once the latter is attained, the primary goal of any integrated pest management system namely increased crop yield (quality and quantity) by using a combination of the management approaches (discussed earlier) to target PPN (Nyczepir & Thomas, 2009) can and will be realised. Therefore, research on alternative control strategies and

management practices and also the integration of the latter to minimize the impact of these parasites on local potato production is critical to assist the producers and the industry. The latter constitute the main aim of this study since prior to this research no work has been done on potato in SA to determine the efficacy of Brassicaceae cover crops and its use as biofumigation agents in reducing RKN populations (Berry & Wiseman, 2003; Berry & Rhodes, 2006; Kruger *et al.*, 2011). The present study was thus initiated since producers in the Christiana area realized that they should incorporate strategies, other than chemical control, to address the RKN problems they experience in their potato plantings.

### 1.8 Objectives of this study

- To assess the status of PPN and NPN in 31 potato fields of five producers in the Christiana area (North-West Province), with the emphasis on RKN.

- To investigate the effects of selected Brassicaceae species as both cover and/green manure crops on both PPN and NPN population levels under natural occurring environmental conditions in a field experiment.

## 1.9 References


Adam, M.A.M., Phillips, M.S. & Blok, V.C. 2007. Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.). *Plant Pathology* 56: 190-197.

Akhtar, M. & Alam, M.M. 1991. Integrated control of plant parasitic nematodes on potato with organic amendments, nematicide and mixed cropping with mustard. *Nematologia Mediterreanea* 19: 169-171.

Batchelor, T.A. 2002. International and European community control on methyl bromide and the status of methyl bromide use and alternatives in the European community. In: Batchelor, T.A. & Bolivar, J.M. Proceedings of International Conference on Alternatives to Methyl Bromide 'The remaining challenges'. European Commission, Brussels, Belgium. pp. 28-32.

Bélair, G. & Parent, L.E. 1996. Using crop rotation to control *Meloidogyne hapla* Chitwood and improve marketable carrot yield. *HortScience* 31: 106-108.

Bello, A. 1998. Biofumigation and integrated crop management. In: Bello, A., González, J.A., Arias, M. & Rodríquez-Kábana, R. Alternatives to methyl bromide for the southern European countries, DGXI, EU, CSIC, Madrid. pp. 99-126.

Bending, G.D. & Lincoln, S.D. 2000. Inhibition of soil nitrifying bacteria communities and their activities by glucosinolate hydrolysis products. *Soil Biology and Biochemistry* 32: 1261-1269.

Berry, S.D., Fargette, M., Spaull, V.M., Morand, S. & Cadet, P. 2008. Detection and quantification of root-knot nematode (*Meloidogyne javanica*), lesion nematode (*Pratylenchus zeae*) and dagger nematode (*Xiphinema elongatum*) parasites of sugarcane using real-time PCR. *Molecular and Cellular Probes* 22: 168-176.

Berry, S.D. & Rhodes, R. 2006. Green manure crops: agronomic characteristics and effect on nematodes. *Proceedings of the South African Sugar Technologists Association* 80: 269-273.

Berry, S.D. & Wiseman, E.J. 2003. The effect of green manure crops on plant parasitic nematodes in the South African sugar industry. *Proceedings of the South African Sugar Technologists Association* 77: 114-117.

Bird, A.F. 1971. Specialized adaptations of nematodes to parasitism. In: Zuckerman, B.M., Mai, W.F. & Rohde, R.A. (eds) Plant parasitic nematodes. Academic Press, New York. pp. 35-49.

Black, V. 2008. Potatoes in South Africa. In: GM potatoes in South Africa – a critical analysis. The African Centre for Biosafety, Johannesburg, South Africa. pp. 12-24.

Blok, V.C., Phillips, M.S. & Fargette, M. 1997. Comparison of sequences from the ribosomal DNA intergenic region of *Meloidogyne mayaguensis* and other major tropical root-knot nematodes. *Journal of Nematology* 29: 16-22.

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

Bongers, T. & Bongers, M. 1998. Functional diversity of nematodes. *Applied Soil Ecology* 10: 239-251.

Bos, L. & Parlevliet, J.E. 1995. Concepts and terminology on plant/pest relationships: Toward consensus in Plant Pathology and Crop Protection. *Annual Review of Phytopathology* 33: 69-102.

Bridge, J. & Starr, J.L. 2007. Plant nematodes of agricultural importance. A color handbook. Academic Press, Boston, USA. 152 pp.

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

Brodie, B.B., Evans, K. & Franco, J. 1993. Nematode parasites of potatoes. In: Evans, K., Trudgill, D.L. & Webster, J.M. (eds) Plant parasitic nematodes in temperate agriculture. CAB International, Wallingford, UK. pp. 87-132.

Brown, E.B. 1969. Assessment of the damage caused to potatoes by potato cyst eelworm, *Heterodera rostochiensis* Woll. *Annals of Applied Biology* 63: 493-502.

Brown, P.D. & Morra, M.J. 1995. Glucosinolate-containing plant tissues as bioherbicides. *Journal of Agricultural Food Chemistry* 43: 3070-3074.

Brown, C.R., Yang, C.P., Mojtahedi, H. & Santo, G.S. 1996. RFLP analysis of resistance to Columbia root-knot nematode derived from *Solanum bulbocastanum* in a BC2 population. *Theoretical and Applied Genetics* 92: 572-576.

Castagnone-Sereno, P. 2006. Genetic variability and adaptive evolution in pathogenetic root-knot nematodes. *Heredity* 96: 282-289.

Castagnone-Sereno, P., Piotte, C., Uijthof, J., Abad, P., Wajnberg, E., Vanlerberghe-Masutti, F., Bongiovanni, M. & Dalmasso, A. 1993. Phylogenetic relationships between amphimictic and parthenogenetic nematodes of the genus *Meloidogyne* as inferred from repetitive DNA analysis. *Heredity* 70: 195-204.

Coetzee, V. 1968. The distribution of the family Heteroderidae (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. *South African Journal of Agricultural Sciences* 11: 775-788.

Cohen, M.F., Yamasaki, H. & Mazzola, M. 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. *Soil Biology and Biochemistry* 37: 1215-1227.

Cook, R. & Starr, J.L. 2006. Resistant cultivars. In: Perry, R.N. & Moens, M. (eds) Plant nematology. CABI Publishing, Wallingford, UK. pp. 370-389.

Coyne, D.L., Fourie, H.H. & Moens, M. 2009. Current and future management strategies in resource-poor farming. In: Perry, R.N., Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 444-466.

CropLife, 2011. Agricultural remedies database. http://www.croplife.co.za/CroplifeSAInitiatives.aspx?AgriculturalRemediesDatabase=true (Accessed on: 20 February 2012).

Curto, G., Dallavalle, E. & Lazzeri, L. 2005. Life cycle duration of *Meloidogyne incognita* and host status of Brassicaceae and Capparaceae selected for glucosinolate content. *Nematology* 7: 203-212.

DAFF (Department of Agriculture, Forestry and Fisheries). 2003. Potato profile. http://www.daff.gov.za (Accessed on: 10 September 2011).

DAFF (Department of Agriculture, Forestry and Fisheries). 2011. Production guidelines: Potatoes. http://www.daff.gov.za (Accessed on: 10 September 2011).

Decraemer, W. & Hunt, D.J. 2006. Structure and classification. In: Perry, R.N. & Moens, M. (eds) Plant nematology. CABI Publishing, Wallingford, UK. pp. 3-32.

De Guiran, G. & Ritter, M. 1979. Life cycle of *Meloidogyne* species and factors influencing their development. In: Lamberti, F. & Taylor, C.E. (eds) Root-knot nematodes (*Meloidogyne* species); systematics, biology and control. Academic Press, London, UK. pp. 172-191.

De Waele, D., Jones, B.L., Bolton, C. & Van den Berg, E. 1989. *Ditylenchus destructor* in hulls and seeds of peanut. *Journal of Nematology* 21: 10-15.

De Waele, D., Wilken, R. & Lindeque, J.M. 1991. Response of potato cultivars to *Ditylenchus destructor* isolated from groundnut. *Revue de Nématologie* 14:123-126.

Duncan, L.W. & Moens, M. 2006. Migratory endoparasitic nematodes. In: Perry, R.N. & Moens, M. (eds) Plant nematology. CABI Publishing, Wallingford, UK. pp. 123-142.

Eisenback, J.D., Hirschmann, H., Sasser, J.N. & Triantaphyllou, A.C. 1981. A general description of several taxonomic approaches to *Meloidogyne* species. In: (eds) A guide to the four most common species of root-knot nematodes (*Meloidogyne* spp.), with a pictorial key. North Carolina State University, Raleigh, USA. pp. 1-4.

Eisenback, J.D. & Hunt, D.J. 2009. General morphology. In: Perry, R.N., Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 18-54.

Evans, A.A.F. & Perry, R.N. 2009. Survival mechanisms. In: Perry, R.N., Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International , Wallingford, UK. pp. 201-222.

FAO (Food and Agricultural Organisation). 2008. International year of the potato. http://www.potato2008.org/en/potato/index.html (Accessed on: 28 August 2010).

FAO (Food and Agricultural Organisation). 2009. How to feed the world in 2050. World summit on food security. Rome, 16-18 November 2009.

FAO (Food and Agricultural Organisation). 2010. Undernourishment around the world in 2010. In: FAO. The state of food insecurity in the world 2010. Rome, 2010. p. 10.

FAO (Food and Agricultural Organisation). 2012. FAOSTAT=Agriculture. www.fao.org (Accessed on: 2 April 2012).

Fargette, M., Phillips, M.S., Blok, V.C., Waugh, R. & Trudgill, D.L. 1996. An RFLP study of relationships between species, populations and resistance-breaking lines of tropical species of *Meloidogyne*. *Fundamental and Applied Nematology* 19: 193-200.

Ferris, H., Bongers, T. & De Goede, R.G.M. 2001. A framework for soil food web diagnostics: extension of nematode faunal analysis concept. *Applied Soil Ecology* 18: 13-29.

Ferris, H. & Matute, M.M. 2003. Structural and functional succession in the nematode fauna of a soil food web. *Applied Soil Ecology* 23: 93-110.

Finlay, M., Dale, B. & Scurrah, M.M. 1998. Breeding for resistance to the potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida*: strategies, mechanisms and genetic resources. In: Marks, R.J. & Brodie, B.B. (eds) Potato Cyst-Nematodes: biology, distribution and control. CABI Publishing, Wallingford, UK. pp. 167-196.

Fourie, H., Leswifi, C., Mc Donald, A.H. & De Waele, D. 2007. Host suitability of vetiver grass to *Meloidogyne incognita* and *M. javanica*. *Nematology* 9: 49-52.

Fourie, H., Mc Donald, A.H. & De Waele, D. 2006. Host suitability of South African and foreign soybean cultivars to *Meloidogyne incognita* race 2. *South African Plant and Soil* 23: 132-137.

Fourie, H., Zijlstra, C. & Mc Donald, A.H. 2001. Identification of Root-knot nematode species occurring in South Africa using the SCAR-PCR technique. *Nematology* 3: 675-580.

GBIF (Global Biodiversity Information Facility). 2011. Classification of species: *Solanum tuberosum* L. http://data.gbif.org/species/13764911/ (Accessed on: 15 August 2011).

Halbrendt, J.M. & LaMondia, J.A. 2004. Crop rotation and other cultural practices. In: Chen, Z.X., Chen, S.Y. & Dickson, D.W. (eds) Nematology advances and perspectives. Volume 2. Nematode management and utilization. CABI Publishing, Wallingford, UK. pp. 909-924.

Hallman, J., Davies, K.G. & Sikora, R. 2009. Biological control using microbial pathogens, endophytes and antagonists. In: Perry, R.N., Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 380-411.

Hartman, K.M. & Sasser, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential host treatise and perineal-pattern morphology. In: Barker, K.R., Carter, C.C. & Sasser, J.N. (eds) Advanced treatise on *Meloidogyne*. Volume II. Methodology. North Carolina University Graphics, Raleigh, North Carolina, USA. pp. 69-78.

Hawkes, J.G. 1990. The potato: Evolution, biodiversity and genetic resources. Belhaven Press, London, UK. p. 259.

Hooper, D.J. 1973. *Ditylenchus destructor*. Commonwealth Institute of Helminthology. Descriptions of plant-parasitic nematodes, Set 2, No. 21: 3p.

Hu, M.X., Zhuo, K. & Liao. J.L. 2011. Multiplex PCR for the simultaneous identification and detection of *Meloidogyne incognita*, *M. enterolobii*, and *M. javanica* using DNA extracted directly from individual galls. *Phytopathology* 101: 1270-1277.

Hunt, D.J., Luc, M. & Manzanilla-López, R.H. 2005. Identification, morphology and biology of plant parasitic nematodes. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 11-52.

Hussey, R.S. & Jansen, G.J.W. 2002. Root-knot nematodes: *Meloidogyne* species. In: Starr, J.L., Cook, R. & Bridge, J. (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, UK. pp. 43-70.

IPC (International Potato Centre). 2011. Potato. http://www.cipotato.org/potato (Accessed on: 10 September 2011).

Jatala, P. 1975. Root-knot nematodes (*Meloidogyne* species) and their effect on potato quality. In: 6th Triennial Conference of the EAPR, 15-19 September 1975, Wageningen, The Netherlands.

Jatala, P., French, E.R. & Gutarra, L. 1975. Interrelationships of *Meloidogyne incognita acrita* and *Pseudomonas solanacearum* on potatoes. *Journal of Nematology* 7: 325.

Jatala, P. & Martin, C. 1977. Interaction of *Meloidogyne incognita acrita* and *Pseudomonas solanacearum* on *Solanum chacoense* and *S. sparsipilum*. *Proceedings of the American Phytopathological Society* 4: 178.

Jatala, P., Kaltenbach, R. & Bocangel, M. 1979. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. *Journal of Nematology* 11: 303 (Abstract).

Jepson, S.B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, UK. 265 p.

Jones, F.G.W. 1970. The control of the potato cyst nematode. *Journal of the Royal Society of Arts* 118: 179-199.

Jones, R.K., Marias, M. & Swart, A. 2011. Aalwurms (Nematodes). In: Denner, F. & Venter, S. (eds) Handleiding vir aartappelproduksie in Suid-Afrika. Landbounavorsingsraad-Roodeplaat Instituut vir Groente en Sierplante. Pretoria, Suid-Afrika. pp. 195-201.

Karssen, G. & Moens, M. 2006. Root-knot nematodes. In: Perry, R.N. & Moens, M. (eds) Plant nematology. CABI Publishing, Wallingford, UK. pp. 59-88.

Keetch, D.P. & Buckley, 1984. A check-list of the plant parasitic nematodes of Southern Africa. Technical Communication No. 795. Department of Agricultural Development, South African Government, Republic of South Africa.

Keetch, D.P. & Milne, D.L. 1982. The control of plant-parasitic nematodes. In: Keetch, D.P. & Heyns, J. (eds) Nematology in southern Africa. Science Bulletin Department of Agriculture and Fisheries Republic of South Africa. Science Bulletin No. 400. pp. 113-129.

Kiple, K.F. & Ornelas, K.C. 2000. Potatoes (White). In: Kiple, K.F. & Ornelas, K.C. (eds) The Cambridge world history of food. Cambridge University Press. pp. 187-200.

Kirkegaard, J.A. & Sarwar, M. 1998. Biofumigation potential of brassica. I. Variation in glucosinolate profiles of diverse field-grown *Brassicas*. *Plant Soil* 201: 71-89.

Kleynhans, K.P.N. 1978. Nematode pests in potatoes. Leaflet of the Department of Agriculture and Technical Services of the Republic of South Africa Potato Series No. G. 13.

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

Kleynhans, K.P.N., Van den Berg, E., Swart, A., Marais, M. & Buckley, N.H. 1996. Plant nematodes in South Africa. Plant Protection Research Institute Handbook No 8, Pretoria, Business Print. 165 p.

Knoetze, R., Malan, A.P., Swart, A. & Pieterse, W. 2004. Present status of potato cyst nematode in South Africa. *African Plant Protection* 10: 129.

Koen, H. 1960. Yet another eelworm species threaten crops. *Farming in South Africa* 36: 33-34.

Koen, H. 1965. How potato-peel nematode spreads. *Farming in South Africa* 41: 31.

Koen, H. 1967. Notes on the host range, ecology and population dynamics of *Pratylenchus brachyurus*. *Nematologica* 13: 118-124.

Koen, H. & Hogewind, W.L. 1967. Symptoms and characteristics of *Pratylenchus brachyurus* infestation on stored potatoes. *South African Journal of Agricultural Sciences* 10: 543-550.

Kruger, N., Fourie, J. & Malan, A. 2011. The role of cover crops in suppressing plant-parasitic nematodes in vineyards. 20th Symposium of the Nematological Society of Southern Africa. Stellenbosch, 15-18 May 2011. (Oral presentation).

Lazzeri, L., Leoni, O., Bernardi, R., Malaguti, L. & Cinti, S. 2004. Plants, techniques and products for optimising biofumigation in full field. *Agroindustria* 3: 281-288.

Lazzeri, L., Tacconi, R. & Palmieri, S. 1993. *In vitro* activity of some glucosinolates and their reaction products toward a population of the nematode *Heterodera schachtii*. *Journal of Agricultural Food Chemistry* 41: 825-829.

Louw, I.W. 1982. Nematode pests of potatoes. In: Keetch, D.P. & Heyns, J. (eds) Nematology in southern Africa. Science Bulletin Department of Agriculture and Fisheries Republic of South Africa. Science Bulletin No. 400. pp. 80-87.

Mai, W.F. 1977. World-wide distribution of potato cyst nematodes and their importance in crop production. *Journal of Nematology* 9: 30-34.

Manzanilla-López, R.H., Evans, K. & Bridge, J. 2004. Plant diseases caused by nematodes. In: Chen, Z.X., Chen, S.Y. & Dickson, D.W. (eds) Nematology advances and perspectives. Volume 2. Nematode management and utilization. CABI Publishing, Wallingford, UK. pp. 637-716.

Martin, G.C. 1972. Potatoes and plant-parasitic nematodes. *Stocks Crops* 3: 27-39.

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

McLeod, R.W. & Steel, C.G. 1999. Effects of brassica-leaf manures and crops on activity and reproduction of *Meloidogyne javanica*. *Nematology* 1: 613-624.

McLeod, R.W., Kirkegaard, J.A. & Steel, C.C. 2001. Invasion, development, growth and egg laying by *Meloidogyne javanica* in Brassicaceae crops. *Nematology* 3: 463-472.

McSorley, R., Dickson, D.W., Brito, J.A. & Hochmuth, R.C. 1994. Tropical rotation crops influence nematode densities and vegetable yield. *Journal of Nematology* 26: 308-314.

Meng, Q.P., Long, H. & Xu, J.H. 2004. PCR assays for rapid and sensitive identification of three major root-knot nematodes, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. *Acta Phytopathologica Sinica 34*: 204-210.

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

Mojtahedi, H., Santo, G.S., Hang, A.N. & Wilson, J.H. 1991. Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. *Journal of Nematology* 23: 170-174.

Morra, M.J. & Kirkegaard, J.A. 2002. Isothiocyanate release from soil-incorporated *Brassica* tissue. *Soil Biology and Biochemistry* 34: 1683-1690.

Morris, R.F. 1971. Distribution and biology of the golden nematode, *Heterodera rostochiensis* in Newfoundland. *Nematologica* 17: 370-376.

Neher, D., Bongers, T. & Ferris, H. 2004. Computation of Nematode community indices. Estes Park, Colorado, Society of Nematologists. 33 p.

Nel, A, Krause, M. & Khelawanlall, N. 2007. A guide for the control of plant pests. 39th Edition. Department of Agriculture, Republic of South Africa. Government Printer, Pretoria. 116 p.

Nicol, J. M., Turner, S.J., Coyne, D.L., den Nijs, L., Hockland, S. & Tahna Maafi, Z. 2011. Current nematode threats to world agriculture. In: Jones, J., Gheysen, G. & Fenoll, C. (eds) Genomics and molecular genetics of plant-nematode interactions. Springer Publishing, New York & London. pp. 21-43.

Ngobeni, L., Fourie, H. & Mc Donald, A.H. 2010. Host suitability of selected South African maize genotypes to the root-knot nematode species *Meloidogyne incognita* race 2 and *Meloidogyne javanica*: A preliminary study. *South African Journal of Plant and Soil* 28: 49-54.

Ntidi, K.N., Fourie, H., Mc Donald, A.H., De Waele, D. & Mienie, M.S. 2012. Plant-parasitic nematodes associated with weeds in developing agriculture in South Africa. *Nematology* (in press).

Nyczepir, A.P. & Thomas, S.H. 2009. Current and future management strategies in intensive crop production systems. In: Perry, R.N. Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 412-443.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. *Mededelingen Landbouwhogeschool Wageningen* 66: 1-46.

Papadopoulou, J. & Triantaphyllou, A.C. 1982. Sex differentiation in *Meloidogyne incognita* and anatomical evidence of sex reversal. *Journal of Nematology* 14: 549-566.

Pattison, A.B., Versteeg, C., Akiew, S. & Kirkegaard, J. 2006. Resistance of Brassicaceae plants to root-knot nematode (*Meloidogyne* spp.) in northern Australia. *International Journal of Pest Management* 52: 53-62.

Perry, R.N. & Moens, M. 2006. Taxonomy and principal genera. In: (eds) Plant nematology. CABI Publishing, Wallingford, UK. p. 1.

Piotte, C., Castagnone-Sereno, P., Uijthof, J., Abad, P., Bongiovanni, M. & Dalmasso, A. 1992. Molecular characterization of species and populations of *Meloidogyne* from various geographic origins with repeated-DNA homologous probes. *Fundamental and Applied Nematology* 15: 271-276.

PSA (Potatoes South Africa). 2010. National annual information. http://www.potatoes.co.za (Accessed on: 2 April 2012).

Potter, M., Davies, K. & Rathjen, A. 1998. Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematodes (*Pratylenchus neglectus*). *Journal of Chemical Ecology* 24: 67-80.

Powers, T.O. & Harris, T.S. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology* 25: 1-6.

Powers, T.O., Platzer, E.G. & Hyman, B.C. 1986. Species-specific restriction site polymorphism in root-knot nematode mitochondrial DNA. *Journal of Nematology* 18: 288-293.

Ramirez-Villapudua, J. & Munnecke, D.E. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. c*onglutinans* and other organisms. *Phytopathology* 78: 289-295.

Riekert, H.F. 1996. Economic feasibility of nematode control in dryland maize in South Africa. *African Crop Science Journal* 4: 477-481.

Riekert, H.F. & Henshaw, G.E. 1998. Effect of soybean, cowpea and groundnut rotations on root-knot nematode build-up and infestation of dryland maize. *African Crop Science Journal* 6: 377-383.

Roberts, P.A. 2002. Concepts and consequences of resistance. In: Starr, J.L., Cook, R. & Bridge, J. (eds) Plant resistance to parasitic nematodes. pp. 23-38.

Santos, G.S. 2001. Root knot nematodes. In: Stevenson, W.R., Loria, R., Franc, G.D. & Weingartnet, D.P. (eds) Compendium of potato diseases. St Paul, Minnesota. APS Press, USA. pp. 51-53.

Sarwar, M., Kirkegaard, J., Wong, P.T.W. & Desmarchelier, J.M. 1998. Biofumigation potential of brassicas, III. *In vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant and Soil* 201: 103-112.

Scurrah, M.I., Niere, B. & Bridge, J. 2005. Nematode parasites of solanum and sweet potatoes. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 193-219.

Sellschop, J.P.F. & Du Toit, J.J. 1948. The production of potatoes. *Farming in South Africa* 23: 645-654.

Sikora, R.A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. *Annual Review of Phytopathology* 30: 245-279 (Abstract).

Sikora, R.A. & Fernández, E. 2005. Nematode parasites of vegetables. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 319-392.

Sikora, R.A., Bridge, J. & Starr, J.L. 2005. Management practices: an overview of integrated nematode management technologies. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 793-824.

Starr, J.L. & Mercer, C.F. 2009. Development of resistant varieties. In: Perry, R.N. Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 326-337.

Stirling, G.R. & Stirling, A.M. 2003. The potential of *Brassica* green manure crops for controlling root-knot nematode (*Meloidogyne javanica*) on horticultural crops in a subtropical environment. *Australian Journal of Experimental Agriculture* 43: 623-603.

Subbarao, K.V., Hubbard, J.C. & Koike, S.T. 1999. Evaluation of broccoli residue incorporation into field soil for verticillium wilt control in cauliflower. *Plant and Disease* 83: 124-129.

Subbotin, S.A., Mundo-Ocampo, M. & Baldwin, J.G. 2010. Systematics of the genus *Globodera*. In: (eds) Systematics of cyst nematodes (Nematoda: Heteroderinae). pp. 107-177.

Swart, A. 2011. Plant quarantine organisms. Agricultural Research Council – Plant Protection Research Institute. Pretoria, South Africa. pp. 1-13.

Taylor, C.E. & Martin, G.C. 1960. Potato production. 8. Pests. *Rhodesia Agricultural Journal* 57: 60-63.

Thomason, I.J. & Caswell, E.P. 1987. Principles of nematode control. In: Brown, R.H. & Kerry, B.R. (eds) Principles and practices of nematode control in crops. Academic Press, Australia. pp. 87-130.

Tigano, M.S., Carneiro, R.M.D.G., Jeyaprakash, A., Dickson, D.W. & Adams, B.J. 2005. Phylogeny of *Meloidogyne* spp. based on 18S rDNA and the intergenic region of mitochondrial DNA sequences. *Nematology* 7: 851-862.

Timper, P., Davis, R.F. & Tillman, P.G. 2006. Reproduction of *Meloidogyne incognita* on winter cover crops used in cotton production. *Journal of Nematology* 38: 83-89.

Toyota, K., Shirakashi, T., Sato, E., Wada, S. & Min, Y. 2008. Development of a real-time PCR method for the potato-cyst nematode *Globodera rostochiensis* and the root-knot nematode *Meloidogyne incognita*. *Soil Science and Plant Nutrition* 54: 72-76.

Triantaphyllou, A.C. 1973. Environmental sex differentiation of nematodes in relation to pest management. *Annual Review of Phytopathology* 11: 441-462.

Trudgill, D.L., Evans, K. & Phillips, M.S. 1998. Potato cyst nematodes: damage mechanisms and tolerance in the potato. In: Marks, R.J. & Brodie, B.B. (eds) Potato cyst nematodes biology, distribution and control. CAB International, Wallingford, UK. pp. 117-133.

Turner, S.J. & Evans, K. 1998. The origins, global distribution and biology of potato cyst nematodes (*Globodera rostochiensis* (Woll.) and *Globodera pallida* (Stone)). In: Marks, R.J. & Brodie, B.B. (eds) Potato Cyst-Nematodes: biology, distribution and control. CAB International, Wallingford, UK. pp. 7-26.

Turner, S.J. & Rowe, J.A. 2006. Cyst nematodes. In: Perry. R. & Moens, M. 2006. Plant nematology. CABI Publishing, Wallingford. pp. 92-120.

Van Biljon, E.R. 2004. Crop rotation as part of an integrated pest management programme for the control of plant parasitic nematodes. Proceedings of the Soilborne Plant Diseases Interest Group Symposium, Stellenbosch, 14-16 September 2004.

Van den Berg, E. 1971. The root lesion nematodes of South Africa (Genus *Pratylenchus* Family Hoplolaimidae). Technology and Communications Department for the Agricultural Technical Services of the Republic of South Africa. No. 99. p. 13.

Van den Berg, E. 1982. The biology of some common plant-parasitic nematodes in South Africa. In: Keetch, D.P. & Heyns, J. Nematology in southern Africa, pp. 100-112. Science Bulletin Department of Agriculture and Fisheries Republic of South Africa. Science Bulletin No. 400.

Wang, H.K., McSorley, R. & Gallaher, R.N. 2004. Relationship of soil management history and nutrient status to nematode community structure. *Nematropica* 34: 83-95.

Wendt, K.R., Swart, A., Vrain, T.C. & Webster, J.M. 1995. *Ditylenchus africanus* sp. n. from South Africa: a morphological and molecular characterization. *Fundamental and Applied Nematology* 18: 141-250.

Whitehead, A.G. & Turner, S.J. 1998. Management and regulatory control strategies for potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*). In: Marks, R.J. & Brodie, B.B. (eds) Potato cyst nematodes biology, distribution and control. CAB International, Wallingford, UK. pp. 135-152.

Widmer, T.L., Mitkowski, N.A. & Abawi, G.S. 2002. Soil organic matter and management of plant-parasitic nematodes. *Journal of Nematology* 34: 289-295.

Williams, L., Morra, M., Brown, P. & McCaffrey, J. 1993. Toxicity of allyl isothiocyanate-amended soil to *Limonius californicas* (Mann.) (Coleoptera: Elateridae) wireworms. *Journal of Chemical Ecology* 19: 1033-1046.

Winslow, R.D. & Willis, R.J. 1972. Nematode diseases of potatoes. In: J.M. Webster. (eds) Economic nematology. Academic Press, London, UK. pp. 17-48.

Xu, J.H., Liu, P.L., Meng, Q.P. & Long, H. 2004. Characterization of *Meloidogyne* species from China using isozyme phenotypes and amplified mitochondrial DNA restriction fragment length polymorphism. *European Journal of Plant Pathology* 110: 309-315.

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

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. & Georgiewa, S.S. 1993. Feeding habits in soil nematode families and genera – An outline for soil ecologists. *Journal of Nematology* 25: 315-331.

Yeates, G.W., Ferris, H., Moens, T. & Van der Putten, W.H. 2009. The role of nematodes in ecosystems. In: Wilson, M.J. & Kakouli-Duarte, T. Nematodes as environmental indicators. pp. 1-44.

Zasada, I.A., Avendano, F., Li, Y.C., Logan, T., Melakeberhan, H., Koenning, S.R. & Tylka, G.L. 2008. Potential of an alkaline-stabilized biosolid to manage nematodes: case studies on soybean cyst and root-knot nematodes. *Plant Diseases* 92: 4-13.

Zasada, I.A. & Ferris, H. 2004. Nematode suppression with brassicaceous amendments: Application based upon glucosinolate profiles. *Soil Biology and Biochemistry* 36: 1017-1024.

Zasada, I.A., Ferris, H., Elmore, C.L., Roncoroni, J.A., MacDonald, J.D., Bolkan, L.R. & Yakabe, L.E. 2003. Field application of brassicaceous amendments for control of soilborne pests and pathogens. Online. Plant Health Progress doi: 10.1094/PHP-2003-1120-01-RS. Field Application of Brassicaceous Amendments for Control of Soilborne Pests and Pathogens/Plant Management Network.

Zhuo, K., Hu, M.X., Liao, J.L. & Rui, K. 2010. First report of *Meloidogyne enterolobii* on arrowroot in China. *Plant Disease* 94: 271.

Zijlstra, C., Donkers-Venne, D.T.H.M. & Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2: 847-853.

## CHAPTER 2

## IDENTIFICATION OF NEMATODE ASSEMBLAGES IN POTATO-BASED CROPPING SYSTEMS IN THE CHRISTIANA AREA (NORTH-WEST PROVINCE, SOUTH AFRICA)

### 2.1 Introduction

Root and tuber crops, such as potato, are the most important food commodities produced in many subtropical and tropical countries (Scurrah *et al*., 2005). The importance of plant-parasitic nematodes (PPN) on such food commodities, particularly potato, are often overlooked because these small, soilborne organisms seldom cause specific above-ground symptoms (Manzanilla-López *et al*., 2004). The frequent lack of distinct symptoms caused by PPN often result in damage to aerial parts of crops which are confused with problems caused by other factors, i.e. soil structure and nutrition deficiencies that can negatively impact the growth and development of crops (Kleynhans *et al*., 1996; Manzanilla-López *et al*., 2004; Bridge & Starr, 2007). The latter scenario generally leads to the slow, invisible build-up and spread of potentially pathogenic PPN populations that ultimately damage crops (Manzanilla-López *et al*., 2004). Correct diagnosis of a malady is, therefore, the starting point for successful management of PPN associated with crops and should be followed by accurate identification of the genera, species and/or families by a trained nematologist.

Ample literature exists about PPN that are associated with potato in various world countries (Scurrah *et al*., 2005; Bridge & Starr, 2007), including South Africa (SA) (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Jones *et al*., 2011). The root-knot nematode (RKN) species, *M. incognita* and *M. javanica* have been listed as the predominant PPN that cause downgrading of potato on local markets. Lesion (*Pratylenchus* spp.) and the golden cyst nematode (GCN; *Globodera rostochiensis*) also affect the crop adversely in some local production areas (Jones *et al*., 2011). However, little is known about the total nematode complex, which includes both PPN and non-parasitic nematodes (NPN) that are associated and adversely affect the crop in local potato-plantings areas.

Besides the presence of damaging PPN, it is also crucial to obtain information about the NPN assemblages, representing beneficial nematodes that occur in local fields. Only then can nematode control strategies be planned and applied successfully to prevent eradication of the beneficial nematodes (Yeates *et al*., 2009). The positive role that NPN plays in agroecosystems has been studied extensively, particularly during the last two decades (Bongers, 1990; Bongers & Ferris, 1999; Ferris *et*

*al*., 2001). However, in SA efforts to address soil health has only been attended to during the past five years with local nematologists also starting to focus on the role of beneficial nematodes.

The current approach by local potato farmers is that they do not produce potato crops that are susceptible to RKN more than once every three or four years on the same field (Sellschop & Du Toit, 1948; Black, 2008). However, to minimise problems caused by RKN as well as other diseases, potato producers in the Christiana area implement an eight year crop rotation cycle during which potato is planted only once (G. Posthumus, pers comm., March 2010). Despite the latter, damage to tubers harvested in this area has increased from 4% to nearly 50% with visible symptoms of RKN infection from the 2001/2002 to the 2009/2010 season (see Table 1.1; Chapter 1) (J. van Vuuren, pers comm., May 2010).

To recommend and/or develop viable and effective crop rotation systems for producers in the Christiana area it is imperative to first identify and determine population levels of the nematode complexes present in fields where potato is included. Once the latter has been done, recommendations about the use of cover and rotation crops, also those that are currently planted by these producers, can be made. At present, grasses such as *Eragrostis* and *Digitaria* species are planted as cover crops, which are mainly utilized as horse or cattle feed rather than a RKN management strategy. In addition, rotation crops such as maize, onion, sunflower and wheat are also included in these cropping sequences**.** As mentioned in the introductory part of this dissertation, information about the host suitability of grasses and grain crops to economically important RKN species is available. However, recommendations for their inclusion can only be made once the RKN species that cause problems to potato plantings in fields of these producers have been identified (Taylor & Martin, 1960; Fourie *et al*., 2007; Jones *et al*., 2011). Therefore, the first part of this study consisted of the comprehensive sampling of 31 fields of five potato-producers in the Christiana area (North-West Province of SA).

## 2.2 Material and methods

### 2.2.1 Study area

This study was conducted near Christiana (S 28°09.312, E 024°59.671), situated in the south eastern part of the North-West Province of SA (Figure 2.1), with an altitude of approximately 1 050-1 400m above sea level (Mucina & Rutherford, 2006). This area is located in the Savanna biome of the Kimberly Thornveld and is characterized by summer and autumn rainfall and dry winters (Mucina &

Rutherford, 2006). Annual rainfall generally ranges between 300-500mm with mean minimum and maximum temperatures between -3.9°C and 37.4°C, respectively (Mucina & Rutherford, 2006). The nematode survey encompassed the sampling of both soil and root samples from six fields of five producers each (Figure 2.2; Tables 2.1–2.5), except for one producer where seven fields were sampled.


Figure 2.1. A map of South Africa indicating where the study area is located near Christiana (North-West Province of South Africa) (Adapted from: http://cybercapetown.com/Maps/SouthAfrica).

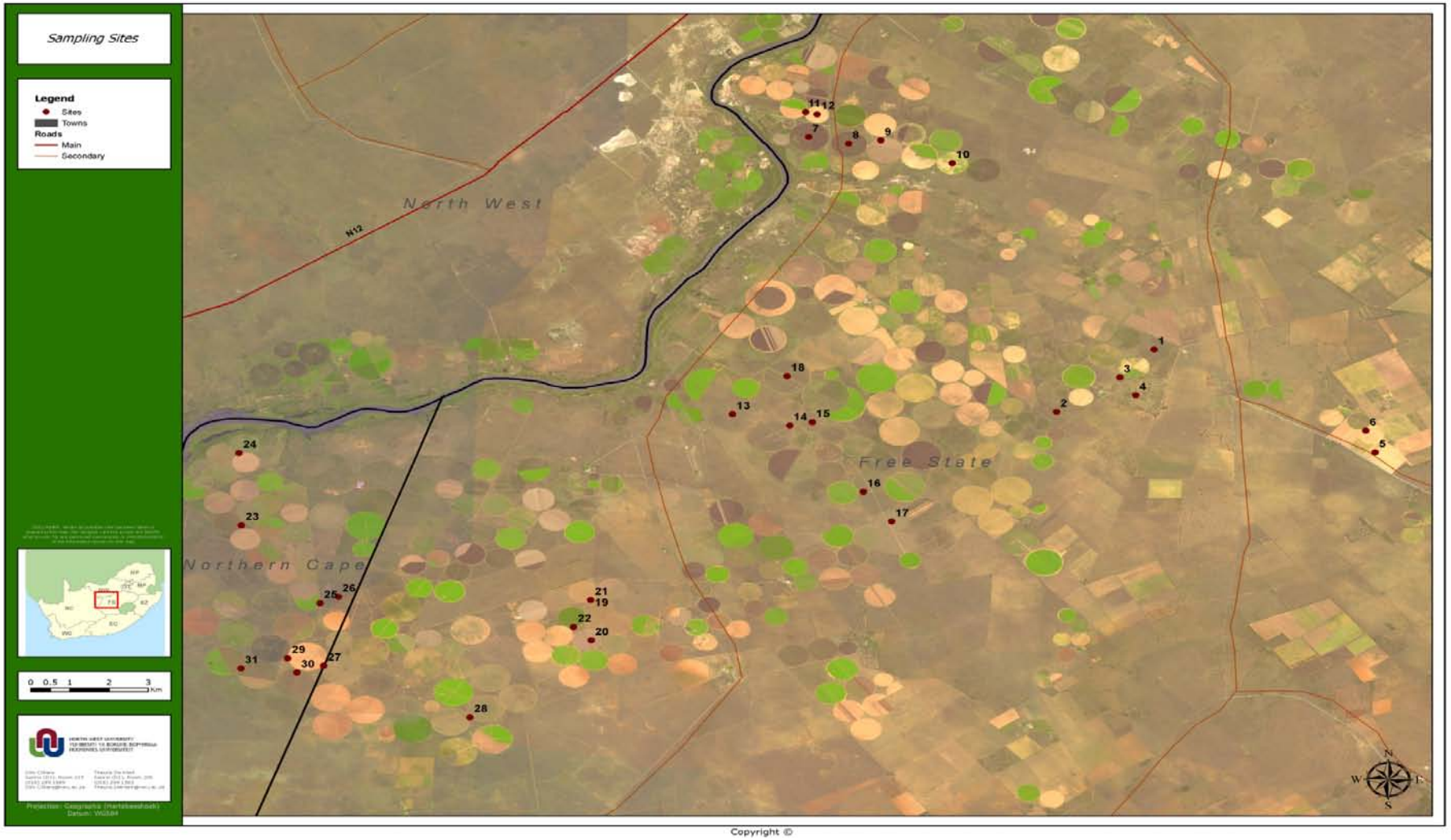


Figure 2.2. A detailed aerial view of the 31 individual fields of five producers in the Christiana area from which nematode samples were collected during the 2010 and 2011 growing seasons (Dirk Cilliers, Unit of Environmental Sciences and Development, NWU, Potchefstroom Campus, compiled the map using Google Earth, 2012).

Table 2.1. Detail information of fields (location, size and soil characteristics) as well as production practices, rotation/cover crops planted and nematicides applied where nematode samples were collected during the 2010 and 2011 growing seasons for producer 1 (Mr. J. Easby).

| Field no | GPS coordinate | Field size (ha) | Soil type | | | Production practice | Cover and/or rotation crop | Crops/Grasses planted | | Potato planting before survey was conducted/cv | Nematicides applied on previous potato crops |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Sand | % Silt | % Organic C | | | 2010 | 2011 | | |
| **1 (TG2)** | S 28°03.607´ E 25°05.149´ | 88 | 93.9 | 3.2 | 0.19 | - 2 x disc - 2 x plough | - *Eragrostis* - Maize - Potato | *Eragrostis* | *Eragrostis* | 2004/Mondial | - EDB®[a] - Temik® |
| **2 (TG3)** | S 28°03.607´ E 25°05.149´ | 88 | 93.7 | 1.2 | 0.22 | - 2 x disc - 2 x plough | - *Eragrostis* - Maize - Potato | *Eragrostis* | Potato/Mondial | 2004/Mondial | - EDB® - Temik® |
| **3 (CG3)** | S 28°03.607´ E 25°05.149' | 88 | 93.9 | 1.1 | 0.17 | - 2 x disc - 2 x plough | - *Eragrostis* - Maize - Potato | *Eragrostis* | Potato/Mondial | 2004/Mondial | - EDB® - Temik® |
| **4 (TK4)** | S 28°03.607´ E 25°05.149´ | 28 | 94 | 1.1 | 0.17 | - 2 x disc - 2 x plough | - *Eragrostis* - Maize - Potato | Potato/Mondial | *Eragrostis* | 2004/Mondial | - EDB® - Temik® |
| **5 (T6T5)** | S 28°03.607´ E 25°05.149' | 36 | 94 | 1.1 | 0.17 | - 2 x disc - 2 x plough | - *Eragrostis* - Maize - Potato | Potato/Mondial | Maize | 2003/Mondial | - EDB® - Temik® |
| **6 (T6T7)** | S 28°03.607´ E 25°05.149´ | 36 | 93.9 | 1.1 | 0.19 | - 2 x disc - 2 x plough | - *Eragrostis* - Maize - Potato | *Eragrostis* | Potato/Sifra | 2005/Mondial | - EDB® - Temik® |

[a]EDB® = ethylenedibromide

Table 2.2. Detail information of fields (location, size and soil characteristics) as well as production practices, rotation/cover crops planted and nematicides applied where nematode samples were collected during the 2010 and 2011 growing seasons for producer 2 (Mr. JF. Van der Merwe).

| Field no | GPS coordinates | Field size (ha) | Soil type | | | Production practice | Cover and/or rotation crop | Crops/Grasses planted | | Potato planting before survey was conducted/cv | Nematicides applied on previous potato crops |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Sand | % Silt | % Organic C | | | 2010 | 2011 | | |
| **7 (VD6)** | S 28.059230° E 25.195532° | 40 | 94 | 1.1 | 0.16 | - Cut - Plough | - *Digitaria* - Maize - Onion - Potato | Potato/Sifra | Fallow | 2004/Mondial | - EDB® [a] - Mocap® |
| **8 (LH10)** | S 28.075963° E 25.171617° | 22 | 94.1 | 1.1 | 0.16 | - Cut - Plough | - *Digitaria* - Potato | Potato/Fabula | *Digitaria* | 2000/Sparta | - EDB® - Nemacur® |
| **9 (LH13)** | S 28.065838° E 25.180370° | 22 | 93.9 | 1.1 | 0.23 | - Cut - Plough | - *Digitaria* - Potato | Potato/Mondial | *Digitaria* | Never | - EDB® - Mocap® |
| **10 (LH15)** | S 28.058582° E 25.159005° | 22 | 96 | 1.1 | 0.15 | - Cut - Plough | - *Digitaria* - Onion - Potato | Onion | Fallow | 2003/Mondial | - EDB® - Nemacur® |
| **11 (LH20)** | S 28.091382° E 25.240698° | 22 | 93.8 | 3.3 | 0.21 | - Cut - Plough | - Maize - Potato - Sunflower - Wheat | Fallow | Potato/Sifra | Never | - EDB® - Mocap® |
| **12 (VS6)** | S 28.003375° E 25.208412° | 22 | 94 | 1.1 | 0.28 | - Cut - Plough | - *Digitaria* - Maize - Potato | Fallow | *Digitaria* | 2007/Mondial | - EDB® - Mocap® |

[a]EDB® = ethylenedibromide

Table 2.3. Detail information of fields (location, size and soil characteristics) as well as production practices, rotation/cover crops planted and nematicides applied where nematode samples were collected during the 2010 and 2011 growing seasons for producer 3 (Mr. J. Greyling).

| Field no | GPS coordinates | Field size (ha) | Soil type | | | Production practice | Cover and/or rotation crop | Crops/Grasses planted | | Potato planting before survey was conducted/cv | Nematicides applied on previous potato crops |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Sand | % Silt | % Organic C | | | 2010 | 2011 | | |
| **13 (A1)** | S 28º07.948´ E 25º00.003´ | 32 | 93.9 | 1.1 | 0.3 | - Plough - Rip | - *Digitaria* - Potato | *Digitaria* | Potato/Mondial | 2005/Mondial | - EDB®[a] - Mocap® - Vydate® |
| **14 (A6)** | S 28º08.949´ E 25º03.277´ | 32 | 91.8 | 1.2 | 0.31 | - Plough - Rip | - *Digitaria* - Maize - Potato | Potato/Mondial | Maize | Never | - EDB® - Mocap® - Nemacur® - Vydate® |
| **15 (B1)** | S 28º08.099´ E 25º03.656´ | 32 | 93.8 | 1.2 | 0.37 | - Plough - Rip | - *Digitaria* - Potato | *Digitaria* | Potato/Sifra | 2005/Mondial | - EDB® - Mocap® - Vydate® |
| **16 (B2)** | S 28º08.099´ E 25º03.650´ | 32 | 91.9 | 1.1 | 0.34 | - Plough - Rip | - *Digitaria* - Maize - Potato | *Digitaria* | *Digitaria* | 2006/Mondial | - EDB® - Mocap® - Vydate® |
| **17 (SP2)** | S 28º07.854´ E 25º02.543´ | 34 | 91.6 | 1.2 | 0.48 | - Plough - Rip | - *Digitaria* - Maize - Potato | *Digitaria* | Potato/Mondial | 2005/Mondial | - EDB® - Mocap® - Vydate® |
| **18 (SP11)** | S 28º05.540´ E 24º58.590´ | 30 | 93.9 | 1.1 | 0.23 | - Plough - Rip | - *Digitaria* - Maize - *Panicum* Potato | *Digitaria* | Potato/Mondial | 2005/Mondial | - EDB® - Mocap® - Vydate® |
| **19 (S5)** | S 28º07.943´ E 24º59.998´ | 20 | 91.9 | 1.1 | 0.36 | - Plough - Rip | - *Digitaria* - *Eragrostis* - Maize - Potato | *Digitaria* | *Digitaria* | 2007/Mondial | - EDB® - Mocap® - Vydate® |

[a]EDB® = ethylenedibromide

Table 2.4. Detail information of fields (location, size and soil characteristics) as well as production practices, rotation/cover crops planted and nematicides applied where nematode samples were collected during the 2010 and 2011 growing seasons for producer 4 (Mr. W. Easby).

| Field no | GPS coordinates | Field size (ha) | Soil type | | | Production practice | Cover and/or rotation crop | Crops/Grasses planted | | Potato planting before survey was conducted/cv. | Nematicides applied on previous potato crops |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Sand | % Silt | % Organic C | | | 2010 | 2011 | | |
| **20 (P1)** | S 27.988130° E 25.110988° | 20 | 91.8 | 1.2 | 0.36 | - Plough<br>- Rip | - Maize<br>- Potato<br>- Onion | Maize | Fallow | 2007/Mondial | - EDB®[a] |
| **21 (P2)** | S 27.995517° E 25.123238° | 30 | 91.9 | 1.1 | 0.33 | - Plough<br>- Rip | - *Digitaria*<br>- Maize<br>- Potato | *Digitaria* | *Digitaria* | 2000/Mondial | - Nemacur® |
| **22 (VH1)** | S 27.998832° E 25.112532° | 60 | 91.8 | 1.2 | 0.29 | - Cut<br>- Plough | - Maize<br>- Potato<br>- Wheat | Fallow | Potato/Mondial | 2005/Mondial | - Nemacur®<br>- Temik® |
| **23 (VH2)** | S 28.00420° E 25.121402° | 50 | 93.8 | 1.2 | 0.26 | - Cut<br>- Plough | - Maize<br>- Potato<br>- Wheat | Potato/Mondial | Maize | 2006/Mondial | - Nemacur®<br>- Temik® |
| **24 (VH3)** | S 28.002718° E 25.130312° | 50 | 93.7 | 1.2 | 0.27 | - Cut<br>- Plough | - Maize<br>- Potato<br>- Wheat | Fallow | Potato/Mondial | 2004/Mondial | - Nemacur®<br>- Temik® |
| **25 (VH5)** | S 28.005967° E 25.148875° | 50 | 94 | 1.1 | 0.23 | - Plough<br>- Rip | - Maize<br>- Potato<br>- Wheat | Fallow | Fallow | 2006/Mondial | - EDB® |

[a]EDB® = ethylenedibromide

Table 2.5. Detail information of fields (location, size and soil characteristics) as well as production practices, rotation/cover crops planted and nematicides applied where nematode samples were collected during the 2010 and 2011 growing seasons for producer 5 (Mr. W. Ras).

| Field no | GPS coordinates | Field size (ha) | Soil type | | | Production practice | Cover and/or rotation crop | Crops/Grasses planted | | Potato planting before survey was conducted/cv | Nematicides applied on previous potato crops |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % Sand | % Silt | % Organic C | | | 2010 | 2011 | | |
| **26 (D4)** | S 28°07.665´ E 25°00.265´ | 60 | 91.8 | 1.2 | 0.25 | - Disc rip - Plough | - *Eragrostis* - Potato | Fallow | Potato/Mondial | 2002/Mondial | - EDB® [a] - Vydate® |
| **27 (GH1)** | S 28°09.106´ E 25°00.323´ | 60 | 93.9 | 1.2 | 0.31 | - Disc rip - Plough | - *Eragrostis* - Potato | *Eragrostis* | *Eragrostis* | 2009/Mondial | - EDB® - Vydate® |
| **28 (GH17)** | S 28°09.563´ E 25°02.731´ | 60 | 93.8 | 1.2 | 0.29 | - Disc rip - Plough | - *Eragrostis* - Potato | Fallow | Potato/Mondial | 2003/Mondial | - EDB® - Vydate® |
| **29 (UK1)** | S 28°08.850´ E 24°59.834´ | 60 | 91.7 | 1.2 | 0.36 | - Disc rip - Plough | - *Eragrostis* - Potato | *Eragrostis* | *Eragrostis* | 2008/Mondial | - EDB® - Vydate® |
| **30 (UK4)** | S 28°09.312´ E 24°59.671´ | 60 | 91.7 | 1.2 | 0.38 | - Disc rip - Plough | - *Eragrostis* - Potato | Fallow | Potato/Mondial | 2002/Mondial | - EDB® - Vydate® |
| **31 (UK5)** | S 28°08.722´ E 24°58.184´ | 60 | 93.7 | 1.2 | 0.44 | - Disc rip - Plough | - *Eragrostis* - Potato | *Eragrostis* | *Eragrostis* | 2006/Mondial | - EDB® - Vydate® |

[a]EDB® = ethylenedibromide

### 2.2.2 Nematode sampling (surveys)

Due to the nature of the crop rotation cycles practiced by potato producers in the Christiana area, not all the fields were planted with potato at the time when nematode samples were collected. Some fields were planted with maize, onion or grass cover crops (Figure 2.3A; Tables 2.1-2.5) while others were left fallow (Figure 2.3B; Tables 2.1-2.5). The time of nematode sampling coincided with flowering of the potato crop during both the 2010 and 2011 growing seasons. This was done to access population levels as well as changes of PPN and NPN and to advise growers on strategies to implement in order to alleviate the PPN problem in their potato crops. Soil samples of each field were also analysed to obtain information on the physical characteristics of the soil and are listed for each of the fields in Tables 2.1-2.5. Due to a technical error, pH measurements for the 31 fields were not analysed.

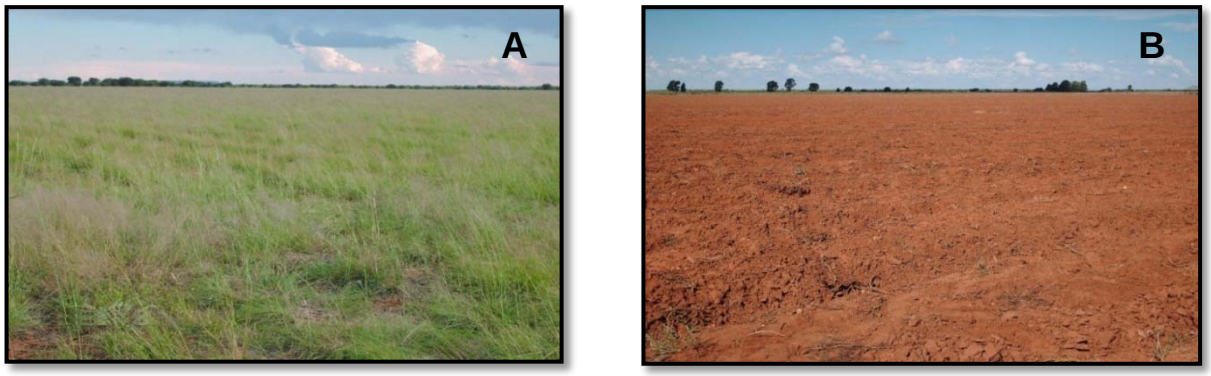


Figure 2.3 (A & B). A field planted with a grass cover crop (A) and a field left fallow (B) in the Christiana area where nematode samples were collected during the 2010 and 2011 growing seasons.

Nematode sampling was done in 31 fields of five potato producers in the Christiana area both during the 2010 and 2011 growing seasons. For the 2010 season, samples were obtained from 16 to 18 March and from 14 to 16 February during 2011. Soil and root samples were collected from fields planted with crops (i.e. potato, maize and onion) as well as those planted with grasses. Only soil samples were, however, collected from fallow fields. Field sizes ranged between 20-88ha and only fields planted with potato, maize or onion at the time of sampling were irrigated from the Vaalriver. Fields planted with grasses were rain fed.

For each field, 40 soil and root (crops and grasses) or only soil samples (fallow fields) were randomly collected at a depth of approximately 30cm using garden spades. This procedure was followed since

the top 15-20cm of soil where the majority of the root systems of crops planted in this area occur within the soil profile is also where PPN generally occur (Hooper *et al*., 2005). Root and corresponding soil samples from each field were placed in plastic bags, marked and transported to the Nematology Laboratory of the North-West University in Potchefstroom (North-West Province) where it were stored at 6-8ºC until nematode extraction was performed. The latter was usually within five days after nematode sampling. Before extraction, soil as well as roots of the 40 samples obtained from each field were mixed thoroughly and divided into 4 sub-samples, which were then each subjected to nematode extractions as described below.

### 2.2.3 Nematode extractions

#### 2.2.3.1 Root samples (50g)

The adapted NaOCl method was used for the extraction of RKN eggs and females from the 50g root sub-samples.

**Principle**

This particular method is based on the use of NaOCl that dissolves the gelatine layer that surrounds the egg masses that has been produced by RKN females on the surface/within root segments (Riekert, 1995).

**Procedure**

a) A 50g root sample was taken from each of the four sub-samples that were collected from each field and cut into 1cm pieces and mixed thoroughly.
b) Each root sample was shaken for 4 minutes in 400ml of a 1% NaOCl solution to break down the gelatinous matrix surrounding the RKN eggs and releases them from the roots.
c) This mixture containing the eggs and J2's was decanted through a range of nested sieves, which from bottom to top consisted of a 10-, 25-, 45-, 63-, 75-, 250- and 710µm-mesh sieves. The latter sieve ensures less clogging on the bottom 10µm-mesh sieve during the extraction process. A vacuum pump (Edwards high vacuum pump, RPM1725/1426, 60/50Hz) was also connected to the 10-µm mesh sieve to apply suction and enhance the movement of the NaOCl-water solution through the range of sieves.
d) Root fragments on the top 710µm-mesh sieve were washed thoroughly with tap water for about 1 minute.

e) Eggs and J2 collected on the 10µm-mesh sieve were collected with running tap water into a 100ml sample bottle for counting.

#### 2.2.3.2 Root samples (5g)

Nematodes were extracted from 5g root samples using the adapted sugar-flotation method (Hooper *et al*., 2005).

- **Sugar-flotation method**

**Principal**

This method relies on the density, size and gravity of nematodes and is based on maceration of root and tuber material.

**Procedure**

a) A 5g sample was taken from the four sub-samples from each of the fields sampled. The roots were cut into 1cm pieces and mixed thoroughly.
b) Each root sample was macerated in 250ml tap water at high speed in a domestic blender for 90 seconds to release nematodes from the root tissue.
c) The water suspension containing the nematodes and root fragments were subsequently decanted on a 710µm-mesh sieve that was nested on a 25µm-mesh sieve.
d) The root pieces on the 710µm-mesh sieve were washed thoroughly with running tap water for 2 minutes**,** the debris discarded and the residue on the 25µm-mesh sieve collected and decanted into a 50ml centrifuge tube for each separate sub-sample.
f) Two cubic cm of kaolin was added to each of the tubes containing the nematodes in suspension, stirred well and centrifuged at 4000rpm for 1 minute.
g) The supernatant that contained mainly debris was decanted and a sucrose solution with a specific gravity of 1.15g/$cm^3$ added to each tube. The mixture containing the nematodes was stirred well and again centrifuged at 1 800g for 1 minute.
h) The supernatant containing the nematodes was decanted onto a 25µm-mesh sieve and rinsed well with tap water to remove the sucrose solution. The residue on the sieve that contains the nematodes was collected in a 100ml sample bottle for counting and identification purposes.

- **The importance of kaolin** (Coolen & D'Herde, 1972)

Kaolin is a clay mineral with a specific gravity of 2.6 and consists of particles that are approximately 2-3µm in size. Although the density of kaolin is greater than that of nematodes, kaolin particles are small and flat. Therefore, these particles sink to the bottom of the centrifuge tube at a slower pace than the nematodes. Kaolin thus spreads out to form a layer over the loose sediment that contains the nematodes and seals it off when the supernatant is decanted. The nematodes thus stays sealed-in under the kaolin layer. After addition of the sucrose solution, the kaolin layer must be broken to enable mixing of the sediment and nematodes with the sucrose solution. This way the nematodes are brought into suspension in the sugar solution and will stay in suspension during and after the centrifuging process. Kaolin also precipitates during the second centrifugation with the sugar solution and prevent re-mixing of the sedimented debris when the sugar solution, containing the nematodes, is decanted on the 25µm-mesh sieve. Finally, a suspension of nematodes in clear water is obtained for counting and identification.

#### 2.2.3.3 Soil samples (200g)

Extraction of nematodes, both PPN and NPN, from 200g of each of the four soil sub-samples, was done using the elutriator method (Seinhorst, 1962).

- **Elutriator method**

**Principle**

The method is based on the difference in size, shape and sedimentation rate between nematodes and soil particles. It also depends on the mobility of nematodes since the nematode-water suspension obtained after elutriation is placed on tissues through which the nematodes move to be collected in clear water for counting and identification purposes. The Elutriator set-up uses an upward stream of tap water that enables the nematodes and fine particles to float in the extraction column, whereas heavier particles concurrently settle in the lower part of the apparatus. The nematode suspension in the extraction column is decanted through a side-outlet and sieved to remove the fine particles. The nematodes and washings are then washed from the sieves and placed on tissues. Nematodes move through the tissues into the water, collected in counting bottles and transferred to counting dishes for counting and identification purposes.

**Procedure**

a) Rubber stoppers were firmly secured to the bottom of the each elutriator and a 5L bucket was placed on the top shelf next to the pipe of each elutriator.

b) Each elutriator was filled to the top outlet pipe with tap water and the pressure dials adjusted to 75ml/min.

c) A large 710µm-mesh sieve was placed on top of a funnel and the funnel then placed into an open 2L Erlenmeyer flask.

d) A 200g soil sub-sample was placed in a 200ml plastic beaker. Soil particles and debris with a diameter of more than 1mm were removed by staying on top of a 710µm-mesh sieve. All the soil was subsequently washed through the sieve into the Erlenmeyer flask.

e) The sieve and funnel were inverted and cleaned with high pressure spray using tap water to remove all debris and dirt (these were now ready to use for the next sub-sample).

f) The Erlenmeyer flask was filled to the brim with tap water. The lid was then wetted with water and placed on top of the flask. Holding the sample carefully and ensuring that the lid was closed, the sample was shaken well by turning the flask upside down 3 times. The flask and lid were then placed upside down on the holder at the top of the elutriator.

g) While holding the lid with one hand, the rubber stopper was gently opened to release the sample containing the nematodes into the elutriator allowing it to flow through the elutriating column for 20 minutes. Throughout the process it was checked that the lid of the Erlenmeyer flask did not block.

h) After 20 minutes the remaining contents from the flask was emptied into the bucket and the bucket was left for a further 10 minutes to collect the rest of the nematode-water suspension that was still inside the elutriator column at the top. The bucket was then placed on the bottom shelf at the end of the elutriator and the clip on the bottom pipe was removed. This way the rest of the nematode-water suspension was transferred from the elutriator into the bucket.

i) The bucket with the nematode-water suspension was left standing for at least 1 hour for the nematodes to settle.

j) The nematodes were then concentrated by passing the suspension in the bucket through a series of six stacked sieves. The bigger nematodes (*Xiphinema, Longidorus*) were retained on the two top sieves (100µm-mesh), while the smaller nematodes (*Pratylenchus, Meloidogyne, Helicotylenchus etc.*) were retained on the four bottom sieves (50µm-mesh).

k) The contents of the bucket were slowly poured onto the top sieve (1$^{st}$ 100µm-mesh). Using a hand-held bottle filled with tap water, the nematodes on this sieve were washed into a clean 200ml beaker. The sieve was then turned upside down and whatever remained on it was washed onto the next

sieve below. Then the top sieve was cleaned with high pressure water spray and was ready for use for the next sample. The contents of the second sieve were washed (as described above) onto the bottom sieve (2nd 100µm-mesh) into the same 200ml beaker. The remaining nematodes and washings on the second sieve was washed onto the third, smaller 50µm sieve below and the second sieve cleaned as describe above for the first sieve. The same procedure as for the first two sieves were followed and the nematodes and washings on the third sieve washed into another a clean 200ml beaker. These steps were repeated until all nematodes and washings on the four 50µm-mesh sieves had been washed into the beaker.

m) Both beakers containing the suspended nematodes were allowed to stand for at least 30 minutes.

n) Brown and blue sieves (both 38µm-mesh) were then lined with 3 ply tissue and the brown sieve placed on top. All the sieves were wetted with tap water.

o) Liquid from the 1st beaker was poured onto the mesh of the brown sieve and the sieve was placed on top of a 150mm brown plastic saucer.

p) Liquid from the 2nd beaker was then poured onto the mesh of the blue sieve and the blue sieve placed on top of a second 150mm brown saucer. Afterwards 50ml distilled water was added to the base of each saucer and both sieves were covered with another brown saucer.

q) After being left for 48 hours, in order for the nematodes to migrate through the tissue and mesh of the sieves into the water, the sieves were lifted and the excess water drained into the bottom of the brown saucers.

r) The nematode-water suspension from the saucer was poured into a clean, 100ml measuring cylinder.

s) The nematode-water suspension in the measuring cylinder was left to stand for 90 minutes before counting and identification of the nematodes suspended was done.

### 2.2.4 Counting of nematodes

PPN that were extracted and of which mature specimens were present were identified to species level using morphological characteristics. The same procedure was followed for NPN, only for the 2011 survey, although the latter were only identified to order or family level. RKN species, however, were identified using molecular techniques, namely the SCAR-PCR method (Chapter 3) (Zijlstra, 2000; Zijlstra *et al*., 2000).

PPN and NPN were counted in a De Grisse counting dish using a stereo microscope at 100x magnification. RKN nematode eggs and J2 from the 5g root as well as NPN from the 200g soil samples

were counted the same way without prior fixing of the nematodes. PPN per 200g soil were also counted with concurrent identification to genus level but fixed afterwards to enable accurate morphological species identification as described below.

### 2.2.5 Species identification by means of morphological features

For species identification, PPN were transferred to anhydrous glycerine and mounted on microscope slides according to the paraffin ring method (see paragraph 2.2.5.1).

#### 2.2.5.1 Transfer of nematodes to anhydrous glycerine

It is crucial to study the finer morphological structures of nematodes for identification at species level. Nematodes were, therefore, fixed in a heated formaldehyde-propionic-acid-water (FPG) solution (100ml of a 40% formalin solution, 10ml propionic acid and 890ml distilled water) [Verbal communication, Dr. Antoinette Swart, Taxonomist ARC-Plant Protection Research Institute (PPRI), National Nematode Collection, Pretoria, South Africa, 22 November 2010] in an incubator at 40ºC for 72 hours and then transferred to a glycerin solution (De Grisse, 1965). The method is described in detail below.

**Procedure**

Nematodes to be fixed were transferred to distilled water in a Syracuse dish (5ml capacity). Most of the water in which the nematode was suspended was carefully removed after the suspension was allowed to stand for at least one hour and the nematodes settled to the bottom of the dish. An FPG solution containing 100ml of a 40% formalin solution, 10ml propionic acid, 890ml distilled water and just enough picric acid to stain the solution citrus yellow was prepared and heated in a water bath to between 60-70ºC. The hot fixative was added to the dish containing the nematodes and placed in a petri dish with a closed lid, which was then placed in a desiccator with an atmosphere of FPG solution. The latter was obtained by adding FPG solution to the bottom of the dessiccator. The desiccator was placed in an oven at 38-40°C for three days. After three days the lid of the petri dish was removed and half of the FPG solution drawn off from the nematode suspension. Solution 1 (200ml of a 95% ethanol solution, 10ml glycerine, 790ml distilled water) was then added to the small dish containing the nematodes. Without replacing the cover of the petri dish the nematodes were put back in the desiccator, with an atmosphere of a 95% ethanol solution, for 12 hours. After 12 hours half of Solution 1 was drawn off and replaced with Solution 2 (950ml of a 95% ethanol solution, 50ml glycerine). The petri dish was then partially covered (so that slow evaporation could take place) and placed in an incubator at 38-40ºC for

two to three days or until all the ethanol had evaporated. The small glass dish with nematodes was removed from the incubator and placed in a desiccator (that contained silica at the bottom) for another two days after which they were ready to be mounted.

- **The paraffin-ring method**

A copper tube with a diameter of 1.5cm was heated, pressed in paraffin wax and touched on the surface of a glass slide. As a result, a paraffin ring was formed on the slide. A small drop of glycerine was placed in the middle of the wax ring. Nematodes were individually fished from the eyeglass using a needle, transferred to the middle of the ring and sealed off by slipping a cover glass over the paraffin ring. The slide was heated over an open/warm plate to melt the paraffin wax and this way sealing in the nematodes. Finally the outside edge of the cover glass was sealed off with colourless acrylic nail hardener (Cutex). The mounted nematode specimens were sent to the nematode taxonomists, Drs. A. Malan, M. Marias and E. Van den Berg (ARC-Plant Protection Research Institute, Pretoria) for identification to species level.

### 2.2.6 Rearing of root-knot nematode (RKN) species for molecular identification

Seedlings of the susceptible tomato cultivar Rodade was grown in 4L pots containing soil samples from the 31 fields of the five producers to rear RKN populations for identification purposes. Soil were collected from each locality and placed in separate, 4L plastic pots in a greenhouse which had no temperature regulation. Susceptible tomato seedlings (cv. Rodade) were planted in each pot to attract RKN J2's and establish RKN populations for each locality. Infected tomato plants with visible galling were removed approximately 56 days later for identification of *Meloidogyne* species using molecular-based methods as described in Chapter 3.

## 2.3 Data analysis

### 2.3.1 Calculation of prominence values

Nematode data obtained as a result of both surveys conducted during 2010 and 2011, respectively, was subjected first to the calculation of prominence values (PV) using Excel (Microsoft Windows XP). PV takes into consideration the population density (PD) as well as frequency of occurrence (%) of the

relevant nematode family/genus/species. For this study, PV were calculated only for nematode genera and families. The following equation (De Waele & Jordaan, 1988) was used to calculate PV:

$$PV = \text{population density} \times \sqrt{\text{Frequency of occurrence}} / 10$$

Population density of each nematode genus/family at each locality (producers' fields) was calculated as follows:

$$\frac{\text{total number of the genus/family present in each field}}{\text{number of samples per field in which the genus/family occurred}}$$

Frequency of occurrence of each nematode genus/family at each locality (producers' fields) was calculated as follows:

$$\frac{\text{number of samples per field in which the genus/family occurred}}{\text{total number of sample obtained per field}} \times 100$$

**2.3.2 Parametric and non-parametric statistics**

Data for PPN (2010 and 2011) and NPN (2011) was transformed to $\log_{10}(x+1)$ to remove the inherent variability before analyses. Due to general non-uniformity of nematode data it was also subjected to the Kruskal-Wallis test for non-parametric data and the Mann-Whitney U, T-test and Anova tests for parametric data (Statistica for Windows Version 10). For these analyses the various cultivation practices, nematicides and rotation sequences applied to the 31 fields were grouped into categories and assigned values (Table 2.6) to enable identification of those parameters that had a significant effect on the population levels of the various nematode genera/family identified during the surveys.

In addition, NPN data obtained during the 2011 season were subjected to a faunal analysis based on calculating Enrichment Indices (EI) and Structural Indices (SI) using the Faunalizer program originally described by Bongers and Ferris (1999) and improved by Ferris *et al*. (2001).

Nematode families present in soil samples were categorised along a coloniser-persister (cp) continuum (Bongers, 1990). This system recognizes that taxa of monophyletic families are adapted similarly to specific environmental conditions and food sources through anatomical and physiological commonalities. These taxa are then assigned a 1-5 linear scale according to their *r* and *K* strategies.

Subsequently, enrichment and structure indices were calculated according to Ferris *et al*. (2001), which are the descriptors of the food web condition at a particular site. These trajectories allow a graphic representation of the faunal profile in order to quantify the soil food web state through the enrichment index (a measure of opportunistic bacterivore and fungivore nematodes) and the structure index (indicator of food web state affected by stress or disturbance). Results obtained by subjecting nematode data to these statistical analyses indicated whether enrichment-opportunist bacterivore nematodes increased rapidly in response to low C/N plant materials or whether population levels of opportunist bacterivore nematodes increased/decreased. Trends in terms of population shifts of fungivore nematodes as well as predator nematodes are also recorded using this simplified food web analysis for NPN (Ferris *et al*., 2001).

Categorisation of these genera and family into functional guilds are based upon designated feeding habits, namely ba = bacterivores, ca = carnivore, fu = fungivores, om = predator (omnivore). Suffix numbers for each guild represents cp values (Bongers, 1990) for the various nematode taxa as reflected in Table 2.23.

Table 2.6. Categories assigned to various rotation sequences, cultivation practices and nematicides applied on 31 fields of five potato producers in the Christiana area (North-West Province).

| Value assigned | Cultivation practices | Nematicides | Rotation sequences |
|---|---|---|---|
| **1** | Plough | EDB®[a] only | Grass and potato |
| **2** | Rip and Plough | Other nematicides | Grass, maize and potato |
| **3** | **-** | EDB® and other nematicides (combined) | Maize, wheat and potato |
| **4** | **-** | **-** | Maize and/or grass and potato |

[a]EDB®=ethylenedibromide

## 2.4 Results

### 2.4.1 Prominence values

#### 2.4.1.1 Plant-parasitic nematode (PPN) data

- **Roots (50g and 5g)**

Table 2.7. ***Meloidogyne*** species data for fields of five producers in the Christiana area sampled during the **2011** growing season as represented by prominence values (PV), frequency of occurrence (FO; %) and population density (PD) per **50g roots** of various crops and grasses.

| Field no | PV | PD | FO |
|---|---|---|---|
| **23** | 701 | 7009 | 100 |
| **5** | 276 | 2755 | 100 |
| **19** | 53 | 1050 | 25 |
| **14** | 12 | 172 | 50 |
| **15** | 7 | 102 | 50 |
| **7** | 5 | 52 | 100 |
| **29** | 4 | 82 | 25 |
| **4** | 4 | 76 | 25 |
| **3** | 1 | 28 | 25 |
| **12** | 0.3 | 6 | 25 |

Extraction of *Meloidogyne* spp. from 50g roots of crops and grasses sampled using the adapted NaOCl method (Riekert, 1995) was done only during the 2011 survey. This was due to unforeseen technical problems experienced with extraction apparatus of the Nematology Laboratory during the 2010 season.

RKN eggs and J2 were extracted from crops and grass roots from 10 of the 31 fields sampled during 2011 and were identified as *M. incognita* (fields 3, 14, 19, 23 and 29) and *M. javanica* (fields 5 and 29) using molecular techniques (see Chapter 3). Mature RKN females from fields 4, 7, 12 and 15 could not be obtained for molecular or morphological identification purposes for the purpose of this study (see Chapter 3).

PV for RKN ranged from 0.3 (field 12) to 701 (field 23), PD from 6 to 7009 and FO from 25% to 100% for the respective fields. Fields 23, 5 and 19 had the highest PV, PD and FO for RKN while field 12 had the lowest (Table 2.7).

Table 2.8. Data for **endo- and semi-endoparasitic** nematode genera/family in fields of the five producers from the Christiana area sampled during the **2010 and 2011** growing seasons as represented by prominence values (PV), frequency of occurrence (FO; %) and population density (PD) per **5g roots** of various crops and grasses.

| **2010 Survey** | | | | | | | | **2011 Survey** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Meloidogyne* spp.** | | | | **Hoplolaimidae** | | | | ***Meloidogyne* spp.** | | | | ***Pratylenchus* spp.** | | | | ***Ditylenchus* spp.** | | | |
| **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** |
| **14** | 45 | 450 | 100 | **5** | 2 | 33 | 25 | **23** | 39 | 451 | 75 | **10** | 5 | 56 | 75 | **29** | 2 | 15 | 100 |
| **31** | 23 | 227 | 100 | **21** | 0.5 | 10 | 25 | **14** | 4 | 43 | 75 | **23** | 1 | 14 | 75 | **31** | 0.9 | 11 | 75 |
| **23** | 16 | 219 | 50 | | | | | **5** | 3 | 44 | 50 | **3** | 0.9 | 14 | 50 | | | | |
| **1** | 15 | 294 | 25 | | | | | **15** | 1 | 21 | 25 | **1** | 0.6 | 9 | 50 | | | | |
| **20** | 9 | 125 | 50 | | | | | **12** | 0.5 | 11 | 25 | **14** | 0.3 | 7 | 25 | | | | |
| **17** | 8 | 83 | 100 | | | | | **3** | 0.5 | 10 | 25 | **18** | 0.2 | 4 | 25 | | | | |
| **6** | 7 | 78 | 75 | | | | | **26** | 0.2 | 4 | 25 | | | | | | | | |
| **21** | 5 | 61 | 75 | | | | | | | | | | | | | | | | |
| **16** | 2 | 42 | 25 | | | | | | | | | | | | | | | | |
| **2** | 1 | 19 | 25 | | | | | | | | | | | | | | | | |

*Meloidogyne* spp. were the predominant endoparasitic genus identified from 5g roots of crops and grasses sampled during both the 2010 and 2011 growing seasons (Table 2.8). RKN J2 were present in 10 and seven of the 31 fields sampled during the 2010 and 2011 growing seasons, respectively, and were identified as *M. incognita* (fields 20 and 3) and *M. javanica* (fields 16 and 26) using molecular techniques (see Chapter 3). Mature RKN females from fields 1, 2, 6, 15, 17, 21 and 31 could not be obtained for molecular or morphological identification purposes for the purpose of this study (see Chapter 3).

During 2010 *Meloidogyne* spp. were followed only by individuals of the Hoplolaimidae family in terms of predominance, while *Pratylenchus* and *Ditylenchus* genera ranked second and third in 2011 after *Meloidogyne* spp. (Table 2.8). PV for *Meloidogyne* spp. ranged from 1 to 45, PD from 19 to 450 and FO from 25% to 100%.

During 2010 individuals of the Hoplolaimidae were present only in roots of crop plants from 2 of the 31 fields sampled and had low PV (0.5 to 2) and PD (10 to 33) with individuals occurring in only 25% of the samples collected.

During 2011 PV for *Meloidogyne* spp. ranged from 0.2 to 39, PD from 4 to 451 and FO from 25% to 75% (Table 2.8). PV, PD and FO for *Pratylenchus* and *Ditylenchus* spp. were substantially lower with the highest being 5 and 2, 56 and 15 and 75% and 100%, respectively.

- **Soil (200g)**

Table 2.9. Data for **endoparasitic** nematode genera/family in fields of the five producers in the Christiana area sampled during the **2010 and 2011** growing seasons as represented by prominence values (PV), frequency of occurrence (FO; %) and population density (PD) per **200g soil**.

| 2010 Survey | | | | | | | | | | | | 2011 Survey | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Meloidogyne* spp.** | | | | ***Pratylenchus* spp.** | | | | **Heteroderidae** | | | | ***Meloidogyne* spp.** | | | | ***Pratylenchus* spp.** | | | |
| **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** |
| **25** | 111 | 1112 | 100 | **25** | 34 | 340 | 100 | **31** | 3 | 32 | 100 | **23** | 344 | 3442 | 25 | **23** | 14 | 137 | 100 |
| **23** | 68 | 682 | 100 | **22** | 17 | 165 | 100 | **29** | 1 | 20 | 25 | **14** | 77 | 762 | 100 | **25** | 9 | 87 | 100 |
| **14** | 63 | 630 | 100 | **20** | 12 | 117 | 100 | | | | | **5** | 32 | 317 | 100 | **3** | 7 | 67 | 100 |
| **29** | 46 | 462 | 100 | **24** | 8 | 90 | 75 | | | | | **22** | 9 | 90 | 100 | **16** | 6 | 60 | 100 |
| **20** | 14 | 137 | 100 | **3** | 7 | 67 | 100 | | | | | **27** | 3 | 37 | 75 | **6** | 5 | 47 | 100 |
| **9** | 12 | 117 | 100 | **8** | 6 | 62 | 100 | | | | | **18** | 3 | 60 | 25 | **18** | 4 | 40 | 100 |
| **8** | 4 | 42 | 100 | **16** | 6 | 62 | 100 | | | | | **24** | 3 | 60 | 25 | **9** | 3 | 37 | 75 |
| **22** | 3 | 32 | 100 | **4** | 6 | 57 | 100 | | | | | **20** | 2 | 23 | 75 | **20** | 2 | 22 | 100 |
| **11** | 3 | 37 | 75 | **9** | 5 | 52 | 100 | | | | | **1** | 1 | 20 | 50 | **1** | 2 | 30 | 50 |
| **24** | 3 | 33 | 75 | **10** | 4 | 47 | 75 | | | | | **16** | 1 | 20 | 50 | **21** | 1 | 17 | 75 |
| **31** | 3 | 30 | 75 | **26** | 3 | 32 | 100 | | | | | **6** | 1 | 20 | 25 | **27** | 1 | 20 | 50 |
| **30** | 2 | 27 | 75 | **7** | 1 | 15 | 50 | | | | | **10** | 1 | 20 | 25 | **5** | 1 | 20 | 25 |
| **21** | 2 | 40 | 25 | **19** | 2 | 22 | 100 | | | | | **15** | 1 | 20 | 25 | **7** | 1 | 20 | 25 |
| **15** | 2 | 23 | 75 | **2** | 2 | 23 | 75 | | | | | **26** | 1 | 20 | 25 | **8** | 1 | 20 | 25 |
| **7** | 1 | 15 | 50 | **21** | 2 | 30 | 25 | | | | | **25** | 0.8 | 10 | 75 | **14** | 1 | 15 | 50 |
| **4** | 1 | 13 | 75 | **11** | 1 | 20 | 50 | | | | | **9** | 0.5 | 10 | 25 | **30** | 1 | 20 | 25 |
| **12** | 0.5 | 10 | 25 | **6** | 0.7 | 10 | 50 | | | | | **29** | 0.5 | 10 | 25 | **31** | 0.7 | 10 | 50 |
| **16** | 0.5 | 10 | 25 | **12** | 0.5 | 10 | 25 | | | | | **31** | 0.5 | 10 | 25 | **17** | 0.5 | 10 | 25 |
| **28** | 0.5 | 10 | 25 | **18** | 0.5 | 10 | 25 | | | | | | | | | **26** | 0.5 | 10 | 25 |
| | | | | **23** | 0.5 | 10 | 25 | | | | | | | | | **28** | 0.5 | 10 | 25 |
| | | | | **30** | 0.5 | 10 | 25 | | | | | | | | | **29** | 0.5 | 10 | 25 |
| | | | | **31** | 0.5 | 10 | 25 | | | | | | | | | | | | |

Nematode species identification could only be done for fields of which 5g root and their corresponding soil (200g) samples contained mature specimens of endoparasitic genera and/or families listed in Table 2.9. For the other fields only juvenile specimens were present and in some cases very low numbers of these genera and/or families occurred, which complicated complete identification of individuals to species level.

*Meloidogyne* spp. were the predominant endoparasitic genus identified from 200g soil samples collected from the 31 fields during both the 2010 and 2011 surveys. RKN J2 were present in 19 and 18 of the 31 fields sampled during the 2010 and 2011 seasons, respectively (Table 2.9). During 2010 RKN species identified from these fields were *M. incognita* (fields 9, 11, 22 and 25) and *M. javanica* (field 28) using molecular techniques (see Chapter 3), while *M. incognita* was identified during 2011 only for field 18. Mature RKN females could not be obtained after *in vivo* rearing of RKN J2 present in soil samples from the fields 1, 4, 6, 7, 8, 10, 12, 15, 21, 24, 27, 30 and 31 in separate pots planted with the tomato cv. Rodade for 56 days.

During 2010 *Meloidogyne* spp. were followed by individuals of *Pratylenchus* spp. and the Heteroderidae family and in 2011 only by individuals of *Pratylenchus* spp. in terms of its predominance (Table 2.9).

PV for *Meloidogyne* spp. ranged from 0.5 to 111 and PD from 10 to 1112 with RKN J2 occurring in 25% to 100% of the samples collected during 2010 (Table 2.9). Individuals of *Pratylenchus* spp. were present in soil from 22 of the 31 fields sampled with lower PV (0.5 to 34) and PD (10 to 340), and occurring in 25% to 100% of the samples collected. Individuals of the Heteroderidae family were present in only two of the 31 fields sampled with very low PV (1 to 3), PD (20 to 32) but FO values of 25% and 100%.

During 2011 PV for *Meloidogyne* spp. ranged from 0.5 to 344, PD from 10 to 3442 with RKN J2 occurring in 25% to 100% of the samples collected (Table 2.9). *Pratylenchus* spp. was present in 21 of the 31 fields sampled with relatively low PV (0.5 to 14) and PD (10 to 137). Individuals of the latter genus occurred in 25% to 100% of the samples collected. *Pratylenchus* spp. identified during both seasons were *P. zeae* (fields 8, 9, 10, 18, 20, 23, 25, 26, 29 and 30) and *P. neglectus* (field 27). No mature male or female specimens were present in soil samples obtained from the other fields where *Pratylenchus* individuals occurred (Table 2.9) and could not be identified to species level using morphological characteristics.

Table 2.10. Data for **semi- endo-/ecto- and ectoparasitic** nematode genera/family in fields of five producers in the Christiana area sampled during the **2010** growing season as represented by prominence values (PV), frequency of occurrence (FO; %) and population density (PD) per **200g soil**.

| **Trichodoridae** | | | | **Hoplolaimidae** | | | | ***Tylenchorhynchus* spp.** | | | | **Criconematidae** | | | | ***Xiphinema* spp.** | | | | ***Longidorus* spp.** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** |
| **25** | 5 | 52 | 100 | **6** | 14 | 142 | 100 | **21** | 59 | 587 | 100 | **4** | 7 | 67 | 100 | **26** | 6 | 55 | 100 | **4** | 0.5 | 10 | 25 |
| **21** | 5 | 45 | 100 | **30** | 12 | 122 | 100 | **28** | 20 | 197 | 100 | **14** | 4 | 35 | 100 | **28** | 2 | 30 | 25 | **12** | 0.5 | 10 | 25 |
| **7** | 4 | 37 | 100 | **19** | 9 | 90 | 100 | **26** | 11 | 112 | 100 | **29** | 2 | 20 | 75 | **30** | 1 | 17 | 75 | **18** | 0.5 | 10 | 25 |
| **29** | 4 | 37 | 100 | **4** | 6 | 60 | 100 | **22** | 7 | 67 | 100 | **31** | 2 | 20 | 75 | **4** | 1 | 20 | 25 | **19** | 0.5 | 10 | 25 |
| **30** | 4 | 37 | 100 | **17** | 3 | 30 | 75 | **24** | 7 | 65 | 100 | **5** | 1 | 15 | 50 | **19** | 0.5 | 10 | 25 | **23** | 0.5 | 10 | 25 |
| **10** | 3 | 33 | 75 | **28** | 2 | 27 | 75 | **2** | 4 | 50 | 75 | **30** | 1 | 15 | 50 | **22** | 0.5 | 10 | 25 | **26** | 0.5 | 10 | 25 |
| **22** | 2 | 22 | 100 | **14** | 2 | 20 | 75 | **19** | 4 | 42 | 100 | **7** | 0.7 | 10 | 25 | **29** | 0.5 | 10 | 25 | | | | |
| **19** | 2 | 23 | 75 | **16** | 2 | 25 | 50 | **18** | 2 | 27 | 75 | **2** | 0.5 | 10 | 25 | | | | | | | | |
| **16** | 2 | 20 | 75 | **12** | 2 | 15 | 100 | **17** | 1 | 20 | 25 | **6** | 0.5 | 10 | 25 | | | | | | | | |
| **8** | 2 | 18 | 100 | **7** | 1 | 20 | 50 | **6** | 0.5 | 10 | 25 | **12** | 0.5 | 10 | 25 | | | | | | | | |
| **3** | 2 | 30 | 25 | **22** | 1 | 17 | 75 | **10** | 0.5 | 10 | 25 | **19** | 0.5 | 10 | 25 | | | | | | | | |
| **20** | 1 | 12 | 100 | **26** | 1 | 20 | 50 | **25** | 0.5 | 10 | 25 | | | | | | | | | | | | |
| **17** | 1 | 20 | 25 | **31** | 1 | 13 | 75 | | | | | | | | | | | | | | | | |
| **24** | 1 | 20 | 25 | **2** | 0.7 | 10 | 50 | | | | | | | | | | | | | | | | |
| **12** | 1 | 15 | 50 | **8** | 0.5 | 10 | 25 | | | | | | | | | | | | | | | | |
| **26** | 1 | 15 | 50 | **10** | 0.5 | 10 | 25 | | | | | | | | | | | | | | | | |
| **6** | 1 | 13 | 75 | | | | | | | | | | | | | | | | | | | | |
| **31** | 1 | 13 | 75 | | | | | | | | | | | | | | | | | | | | |
| **11** | 1 | 10 | 100 | | | | | | | | | | | | | | | | | | | | |
| **4** | 0.8 | 10 | 75 | | | | | | | | | | | | | | | | | | | | |
| **2** | 0.7 | 10 | 50 | | | | | | | | | | | | | | | | | | | | |
| **9** | 0.5 | 10 | 25 | | | | | | | | | | | | | | | | | | | | |
| **23** | 0.5 | 10 | 25 | | | | | | | | | | | | | | | | | | | | |
| **27** | 0.5 | 10 | 25 | | | | | | | | | | | | | | | | | | | | |
| **28** | 0.8 | 10 | 75 | | | | | | | | | | | | | | | | | | | | |

Nematode species identification could only be done for fields of which 5g root and their corresponding soil (200g) samples contained mature specimens of both ectoparasitic and semi-endo-/ectoparasitic genera and/or families listed in Table 2.10. For the other fields only juvenile specimens were present and in some cases very low numbers of these genera and/or families occurred, which complicated complete identification of individuals to species level.

Semi-endo-/ectoparasitic genera present in 200g soil samples from the 31 fields surveyed during 2010 were individuals belonging to the Hoplolaimidae family (Table 2.10). Ectoparasites were represented by individuals from the Trichodoridae and Criconematidae families as well as individuals of the *Longidorus*, *Tylenchorhynchus*, and *Xiphinema* genera.

*Tylenchorhynchus* spp. was the predominant genus and was present in 12 of the 31 fields sampled, followed by Hoplolaimidae, Criconematidae, *Xiphinema*, Trichodoridae and *Longidorus* spp. PV for *Tylenchorhynchus* spp. ranged from 0.5 to 59 and PD from 10 to 587. Individuals of this genus were present in 25% to 100% of the samples collected and represented *T. brevilineatus* and *T. ventralis* (field 28). Mature specimens of *Tylenchorhynchus* spp, occurring in the soil from the other fields could not be found for morphological identification. Individuals of the Hoplolaimidae family were present in 16 of the 31 fields sampled with PV ranging from 0.5 to 14, PD from 10 to 142, occurring in 25% to 100% of the samples collected. Individuals of this family were represented by *Scutellonema brachyurus* (field 8) and *S. truncatum* (field 30), *Rotylenchus unisex* (field 30) as well as *R*. *caudaphasmidis* (field 30). Mature specimens of Hoplolaimidae occurring in the soil from the other fields could not be found for morphological identification.

Individuals belonging to the family Criconematidae occurred in 11 of the 31 fields sampled with PV ranging from 0.5 to 7, PD from 10 to 67 and FO from 25% to 100%. The Criconematidae family was represented by *Criconema mutabile* (field 7), *Criconemoides sphaerocephalus* (fields 4, 6, 7, 11, 14 and 15) and *Hemicriconemoides brachyurus* (fields 29 and 31). Individuals from the Trichodoridae family represented the genera *Paratrichodorus* and *Nanidorus* and occurred in 25 of the 31 fields sampled. For Trichodoridae (data pooled for the two genera) PV ranged from 0.8 to 5, PD from 10 to 52 and FO from 75% to 100%. The latter genera were represented by *Nanidorus minor* (fields 10, 19, 23 and 30) and *Paratrichodorus lobatus* (field 25). *Xiphinema* and *Longidorus* spp. occurred in seven and six of the 31 fields sampled, respectively. PV for *Xiphinema* spp. ranged from 0.5 to 6, PD from 10 to 55 and FO from 25% to 100%, while PV for *Longidorus* spp. was 0.5, PD 10 and the FO 25%.

Table 2.11. Data for **semi- endo-/ecto- and ectoparasitic** nematode genera/family in fields of five producers in the Christiana area sampled during the **2011** growing season as represented by prominence values (PV), frequency of occurrence (FO; %) and population density (PD) per **200g soil**.

| **Trichodoridae** | | | | **Criconematidae** | | | | ***Tylenchorhynchus* spp.** | | | | **Hoplolaimidae** | | | | ***Xiphinema* spp.** | | | | ***Psilenchus* spp.** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** |
| **14** | 105 | 104 | 100 | **6** | 2 | 20 | 100 | **21** | 6 | 62 | 100 | **26** | 2 | 30 | 50 | **27** | 1 | 13 | 75 | **21** | 6 | 66 | 75 |
| **16** | 8 | 75 | 100 | **3** | 1 | 16 | 75 | **3** | 6 | 55 | 100 | **22** | 2 | 30 | 25 | **6** | 0.5 | 10 | 25 | **13** | 4 | 50 | 50 |
| **23** | 5 | 57 | 75 | **7** | 1 | 20 | 50 | **29** | 3 | 27 | 100 | **2** | 1 | 15 | 50 | **12** | 0.5 | 10 | 25 | | | | |
| **10** | 5 | 50 | 100 | **14** | 1 | 20 | 50 | **5** | 3 | 30 | 75 | **5** | 0.7 | 10 | 50 | **16** | 0.5 | 10 | 25 | | | | |
| **5** | 5 | 45 | 100 | **15** | 1 | 15 | 50 | **14** | 2 | 20 | 100 | **3** | 0.5 | 10 | 25 | **17** | 0.5 | 10 | 25 | | | | |
| **1** | 2 | 20 | 100 | **19** | 1 | 20 | 25 | **27** | 2 | 23 | 75 | **6** | 0.5 | 10 | 25 | **19** | 0.5 | 10 | 25 | | | | |
| **3** | 2 | 25 | 50 | **5** | 0.7 | 10 | 50 | **31** | 1 | 20 | 50 | **15** | 0.5 | 10 | 25 | **21** | 0.5 | 10 | 25 | | | | |
| **29** | 2 | 25 | 50 | **2** | 0.5 | 10 | 25 | **9** | 1 | 20 | 25 | **16** | 0.5 | 10 | 25 | | | | | | | | |
| **18** | 2 | 15 | 100 | **29** | 0.5 | 10 | 25 | **23** | 0.5 | 10 | 25 | **20** | 0.5 | 10 | 25 | | | | | | | | |
| **2** | 1 | 20 | 50 | **31** | 0.5 | 10 | 25 | **26** | 0.5 | 10 | 25 | | | | | | | | | | | | |
| **7** | 1 | 20 | 50 | | | | | | | | | | | | | | | | | | | | |
| **25** | 1 | 20 | 50 | | | | | | | | | | | | | | | | | | | | |
| **8** | 1 | 15 | 50 | | | | | | | | | | | | | | | | | | | | |
| **19** | 1 | 13 | 75 | | | | | | | | | | | | | | | | | | | | |
| **27** | 1 | 20 | 25 | | | | | | | | | | | | | | | | | | | | |
| **6** | 0.8 | 10 | 75 | | | | | | | | | | | | | | | | | | | | |
| **9** | 0.8 | 10 | 75 | | | | | | | | | | | | | | | | | | | | |
| **20** | 0.8 | 10 | 75 | | | | | | | | | | | | | | | | | | | | |
| **31** | 0.8 | 10 | 75 | | | | | | | | | | | | | | | | | | | | |
| **21** | 0.7 | 10 | 50 | | | | | | | | | | | | | | | | | | | | |
| **15** | 0.5 | 10 | 25 | | | | | | | | | | | | | | | | | | | | |

Nematode species identification could only be done for fields of which 5g root and their corresponding soil (200g) samples contained mature specimens of both ectoparasitic and semi-endo-/ectoparasitic genera and/or families listed in Table 2.11. For the other fields, only juvenile specimens were present and in some cases very low numbers of these genera and/or families occurred, which complicated complete identification of individuals to species level.

Individuals from the Hoplolaimidae family were the only semi-ectoparasitc group identified from 200g soil samples of the 31 fields surveyed during 2011 and were present in 9 of the 31 fields sampled (Table 2.11). Ectoparasitic nematodes present were individuals belonging to the Criconematidae and Trichodoridae families as well as those from the genera *Psilenchus* and *Xiphinema*. Trichodoridae spp. (numbers pooled) were predominant, followed by *Psilenchus* spp., *Tylenchorhynchus* spp., Hoplolaimidae, Criconematidae and *Xiphinema* spp.

Trichodoridae individuals were present in 21 of the 31 fields sampled, with PV ranging from 0.5 to 105, PD from 10 to 104 and FO from 25% to 100% (Table 2.11). *Psilenchus* spp. individuals were present in only 2 of the 31 fields with PV ranging between 4 and 6, PD between 50 and 66 and occurred in 50% to 75% of the samples collected. *Tylenchorhynchus* spp. occurred in 10 of the 31 fields sampled with PV ranging from 0.5 to 6, PD from 10 to 62 and individuals being present in 25% to 100% of the samples collected. Individuals of the Criconematidae family also occurred in 10 of the 31 fields sampled, with PV ranging from 0.5 to 2, PD from 10 to 20 and FO from 25% to 100%. Individuals of the Hoplolaimidae family occurred in 9 of the 31 fields sampled, with PV ranging from 0.5 to 2, PD from 10 to 30 and FO from 25% to 50%. *Xiphinema* spp. occurred in seven of the 31 fields sampled, with PV ranging from 0.5 to 1, PD from 10 to 13 and FO from 25% to 75%.

### 2.4.1.2 Non-parasitic nematode (NPN) data

- **Soil (200g)**

Table 2.12. Data for **bacterivorous** nematode genera in fields of five producers in the Christiana area sampled during the **2011** growing season as represented by prominence values (PV), frequency of occurrence (FO; %) and population density (PD) per **200g soil**.

| ***Rhabditis* spp.** | | | | ***Acrobeles* spp.** | | | | ***Cephalobus* spp.** | | | | ***Eucephalobus* spp.** | | | | ***Prismatolaimus* spp.** | | | | ***Acrobeloides* spp.** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** |
| **7** | 1104 | 11042 | 100 | **10** | 24 | 282 | 75 | **12** | 13 | 131 | 100 | **16** | 8 | 150 | 25 | **25** | 3 | 39 | 50 | **11** | 1 | 20 | 25 |
| **12** | 661 | 6610 | 100 | **12** | 23 | 225 | 100 | **7** | 13 | 129 | 100 | **10** | 6 | 70 | 75 | | | | | | | | |
| **28** | 180 | 2084 | 75 | **29** | 21 | 205 | 100 | **15** | 8 | 118 | 50 | **8** | 1 | 24 | 25 | | | | | | | | |
| **30** | 159 | 1594 | 100 | **7** | 19 | 193 | 100 | **5** | 6 | 75 | 75 | **13** | 1 | 24 | 25 | | | | | | | | |
| **3** | 80 | 798 | 100 | **3** | 14 | 135 | 100 | **10** | 4 | 38 | 100 | | | | | | | | | | | | |
| **17** | 68 | 681 | 100 | **9** | 13 | 133 | 100 | **3** | 4 | 41 | 75 | | | | | | | | | | | | |
| **2** | 61 | 611 | 100 | **20** | 13 | 150 | 75 | **9** | 3 | 50 | 25 | | | | | | | | | | | | |
| **15** | 54 | 541 | 100 | **2** | 13 | 150 | 75 | **4** | 1 | 24 | 25 | | | | | | | | | | | | |
| **4** | 49 | 488 | 100 | **23** | 12 | 175 | 50 | **1** | 1 | 22 | 25 | | | | | | | | | | | | |
| **22** | 41 | 408 | 100 | **8** | 9 | 100 | 75 | **22** | 0.9 | 18 | 25 | | | | | | | | | | | | |
| **1** | 38 | 382 | 100 | **21** | 7 | 83 | 75 | **6** | 0.5 | 11 | 25 | | | | | | | | | | | | |
| **13** | 38 | 534 | 50 | **31** | 6 | 126 | 25 | | | | | | | | | | | | | | | | |
| **10** | 28 | 277 | 100 | **18** | 4 | 63 | 50 | | | | | | | | | | | | | | | | |
| **11** | 24 | 236 | 100 | **22** | 4 | 61 | 50 | | | | | | | | | | | | | | | | |
| **27** | 22 | 223 | 100 | **16** | 4 | 44 | 75 | | | | | | | | | | | | | | | | |
| **6** | 20 | 200 | 100 | **25** | 4 | 76 | 25 | | | | | | | | | | | | | | | | |
| **21** | 18 | 208 | 75 | **11** | 3 | 56 | 25 | | | | | | | | | | | | | | | | |
| **16** | 17 | 165 | 100 | **15** | 3 | 32 | 75 | | | | | | | | | | | | | | | | |
| **26** | 12 | 150 | 75 | **17** | 3 | 37 | 50 | | | | | | | | | | | | | | | | |
| **23** | 10 | 100 | 100 | **6** | 3 | 50 | 25 | | | | | | | | | | | | | | | | |
| **8** | 10 | 138 | 50 | **30** | 3 | 50 | 25 | | | | | | | | | | | | | | | | |
| **18** | 7 | 83 | 75 | **14** | 2 | 45 | 25 | | | | | | | | | | | | | | | | |
| **14** | 7 | 79 | 75 | **5** | 2 | 24 | 75 | | | | | | | | | | | | | | | | |
| **19** | 7 | 66 | 100 | **4** | 1 | 24 | 25 | | | | | | | | | | | | | | | | |
| **5** | 6 | 66 | 75 | **13** | 1 | 24 | 25 | | | | | | | | | | | | | | | | |
| **31** | 6 | 56 | 100 | **24** | 1 | 22 | 25 | | | | | | | | | | | | | | | | |
| **24** | 3 | 36 | 75 | **1** | 1 | 20 | 25 | | | | | | | | | | | | | | | | |
| **9** | 2 | 26 | 50 | **19** | 1 | 20 | 25 | | | | | | | | | | | | | | | | |
| **20** | 1 | 24 | 25 | | | | | | | | | | | | | | | | | | | | |

NPN assemblages were identified to genus and family level (Tables 2.12 and 2.13) only during the 2011 survey.

Non-parasitic, bacterivorous nematodes in 200g soil samples from the 31 fields sampled during 2011 were represented by *Acrobeles*, *Acrobeloides*, *Cephalobus, Eucephalobus*, *Prismatolaimus* and *Rhabditis* spp. (Table 2.12).

*Rhabditis* spp. was the predominant genus of which individuals were present in 29 of the 31 fields sampled, followed by *Acrobeles*, *Cephalobus*, *Eucephalobus*, *Prismatolaimus* and *Acrobeloides* spp. (Table 2.12).

The PV for *Rhabditis* spp. was substantially higher than that for any of the other bacterivorous genera and ranged from 1 to 1104 and PD from 24 to 11042 (Table 2.12). Individuals of the latter genera were present in 25% to 100% of the samples collected.

*Acrobeles* spp. individuals occurred in 28 of the 31 fields sampled with PV that ranged from 1 to 24, PD from 20 to 282 and individuals being present in 25% to 100% of the samples.

Individuals of *Cephalobus* and *Eucephalobus* spp. occurred in 11 and 4 of the 31 fields sampled respectively. *Cephalobus* spp. had PV that ranged from 0.5 to 31, PD from 11 to 131 and FO from 25% to 100%, while PV for *Eucephalobus* spp. ranged from 1 to 8, PD from 24 to 150 and FO from 25% to 75%.

The least dominant bacterivorous genera were *Prismatolaimus and Acrobeloides* spp. with low PV (3 and 1), PD (39 and 20) and FO (50% and 25%), respectively.

Table 2.13. Data for **fungivorous and predator** nematode genera/family in fields of five producers in the Christiana area sampled during the **2011** growing season as represented by prominence values (PV), frequency of occurrence (FO; %) and population density (PD) per **200g soil**.

| **Fungivores** | | | | | | | | | | | | **Predators** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Tylenchus* spp.** | | | | ***Aphelenchus* spp.** | | | | ***Aphelenchoides* spp.** | | | | **Dorylaimidae** | | | | **Mononchidae** | | | |
| **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** | **Field no** | **PV** | **PD** | **FO** |
| **18** | 37 | 369 | 100 | **10** | 12 | 175 | 50 | **29** | 13 | 127 | 100 | **10** | 21 | 300 | 50 | **2** | 13 | 251 | 25 |
| **16** | 14 | 157 | 75 | **3** | 5 | 73 | 50 | | | | | **18** | 16 | 163 | 100 | **9** | 5 | 52 | 100 |
| **27** | 13 | 130 | 100 | **30** | 3 | 50 | 25 | | | | | **11** | 15 | 145 | 100 | | | | |
| **15** | 9 | 91 | 100 | **6** | 2 | 19 | 75 | | | | | **2** | 14 | 167 | 75 | | | | |
| **11** | 8 | 167 | 25 | **28** | 1 | 24 | 25 | | | | | **29** | 12 | 122 | 100 | | | | |
| **3** | 7 | 143 | 25 | | | | | | | | | **23** | 11 | 113 | 100 | | | | |
| **23** | 7 | 100 | 50 | | | | | | | | | **15** | 9 | 103 | 75 | | | | |
| **20** | 6 | 67 | 75 | | | | | | | | | **16** | 7 | 105 | 50 | | | | |
| **10** | 5 | 100 | 25 | | | | | | | | | **30** | 7 | 100 | 50 | | | | |
| **13** | 5 | 100 | 25 | | | | | | | | | **3** | 6 | 85 | 50 | | | | |
| **7** | 4 | 53 | 50 | | | | | | | | | **22** | 6 | 57 | 100 | | | | |
| **14** | 3 | 49 | 50 | | | | | | | | | **5** | 6 | 75 | 50 | | | | |
| **12** | 3 | 50 | 25 | | | | | | | | | **20** | 5 | 58 | 75 | | | | |
| **8** | 3 | 37 | 50 | | | | | | | | | **21** | 4 | 43 | 100 | | | | |
| **25** | 3 | 37 | 50 | | | | | | | | | **13** | 4 | 76 | 25 | | | | |
| **1** | 1 | 21 | 50 | | | | | | | | | **28** | 4 | 76 | 25 | | | | |
| **9** | 1 | 27 | 25 | | | | | | | | | **31** | 4 | 76 | 25 | | | | |
| **5** | 1 | 24 | 25 | | | | | | | | | **4** | 3 | 50 | 25 | | | | |
| **6** | 1 | 24 | 25 | | | | | | | | | **8** | 3 | 50 | 25 | | | | |
| **17** | 1 | 24 | 25 | | | | | | | | | **9** | 3 | 50 | 25 | | | | |
| **22** | 1 | 24 | 25 | | | | | | | | | **17** | 3 | 50 | 25 | | | | |
| | | | | | | | | | | | | **19** | 3 | 33 | 50 | | | | |
| | | | | | | | | | | | | **1** | 2 | 45 | 25 | | | | |
| | | | | | | | | | | | | **14** | 2 | 24 | 50 | | | | |
| | | | | | | | | | | | | **25** | 2 | 24 | 50 | | | | |
| | | | | | | | | | | | | **24** | 1 | 22 | 25 | | | | |

Non-parasitic, fungivorous nematode genera that were present in 200g soil samples collected from 31 fields during 2011 were *Aphelenchus*, *Tylenchus* and *Aphelenchoides* species. Predator nematodes in the same samples were represented by the Dorylaimidae and Mononchidae families (Table 2.13).

*Tylenchus* was the predominant fungivore genus of which individuals were present in 21 of the 31 fields sampled, followed by *Aphelenchus* and *Aphelenchoides* spp. (Table 2.12). *Tylenchus* spp. had PV that ranged from 1 to 37, PD from 24 to 369 and occurred in 25% to 100% of the samples collected.

Individuals of *Aphelenchoides* occurred in only one of the 31 fields sampled and had a PV of 13, PD of 127 with individuals being present in all four samples (100%) from this field. *Aphelenchus* spp. was the least dominant and occurred in five of the 31 fields sampled with PV that ranged from 1 to 12, PD from 24 to 175 and FO from 25% to 50%.

For the predator nematode families identified individuals of the Dorylaimidae family were predominant and occurred in 26 of the 31 fields sampled with PV ranging from 1 to 21, PD form 22 to 300 and FO form 25% to 100% (Table 2.13).

Individuals of the Mononchidae family only occurred in 2 of the 31 fields sampled with PV of 5 and 13, PD of 52 and 251 and FO of 100% and 25%, respectively.

### 2.4.2 Parametric and non-parametric statistics

#### 2.4.2.1 Plant-parasitic nematode (PPN) data

- **Roots (50g and 5g)**

Table 2.14. Population levels [$log_{10}(x+1)$ transformed] for ***Meloidogyne*** species per **50g roots** for cultivation practices, nematicides and crop rotation sequences when data were pooled for 31 fields of five producers for the **2011** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Nematode genus | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| ***Meloidogyne* spp.** | 1.6 ±3<br>(4 ±19) | 1 ±2<br>(2 ±6) | 0 | 2.2 ±4.4<br>(8 ±80) | 1.3 ±2.2<br>(3 ±8) | 0.7 ±1.5<br>(1 ±3) | 1.7 ±2.7<br>(5 ±14) | 2.2 ±4.4<br>(8 ±80) | 0 |
| ***P* values** | **Mann-Whitney U:**<br>0.7668 | | **Kruskal-Wallis:**<br>0.6653 | | | **Kruskal-Wallis:**<br>0.5481 | | | |
| | **T-test:**<br>0.531 | | **Anova:**<br>0.5925 | | | **Anova:**<br>0.5155 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato

Table 2.15. Population levels [$log_{10}(x+1)$ transformed] for **endo- and semi-endoparasitic** nematode genera/family per **5g roots** as well as for cultivation practices, nematicides and crop rotation sequences when data were pooled for 31 fields of five producers for the **2010** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Nematode genus/family | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| ***Meloidogyne* spp.** | 1 ±1.8 (2 ±5) | 1.4 ±2 (3 ±6) | 2 ±2.9 (6 ±17) | 2.1 ±2.5 (7 ±11) | 1 ±1.8 (2 ±5) | 0.4 ±1.3 (1 ±3) | 1.9 ±2 (6 ±6) | 1.1 ±2.3 (2 ±9) | 1.4 ±2.4 (3 ±10) |
| ***P* values** | **Mann-Whitney U:** 0.678 | | **Kruskal-Wallis**: 0.4839 | | | **Kruskal-Wallis**: 0.2498 | | | |
| | **T-test:** 0.521 | | **Anova:** 0.4486 | | | **Anova:** 0.3264 | | | |
| **Hoplolaimidae** | 0.14 ±0.6 (0.1 ±0.8) | 0 | 0 | 0 | 0.09 ±0.4 (0.09 ±0.5) | 0 | 0.1 ±0.6 (0.1 ±0.8) | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.7670 | | **Kruskal-Wallis**: 0.8869 | | | **Kruskal-Wallis**: 0.7273 | | | |
| | **T-test:** 0.3090 | | **Anova:** 0.8936 | | | **Anova:** 0.7476 | | | |
| **Total PPN[d]** | 1.1 ±1.8 (2 ±5) | 1.4 ±2 (3 ±6) | 2 ±2.9 (6 ±17) | 2.1 ±2.5 (7 ±11) | 1.1 ±1.8 (2 ±5) | 0.4 ±1.3 (0.5 ±3) | 2.1 ±1.9 (7 ±6) | 1.1 ±2.3 (2 ±9) | 1.4 ±2.4 (3 ±10) |
| ***P* values** | **Mann-Whitney U:** 0.8278 | | **Kruskal-Wallis**: 0.5570 | | | **Kruskal-Wallis**: 0.1572 | | | |
| | **T-test:** 0.6684 | | **Anova:** 0.5016 | | | **Anova:** 0.2206 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato; [d]Total PPN=*Meloidogyne* spp. + Hoplolaimidae

Table 2.16. Population levels [$\log_{10}(x+1)$ transformed] for **endoparasitic** nematode genera/family per **5g roots** as well as for cultivation practices, nematicides and crop rotation sequences when data were pooled for 31 fields of five producers for the **2011** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Nematode genera | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| ***Meloidogyne* spp.** | 0.7 ±1.6 (1 ±4) | 0.3 ±1 (0.3 ±2) | 0 | 1.4 ±3 (3 ±19) | 0.4 ±1 (0.5 ±2) | 0.2 ±0.6 (0.2 ±0.8) | 0.6 ±1.2 (0.8 ±2) | 1.4 ±3 (3 ±19) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.7072 | | **Kruskal-Wallis:** 0.7144 | | | **Kruskal-Wallis:** 0.6955 | | | |
| | **T-test:** 0.4667 | | **Anova:** 0.3176 | | | **Anova:** 0.4255 | | | |
| ***Pratylenchus* spp.** | 0.6 ±1.1 (0.8 ±2) | 0.1 ±0.3 (0.1 ±0.3) | 0 | 0.6 ±1.2 (0.8 ±2) | 0.3 ±0.8 (0.3 ±1) | 0.3 ±1.2 (0.3 ±2) | 0.4 ±0.6 (0.5 ±0.8) | 0.6 ±1.2 (0.8 ±2) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.4177 | | **Kruskal-Wallis:** 0.7343 | | | **Kruskal-Wallis:** 0.6047 | | | |
| | **T-test:** 0.0957 | | **Anova:** 0.7328 | | | **Anova:** 0.8615 | | | |
| ***Ditylenchus* spp.** | 0 | 0.3 ±1 (0.3 ±2) | 0 | 0 | 0.2 ±0.7 (0.2 ±1) | 0.5 ±1 (0.6 ±2) | 0 | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.5665 | | **Kruskal-Wallis:** 0.7804 | | | **Kruskal-Wallis:** 0.2470 | | | |
| | **T-test:** 0.1710 | | **Anova:** 0.7946 | | | **Anova:** 0.2602 | | | |
| **Total PPN[d]** | 1.2 ±1.8 (2 ±5) | 0.7 ±1.2 (1 ±2) | 0 | 1.4 ±3 (3 ±19) | 1 ±1.3 (2 ±3) | 1.1 ±1.4 (2 ±3) | 1 ±1.2 (2 ±2) | 1.4 ±3 (3 ±19) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.7518 | | **Kruskal-Wallis:** 0.5199 | | | **Kruskal-Wallis:** 0.5185 | | | |
| | **T-test:** 0.4315 | | **Anova:** 0.5472 | | | **Anova:** 0.6469 | | | |

[a]1=plough: 2=rip & plough; [b]1=EDB® only :2=other nematicides than EDB® :3=EDB® & other nematicides; [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato ; [d]Total PPN=*Meloidogyne* spp. + *Pratylenchus* spp. + *Ditylenchus* spp.

The adapted NaOCl method was only used to extract eggs and J2 from 50g roots of crops and grasses sampled during 2011 (Table 2.14).

When RKN numbers were pooled for the 31 fields, none of the respective cultivation practices, nematicides or crop rotation sequences applied had a significant ($P \leq 0.05$) effect on RKN numbers/50g roots of crops and grasses sampled (Table 2.14). This trend was true when the $\log_{10}(x+1)$ transformed data were subjected to both non-parametric (Kruskal-Wallis) and parametric (Mann-Whitney U, T-test and Anova) statistical analyses.

Only individuals of the *Meloidogyne* genus and Hoplolaimidae family were present in 5g root samples collected from crops planted on 31 fields of five producers during 2010 (Table 2.15).

The respective cultivation practices, nematicides and crop rotation sequences applied on the 31 fields did not have a significant ($P \leq 0.05$) effect on population levels of either *Meloidogyne* or Hoplolaimidae or the total PPN, represented by pooled population levels [$\log_{10}(x+1)$ transformed] of the latter genus and family when data were subjected to parametric and non-parametric statistics.

During 2011 individuals of *Ditylenchus*, *Meloidogyne* and *Pratylenchus* spp. were present in 5g root samples from crops planted on 31 fields of five producers (Table 2.16).

No significant ($P \leq 0.05$) effect(s) were obtained for the cultivation practices, nematicides or crop rotation sequences applied in terms of nematode numbers of the three endoparasitic nematode genera as well as total PPN when data were subjected to both non-parametric (Kruskal-Wallis) and parametric (Mann-Whitney U, T-test and Anova) statistical analyses.

- **Soil (200g)**

Table 2.17. Population levels [$log_{10}(x+1)$ transformed] for **endoparasitic** nematode genera/family per **200g soil** as well as cultivation practices, nematicides and crop rotation sequences when data were pooled for 31 fields of five producers during the **2010** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Nematode genera/family | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| ***Meloidogyne* spp.** | 2 ±2.1 (6 ±7) | 2.4 ±2.5 (10 ±11) | 6 ±1.5a (402 ±3) | 4 ±1.8ab (53 ±5) | 1.6 ±2b (4 ±6) | 2.1 ±2.2ab (7 ±8) | 1 ±1.8a (2 ±5) | 5 ±1.9b (147 ±6) | 4 ±0.8ab (53 ±1) |
| ***P* values** | **Mann-Whitney U:** 0.9056 | | **Kruskal-Wallis:** 0.0128 | | | **Kruskal-Wallis:** 0.0105 | | | |
| | **T-test:** 0.6849 | | **Anova:** 0.0073 | | | **Anova:** 0.0125 | | | |
| ***Pratylenchus* spp.** | 2.7 ±1.6 (14 ±4) | 1.7 ±2 (4 ±6) | 5.3 ±0.7a (199 ±1) | 3.2 ±1.8ab (23 ±5) | 1.8 ±1.6b (5 ±4) | 1.4 ±1.7 (3 ±4) | 1.9 ±1.6 (6 ±4) | 4.1 ±2 (59 ±6) | 3.7 ±1.2 (39 ±2) |
| ***P* values** | **Mann-Whitney U:** 0.1138 | | **Kruskal-Wallis**: 0.0232 | | | **Kruskal-Wallis**: 0.0580 | | | |
| | **T-test:** 0.1368 | | **Anova:** 0.0154 | | | **Anova:** 0.0625 | | | |
| **Heteroderidae** | 0 | 0.3 ±1 (0.3 ±2) | 0 | 0 | 0.2 ±0.8 (0.2 ±1) | 0.5 ±1.2 (0.6 ±2) | 0 | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.5665 | | **Kruskal-Wallis:** 0.7804 | | | **Kruskal-Wallis:** 0.3619 | | | |
| | **T-test:** 0.1913 | | **Anova:** 0.8106 | | | **Anova:** 0.4391 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato

Table 2.18. Population levels [$\log_{10}(x+1)$ transformed] for **endoparasitic** nematode genera/family per **200g soil** as well as cultivation practices, nematicides and crop rotation sequences when data were pooled for 31 fields of five producers during the **2011** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| 2011 Survey | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nematode genera** | **Cultivation practices[a]** | | **Nematicides[b]** | | | **Crop rotation sequences[c]** | | | |
| | **1** | **2** | **1** | **2** | **3** | **1** | **2** | **3** | **4** |
| ***Meloidogyne* spp.** | 1.9 ±2.5 (6 ±11) | 1.6 ±1.8 (4 ±5) | 2.5 ±0.5 (11 ±0.6) | 3.8 ±3.4 (44 ±29) | 1.3 ±1.8 (3 ±5) | 1 ±1.1 (2 ±2) | 1.5 ±2.3 (3 ±9) | 4.4 ±2.7 (80 ±14) | 1.5 ±1.4 (3 ±3) |
| ***P* values** | **Mann-Whitney U:** 0.8899 | | **Kruskal-Wallis**: 0.8650 | | | **Kruskal-Wallis**: 0.9540 | | | |
| | **T-test:** 0.7507 | | **Anova:** 0.0788 | | | **Anova:** 0.0597 | | | |
| ***Pratylenchus* spp.** | 1.6 ±1.8 (4 ±5) | 1.9 ±1.3 (6 ±3) | 3.8 ±0.9 (44 ±1) | 1.9 ±2.3 (6 ±9) | 1.6 ±1.4 (4 ±3) | 1.5 ±1 (3 ±2) | 1.7 ±1.6 (4 ±4) | 2.3 ±2.7 (9 ±14) | 1.6 ±1.6 (4 ±4) |
| ***P* values** | **Mann-Whitney U:** 0.5799 | | **Kruskal-Wallis**: 0.1929 | | | **Kruskal-Wallis**: 0.9524 | | | |
| | **T-test:** 0.4530 | | **Anova:** 0.1529 | | | **Anova:** 0.8383 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato

Individuals of the Heteroderidae family as well as from the *Meloidogyne* and *Pratylenchus* genera were present in 200g soil samples obtained from five producers in the Christiana area during 2010 (Table 2.17).

Significant differences ($P \leq 0.05$) were obtained for both *Meloidogyne* and *Pratylenchus* spp. in terms of nematicides applied and only for *Meloidogyne* spp. in terms of crop rotation sequences implemented (Table 2. 17). Nematicides application of EDB® combined with other nematicides (category 3) resulted in significantly lower ($P \leq 0.05$) RKN individuals/5g roots compared to EDB® application only (category 1), but it did not differ significantly ($P \leq 0.05$) from category 2, which included application of nematicides other than EDB®. Categories 1 and 2 as well as 2 and 3 did, however, not differ significantly from each other with regard to nematicide application for both RKN and *Pratylenchus* spp.

For cropping sequences, grass, maize and potato included together in a cycle (category 2) resulted in the lowest RKN population levels/200g soil and differed significantly ($P \leq 0.05$) from maize, wheat and potato (category 3) but not from cycles including grass and potato (category 1) and maize and/or grass and potato (category 4). No significant differences were obtained for RKN between categories 1 and 4 with regard to cropping sequences.

No significant correlation ($P \leq 0.05$), were obtained for the cultivation practices, nematicides or crop rotation sequences applied on the 31 fields using both non-parametric (Kruskal-Wallis) and parametric (Mann-Whitney U, T-test and Anova) statistical analyses for Heteroderidae (Table 2.17).

During the 2011 survey *Meloidogyne* and *Pratylenchus* spp. were identified from 200g soil samples (Table 2.18). No significant effect ($P \leq 0.05$) was obtained for both *Meloidogyne* and *Pratylenchus* spp. numbers with regard to the different cultivation practices, nematicides or crop rotation sequences applied on the 31 fields. The latter trend was evident for data subjected to both non-parametric (Kruskal-Wallis) and parametric (Mann-Whitney U, T-test and Anova) statistical analyses.

Table 2.19. Population levels [$log_{10}(x+1)$ transformed] for **semi- endo-/ecto- and ectoparasitic** nematode genera/family per **200g soil** for cultivation sequences, nematicides, crop rotation sequences when data were pooled for 31 fields of five producers for the **2010** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Nematode genera/families | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| **Hoplolaimidae** | 2.9 ±2 (17 ±6) | 2.5 ±2.1 (11 ±7) | 6 ±1.5a (402 ±3) | 4 ±1.8ab (53 ±5) | 2.2 ±1.8b (8 ±5) | 1.8 ±1.9b (5 ±6) | 2.2 ±1.8b (8 ±5) | 5.1 ±1.9a (163 ±6) | 4 ±0.8b (53 ±1) |
| ***P* values** | **Mann-Whitney U:** 0.4291 | | **Kruskal-Wallis:** 0.0318 | | | **Kruskal-Wallis:** 0.0508 | | | |
| | **T-test:** 0.5992 | | **Anova:** 0.0108 | | | **Anova:** 0.0373 | | | |
| **Trichodoridae** | 2 ±1.1 (6 ±2) | 2 ±1.4 (6 ±3) | 3.3 ±1 (26 ±2) | 2.5 ±1.2 (11 ±2) | 1.8 ±1.2 (5 ±2) | 2 ±1.3 (6 ±2) | 1.6 ±1.3 (4 ±3) | 2.5 ±1.2 (11 ±2) | 2.6 ±0.2 (12 ±0.2) |
| ***P* values** | **Mann-Whitney U:** 0.7220 | | **Kruskal-Wallis**: 0.2015 | | | **Kruskal-Wallis**: 0.3843 | | | |
| | **T-test:** 0.9121 | | **Anova:** 0.2304 | | | **Anova:** 0.4204 | | | |
| ***Tylenchorhynchus* spp.** | 1 ±1.6 (2 ±4) | 1.6 ±2.2 (4 ±8) | 0.6 ±0.9a (0.8 ±1) | 3.7 ±2.6b (39 ±12) | 1 ±1.7a (2 ±4) | 1.1 ±2 (2 ±6) | 1.5 ±2 (3 ±6) | 2.4 ±2.1 (10 ±7) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.4890 | | **Kruskal-Wallis**: 0.0984 | | | **Kruskal-Wallis**: 0.3428 | | | |
| | **T-test:** 0.3544 | | **Anova:** 0.0285 | | | **Anova:** 0.5426 | | | |
| **Criconematidae** | 0.8 ±1.2 (1 ±2) | 0.8 ±1.3 (1.2 ±2) | 0 | 0 | 1 ±1.3 (1.7 ±2) | 0.7 ±1.2 (1 ±2) | 1.1 ±1.4 (2 ±3) | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.8743 | | **Kruskal-Wallis:** 0.1547 | | | **Kruskal-Wallis:** 0.1835 | | | |
| | **T-test:** 0.9791 | | **Anova:** 0.2227 | | | **Anova:** 0.3357 | | | |

| Nematode genera/families | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| ***Xiphinema* spp.** | 0.2 ±0.5 (0.2 ±0.6) | 0.7 ±1.2 (1 ±2) | 0 | 0.3 ±0.6 (0.3 ±0.8) | 0.5 ±1 (0.6 ±2) | 1 ±1.4 (1.7 ±3) | 0.2 ±0.6 (0.2 ±0.8) | 0.3 ±0.6 (0.3 ±0.8) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.3632 | | **Kruskal-Wallis**: 0.7387 | | | **Kruskal-Wallis**: 0.4406 | | | |
| | **T-test:** 0.1581 | | **Anova:** 0.7425 | | | **Anova:** 0.3298 | | | |
| ***Longidorus* spp.** | 0.2 ±0.5 (0.2 ±0.6) | 0.2 ±0.5 (0.2 ±0.6) | 0 | 0.3 ±0.6 (0.3 ±0.8) | 0.2 ±0.5 (0.2 ±0.6) | 0.2 ±0.4 (0.2 ±0.5) | 0.4 ±0.6 (0.5 ±0.8) | 0.3 ±0.6 (0.3 ±0.8) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.9684 | | **Kruskal-Wallis**: 0.7596 | | | **Kruskal-Wallis:** 0.6267 | | | |
| | **T-test:** 0.9327 | | **Anova:** 0.7717 | | | **Anova:** 0.6543 | | | |
| **Total PPN[d]** | 4.3 ±1.5 (72 ±3) | 4.6 ±2 (98 ±6) | 6.4 ±1.2ab (601 ±2) | 6 ±0.7a (402 ±1) | 4 ±1.7b (53 ±4) | 4 ±2 (53 ±6.4) | 4.1 ±1.8 (59 ±5) | 6.1 ±1 (445 ±2) | 4.7 ±0.8 (109 ±1) |
| ***P* values** | **Mann-Whitney U:** 0.3529 | | **Kruskal-Wallis**: 0.0083 | | | **Kruskal-Wallis**: 0.1969 | | | |
| | **T-test:** 0.6308 | | **Anova:** 0.0275 | | | **Anova:** 0.3292 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato; [d]Total PPN=*Meloidogyne* spp. + *Pratylenchus* spp.+ Heteroderidae + Hoplolaimidae + Trichodoridae + *Tylenchorhynchus* spp. + Criconematidae + *Xiphinema* spp. + *Longodorus* spp.

For the semi-endo-/ectoparasitic nematode genera/family per 200g soil, individuals of the Hoplolaimidae, Criconematidae and Trichodoridae families as well as those from the *Tylenchorhynchus*, *Xiphinema* and *Longidorus* genera were present (Table 2.19). There was a significant ($P \leq 0.05$) effect evident for Hoplolaimidae, *Tylenchorhynchus* spp. and total PPN with regard to the different nematicides applied.

Hoplolaimidae as well as total PPN numbers were the lowest in soil of fields where EDB® and other nematicides were used in combination (category 3) and differed significantly ($P \leq 0.05$) from those where only EDB® (category 1) was applied, but not from those where nematicides other than EDB® (category 2) were applied. The latter scenario was true for both the non-parametric Kruskal-Wallis and parametric Anova tests.

*Tylenchorhynchus* spp. numbers were significantly lower in fields where EDB® only (category 1) as well as where EDB® and other nematicides were applied in combination (category 3) and differed significantly from those for fields treated with nematicides other than EDB® (category 2). The latter trend was, however, evident for the parametric Anova and not for the non-parametric Kruskal-Wallis test. The latter implies that this significance should rather be ignored since the latter non-parametric test is more strict than the Anova and caters for uneven distributed data such as those generally obtained for nematodes.

For crop rotation practices, significant ($P \leq 0.05$) differences were obtained among the various sequences practiced and Hoplolaimidae numbers/200g soil, but this was only true for the Anova test and is thus ignored as explained above.

In terms of cultivation practices, no significant correlation ($P \leq 0.05$) were obtained for the 31 fields either for *Xiphinema* or *Longidorus* spp. as well as for individuals from the Trichodoridae and Criconematidae families and total PPN using both non-parametric (Kruskal-Wallis) and parametric (Mann-Whitney U, T-test and Anova) statistical analyses.

Table 2.20. Population levels [$log_{10}(x+1)$ transformed] for **semi-endo-/ecto- and ectoparasitic** nematode genera/family per **200g soil** for cultivation sequences, nematicides, crop rotation sequences when data were pooled for 31 fields of five producers for the **2011** growing seasons (means in parenthesis represents the values that were back transformed and not the real means).

| Nematode genera/families | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| **Trichodoridae** | 1.9 ±1.5 (6 ±3) | 1.9 ±1.8 (6 ±5) | 2.2 ±0.2 (8 ±0.2) | 1.4 ±1.8 (3 ±5) | 1.9 ±1.7 (6 ±4) | 1.4 ±1.3 (3 ±3) | 2.5 ±1.9 (11 ±6) | 1.5 ±1.8 (3 ±5) | 1.4 ±1.2 (3 ±2) |
| ***P* values** | **Mann-Whitney U:** 0.7072 | | **Kruskal-Wallis**: 0.6889 | | | **Kruskal-Wallis**: 0.3657 | | | |
| | **T-test:** 0.9329 | | **Anova:** 0.7919 | | | **Anova:** 0.4324 | | | |
| ***Tylenchorhynchus* spp.** | 0.7 ±1.3 (1 ±3) | 1 ±1.5 (2 ±3) | 0 | 1.3 ±2 (3 ±6) | 0.8 ±1.4 (1 ±3) | 1.1 ±1.3 (2 ±3) | 1.1 ±1.7 (2 ±4) | 0.3 ±0.6 (0.3 ±0.8) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.5799 | | **Kruskal-Wallis**: 0.4821 | | | **Kruskal-Wallis**: 0.4857 | | | |
| | **T-test:** 0.5274 | | **Anova:** 0.5619 | | | **Anova:** 0.5001 | | | |
| **Criconematidae** | 0.7 ±1.1 (1 ±2) | 0.5 ±0.9 (0.6 ±1) | 0 | 0 | 0.8 ±1 (1 ±2) | 0.4 ±0.8 (0.5 ±1) | 1 ±1.2 (2 ±2) | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.7668 | | **Kruskal-Wallis**: 0.1951 | | | **Kruskal-Wallis**: 0.1830 | | | |
| | **T-test:** 0.8505 | | **Anova:** 0.2254 | | | **Anova:** 0.1724 | | | |
| **Hoplolaimidae** | 0.5 ±.0.8 (0.6 ±1) | 0.4 ±0.8 (0.5 ±1) | 0.6 ±0.9 (0.8 ±1) | 0.5 ±1 (0.6 ±2) | 0.4 ±0.8 (0.5 ±1) | 0.4 ±0.9 (0.5 ±1) | 0.6 ±0.8 (0.8 ±1) | 0.5 ±1 (0.6 ±2) | 0.4 ±0.7 (0.5 ±1) |
| ***P* values** | **Mann-Whitney U:** 0.6494 | | **Kruskal-Wallis**: 0.9016 | | | **Kruskal-Wallis**: 0.8884 | | | |
| | **T-test:** 0.5137 | | **Anova:** 0.9621 | | | **Anova:** 0.9599 | | | |

| Nematode genera/families | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| ***Xiphinema* spp.** | 0.1 ±0.4 (0.1 ±0.5) | 0.4 ±0.7 (0.5 ±1) | 0 | 0.3 ±0.6 (0.3 ±0.8) | 0.3 ±0.6 (0.3 ±0.8) | 0.2 ±0.7 (0.2 ±1) | 0.5 ±0.6 (0.6 ±0.8) | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.3869 | | **Kruskal-Wallis**: 0.7413 | | | **Kruskal-Wallis**: 0.1174 | | | |
| | **T-test:** 0.2316 | | **Anova:** 0.7694 | | | **Anova:** 0.2677 | | | |
| ***Psilenchus* spp.** | 0 | 0.4 ±1.2 (0.5 ±2) | 0 | 1 ±2 (2 ±6) | 0.1 ±0.6 (0.1 ±0.8) | 0.3 ±1 (0.3 ±2) | 0.3 ±1.1 (0.3 ±2) | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.5665 | | **Kruskal-Wallis:** 0.2466 | | | **Kruskal-Wallis:** 0.8836 | | | |
| | **T-test:** 0.1685 | | **Anova:** 0.2037 | | | **Anova:** 0.9009 | | | |
| **Total PPN[d]** | 6 ±0.7a (402 ±1) | 6.4 ±0.6b (600 ±0.8) | 6.2 ±0.2 (492 ±0.2) | 6.6 ±0.4 (734 ±0.5) | 6.2 ±0.7 (492 ±1) | 6.3 ±0.5 (543 ±0.6) | 6.3 ±0.8 (543 ±1.2) | 6.6 ±0.5 (734 ±0.6) | 5.4 ±0.7 (220 ±1) |
| ***P* values** | **Mann-Whitney U:** 0.0328 | | **Kruskal-Wallis**: 0.2537 | | | **Kruskal-Wallis**: 0.1938 | | | |
| | **T-test:** 0.0893 | | **Anova:** 0.5548 | | | **Anova:** 0.2007 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato; [d]Total PPN= *Meloidogyne* spp. + *Pratylenchus* spp.+ Trichodoride + *Tylenchorhynchus* spp. + Criconematidae + Hoplomaimidae + *Xiphinema* spp. + *Psilenchus* spp.

During the 2011 survey, for the semi-endo-/ectoparasitic nematodes *Tylenchorhynchus, Xiphinema* and *Psilenchus* spp. as well as Trichodoridae, Criconematidae and Hoplolaimidae were present in 200g soil samples (Table 2.20). A significant difference ($P \leq 0.05$) was evident only between the total PPN and cultivation practices, but only for the Mann-Whitney U test and is therefore ignored and not discussed further (Table 2.20). No significant ($P \leq 0.05$) effects were evident with regard to any of the cultivation practices, nematicides or cropping sequences applied for any of the PPN present in soil samples obtained during the 2011 season.

### 2.4.2.2 Non-parasitic nematode (NPN) data

- **Soil (200g)**

Table 2.21. Population levels [$log_{10}(x+1)$ transformed] for **non-parasitic nematode** genera/families per **200g soil** for cultivation sequences, nematicides and crop rotation sequences applied, data were pooled for 31 fields of five producers for the **2010** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Nematode genera/families | Cultivation practices[a] | | Nematicides[b] | | | Crop rotation sequences[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| **Total NPN** | 6.2 ±0.5 (492 ±0.6) | 6.2 ±0.4 (492 ±0.5) | 6 ±0.7 (402 ±1) | 6.3 ±0.4 (543 ±0.5) | 6.2 ±0.4 (491 ±0.5) | 6 ±0.3a (402 ±0.3) | 6.5 ±0.3b (664 ±0.3) | 6.4 ±0.4ab (601 ±0.5) | 5.7 ±0.2a (298 ±0.2) |
| ***P* values** | **Mann-Whitney U:** 0.6073 | | **Kruskal-Wallis**: 0.8981 | | | **Kruskal-Wallis**: 0.0046 | | | |
| | **T-test:** 0.7509 | | **Anova:** 0.7800 | | | **Anova:** 0.0015 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato

Table 2.22. Population levels [$log_{10}(x+1)$ transformed] for **non-parasitic** nematode genera/families per **200g soil** for cultivation sequences, nematicides and crop rotation sequences applied, data were pooled for 31 fields of five producers for the **2011** growing season (means in parenthesis represents the values that were back transformed and not the real means).

| **Bacterivores** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nematode genera/families** | **Cultivation practices[a]** | | **Nematicides[b]** | | | **Crop rotation sequences[c]** | | | |
| | **1** | **2** | **1** | **2** | **3** | **1** | **2** | **3** | **4** |
| ***Rhabditis* spp.** | 5.6 ±1.8 (269 ±5) | 1.5 ±2.2 (3 ±8) | 1 ±1.3a (2 ±3) | 1.7 ±1.1a (4 ±2) | 5.4 ±1.9b (220 ±6) | 4.9 ±2.2 (133 ±8) | 5.5 ±1.4 (243 ±3) | 3.5 ±2.5 (32 ±11) | 3.9 ±1.8 (48 ±5) |
| ***P* values** | **Mann-Whitney U:** 0.2279 | | **Kruskal-Wallis:** 0.0591 | | | **Kruskal-Wallis:** 0.3511 | | | |
| | **T-test:** 0.1297 | | **ANOVA:** 0.0092 | | | **ANOVA:** 0.2257 | | | |
| ***Acrobeles* spp.** | 3.8 ± 1.3 (43 ±3) | 2.8 ±1.4 (15 ±2) | 3.8 ±1.2 (43 ±2) | 3.5 ±1.1 (32 ±2) | 3.2 ±1.5 (23 ±3) | 3 ±1.9 (19 ±6) | 3.3 ±1.2 (26 ±2) | 3.2 ±1 (23 ±2) | 3.9 ±1 (48 ±2) |
| ***P* values** | **Mann-Whitney U:** 0.1490 | | **Kruskal-Wallis:** 0.8429 | | | **Kruskal-Wallis:** 0.8992 | | | |
| | **T-test:** 0.0745 | | **Anova:** 0.8147 | | | **Anova:** 0.8137 | | | |
| ***Cephalobus* spp.** | 2 ±1.8a (6 ±5) | 0.2 ±1b (0.2 ±2) | 0 | 0.4 ±0.9 (0.5 ±1) | 1.3 ±1.8 (2.7 ±5) | 1 ±1.7 (2 ±4) | 1.3 ±1.8 (3 ±5) | 0.4 ±0.9 (0.5 ±1) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.0063 | | **Kruskal-Wallis:** 0.4144 | | | **Kruskal-Wallis:** 0.4719 | | | |
| | **T-test:** 0.0020 | | **Anova:** 0.4147 | | | **Anova:** 0.5212 | | | |

| Bacterivores cont. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nematode genera/families** | **Cultivation practices** | | **Nematicides** | | | **Crop rotation sequences** | | | |
| | **1** | **2** | **1** | **2** | **3** | **1** | **2** | **3** | **4** |
| ***Eucephalobus* spp.** | 0.4 ±1.1 (0.5 ±2) | 0.3 ±1 (0.3 ±2) | 0 | 0 | 0.4 ±1.1 (0.5 ±2) | 0.6 ±1.3 (0.8 ±3) | 0.3 ±1 (0.3 ±2) | 0 | 0.6 ±1.1 (0.8 ±2) |
| ***P* values** | **Mann-Whitney U:** 0.9684 | | **Kruskal-Wallis:** 0.5883 | | | **Kruskal-Wallis:** 0.5517 | | | |
| | **T-test:** 0.9066 | | **Anova:** 0.6375 | | | **Anova:** 0.7659 | | | |
| ***Prismatolaimus* spp.** | 0 | 0.4 ±1 (0.5 ±2) | 1.5 ±2.1ab (3 ±7) | 0a | 0.1 ±0.6b (0.1 ±0.8) | 0.3 ±1 (0.3 ±2) | 0 | 0.7 ±1.5 (1 ±3) | 0 |
| ***P* values** | **Mann-Whitney U:** 0.5665 | | **Kruskal-Wallis:** 0.0371 | | | **Kruskal-Wallis:** 0.3386 | | | |
| | **T-test:** 0.1674 | | **Anova:** 0.0310 | | | **Anova:** 0.3521 | | | |
| ***Acrobeloides* spp.** | 1.3 ±0.5 (3 ±0.6) | 0 | 0 | 0 | 0.08 ±0.4 (0.08 ±0.5) | 0a | 0a | 0a | 0.7 ±1.2b (1 ±2) |
| ***P* values** | **Mann-Whitney U:** 0.7668 | | **Kruskal-Wallis:** 0.8869 | | | **Kruskal-Wallis:** 0.0293 | | | |
| | **T-test:** 0.3096 | | **Anova:** 0.8936 | | | **Anova:** 0.0199 | | | |
| **Fungivores** | | | | | | | | | |
| ***Tylenchus* spp.** | 2.2 ±1.3 (8 ±3) | 2.4 ±2.1 (10 ±7) | 3.4 ±0.6 (29 ±0.8) | 1.4 ±1.9 (3 ±6) | 2.4 ±1.8 (10 ±5) | 2.1 ±2 (7 ±6) | 2.2 ±1.9 (8 ±6) | 2.2 ±1.7 (8 ±4) | 3.6 ±.0.5 (35 ±0.7) |
| ***P* values** | **Mann-Whitney U:** 0.7518 | | **Kruskal-Wallis:** 0.3859 | | | **Kruskal-Wallis:** 0.5114 | | | |
| | **T-test:** 0.8245 | | **Anova:** 0.4182 | | | **Anova:** 0.6606 | | | |

| Fungivores cont. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nematode genera/families** | **Cultivation practices** | | **Nematicides** | | | **Crop rotation sequences** | | | |
| | **1** | **2** | **1** | **2** | **3** | **1** | **2** | **3** | **4** |
| ***Aphelenchus* spp.** | 0.7 ±1.5 (1 ±3) | 0.3 ±0.8 (0.3 ±1) | 0 | 0 | 0.6 ±1.3 (0.8 ±3) | 1 ±1.6 (2 ±4) | 0.5 ±1.2 (0.6 ±2) | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.6494 | | **Kruskal-Wallis:** 0.5038 | | | **Kruskal-Wallis:** 0.4845 | | | |
| | **T-test:** 0.3249 | | **Anova:** 0.5480 | | | **Anova:** 0.5385 | | | |
| ***Aphelenchoides* spp.** | 0 | 0.3 ±1.2 (0.3 ±2) | 0 | 0 | 0.2 ±1 (0.2 ±2) | 0.5 ±1.5 (0.6 ±3) | 0 | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.7820 | | **Kruskal-Wallis:** 0.8869 | | | **Kruskal-Wallis:** 0.5724 | | | |
| | **T-test:** 0.3414 | | **Anova:** 0.8936 | | | **Anova:** 0.5949 | | | |
| **Predators** | | | | | | | | | |
| **Dorylaimidae** | 2.9 ±1.8 (17 ±5) | 3.2 ±1.2 (23 ±2) | 3.2 ±0.9 (23 ±1) | 3.6 ±1.2 (35 ±2) | 3 ±1.6 (19 ±4) | 3.2 ±1.4 (23 ±3) | 2.9 ±1.5 (17 ±3) | 3.3 ±1.3 (26 ±3) | 3.8 ±1.1 (43 ±2) |
| ***P* values** | **Mann-Whitney U:** 0.6212 | | **Kruskal-Wallis:** 0.7757 | | | **Kruskal-Wallis:** 0.7913 | | | |
| | **T-test:** 0.5238 | | **Anova:** 0.7286 | | | **Anova:** 0.8194 | | | |
| **Mononchidae** | 0.5 ±1.4 (0.6 ±3) | 0 | 0 | 0 | 0.3 ±1.1 (0.3 ±2) | 0.4 ±1.2 (0.5 ±2) | 0.3 ±1.1 (0.3 ±2) | 0 | 0 |
| ***P* values** | **Mann-Whitney U:** 0.5400 | | **Kruskal-Wallis:** 0.7804 | | | **Kruskal-Wallis:** 0.8836 | | | |
| | **T-test:** 0.1400 | | **Anova:** 0.7917 | | | **Anova:** 0.8938 | | | |

| Predators cont. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nematode genera/families** | **Cultivation practices[a]** | | **Nematicides[b]** | | | **Crop rotation sequences[c]** | | | |
| | **1** | **2** | **1** | **2** | **3** | **1** | **2** | **3** | **4** |
| **Total NPN[d]** | 6.2 ±1.4 (491 ±3) | 5.8 ±1 (329 ±2) | 4.8 ±0.8 (120 ±1) | 5.3 ±1.1 (199 ±2) | 6.2 ±1.2 (491 ±2) | 6.1 ±1 (444 ±2) | 6.1 ±1.1 (444 ±2) | 5 ±1.1 (147 ±2) | 5.5 ±0.4 (243 ±0.5) |
| ***P* values** | **Mann-Whitney U:** 0.3845 | | **Kruskal-Wallis:** 0.1117 | | | **Kruskal-Wallis:** 0.2770 | | | |
| | **T-test:** 0.3021 | | **Anova:** 0.1228 | | | **Anova:** 0.1938 | | | |

[a]1=plough :2=rip & plough; [b]1=EDB® only :2=nematicides other than EDB® :3=EDB® & other nematicides (combined); [c]1=grass and potato :2=grass, maize & potato :3=maize, wheat & potato :4=maize and/or grass and potato; [d]Total NPN= *Rhabditis* spp. + *Acrobeles* spp. + *Cephalobus* spp. + *Eucephalobus* spp. + *Prismatolaimus* spp. + *Acrobeloides* spp. + *Tylenchus* spp. + *Aphelenchus* spp. + *Aphelenchoides* spp. + Dorylaimidae + Mononchidae

During the 2010 survey only the total number of NPN were obtained since no identification was made to genus and/or family level during counts (Table 2.21).

A significant ($P \leq 0.05$) effect was evident for crop rotation sequences and total NPN numbers/200g soil (Table 2.21). Cropping sequences that included grasses, maize and potato (category 2) resulted in the highest total NPN/200g soil, followed by maize, wheat and potato sequences from which it did not differ significantly ($P \leq 0.05$) (category 3). It did, however, differ significantly ($P \leq 0.05$) from cropping sequences represented by categories 1 (grass and potato) and 4 (maize and/or grass and potato).

For the 2011 season NPN were, however, counted to genera and/or family level with *Rhabditis, Acrobeles*, *Cephalobus*, *Eucephalobus*, *Prismatolaimus* and *Acrobeloides* spp. being identified from 200g soil samples (Table 2.22). A significant difference was evident ($P \leq 0.05$) between *Rhabditis* spp. and nematicides applied with significantly ($P \leq 0.05$) higher numbers of these genera being present in soil of fields where EDB® and other nematicides (category 3) were applied compared to those where only EDB® (category 1) and nematicides other than EDB® (category 2) were applied. This trend was, however, only evident for the parametric Anova test and not for the non-parametric Kruskal-Wallis test.

Significant differences ($P \leq 0.05$) were also obtained between *Prismatolaimus* spp. and nematicides applied with significantly ($P \leq 0.05$) higher numbers of this genus for fields where only EDB® (category 1) were applied compared to those where nematicides other than EDB® (category 2) and EDB® in combination with other nematicides (category 3) were applied (Table 2.22). Although the latter was evident of data subjected to both parametric and non-parametric statistical tests, population levels of this genera were very low.

For *Acrobeloides* spp., significant ($P \leq 0.05$) differences were obtained for crop rotation sequences implemented but since population levels for this genus were very low and ranged between zero and 0.7 this data are not discussed further.

For *Cephalobus* spp. a significant difference ($P \leq 0.05$) was obtained for cultivation practices with numbers of this genus being higher for fields that were only ploughed compared to those that were ripped and ploughed (Table 2.22). The latter was evident for both the parametric and non-parametric tests. Fungivore nematodes were represented by *Tylenchus, Aphelenchus* and

*Aphelenchoides* spp. No significant differences ($P \leq 0.05$) were, however, obtained between either of the latter genera and cultivation practices, nematicides or crop rotation sequences applied. Predator nematodes were represented by individuals of the Dorylaimidae and Mononchidae families (Table 2.22). No significant correlation ($P \leq 0.05$) was obtained for the respective cultivation practices, nematicides or crop rotation sequences applied on the 31 fields using both non-parametric (Kruskal-Wallis) and parametric (Mann-Whitney U, T-test and Anova) statistical analyses either for Dorylaimidae, Mononchidae or total NPN.

In terms of associations between soil type characteristics such as percentage soil, clay and organic material and PPN as well as NPN no significant ($P \leq 0.05$) effects were evident and data are thus not shown.

### 2.4.3 Faunal analyses for non-parasitic nematodes (NPN)

Table 2.23. Functional guilds, to which non-parasitic nematode genera/families (identified in soil samples from the 31 fields of five producers in Christiana area), were assigned according to their feeding habits and life stage characteristics (Bongers, 1990).

| Genus | Guild |
|---|---|
| ***Rhabditis*** | $Ba_1$ |
| ***Acrobeles*** | $Ba_2$ |
| ***Acrobeloides*** | $Ba_2$ |
| ***Cephalobus*** | $Ba_2$ |
| ***Chiloplacus*** | $Ba_2$ |
| ***Eucephalobes*** | $Ba_2$ |
| ***Prismatolaimus*** | $Ba_3$ |
| ***Aphelenchus*** | $Fu_2$ |
| ***Tylenchus**** | $Fu_2$ |
| ***Aphelenchoides*** | $Fu_2$ |
| **Doryliamidae** | $Om_4$ |
| **Mononchidae** | $Ca_4$ |

*Personal communication, Dr. A. Swart, Nematode Taxonomist, ARC-PPRI, Pretoria, 15 May 2010

ba: bacterivores, ca: carnivore, fu: fungivores, om: predator (omnivore). Suffix numbers represent cp values for the various taxa.

The proposed faunal profile into which soil can be categorised according to the presence, abundance and diversity of NPN, soil nematodes (Ferris *et al*., 2001)(Figure 2.4) was also consulted continuously throughout the results section of this study.

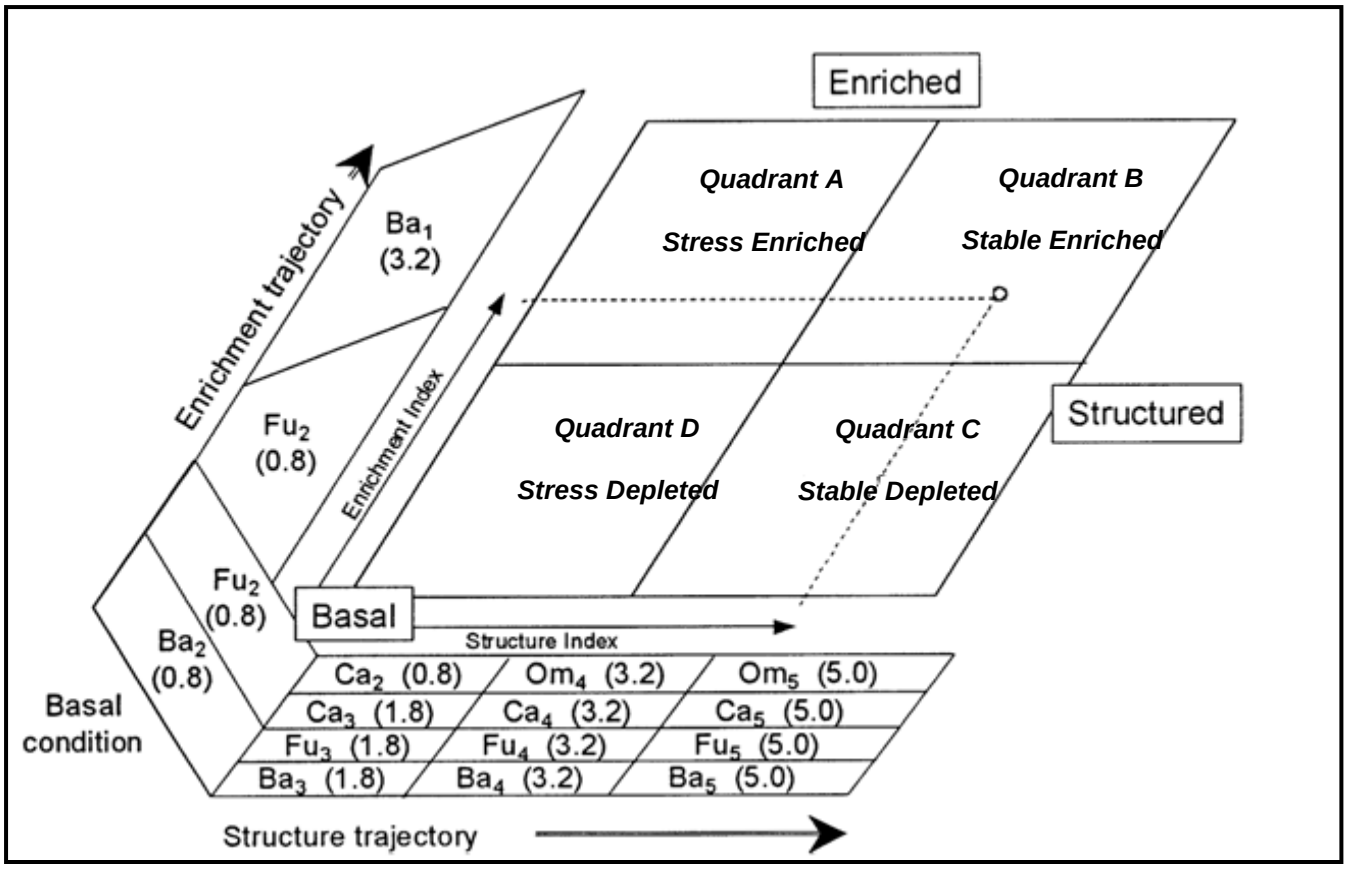


Figure 2.4. Functional guilds of non-parasitic, soil nematodes characterized by their respective feeding habit (trophic group) and by life history characteristics as expressed along a colonizer–persister (cp) scale (after Bongers & Bongers, 1998) (Graphical illustration from Ferris *et al*., 2001).

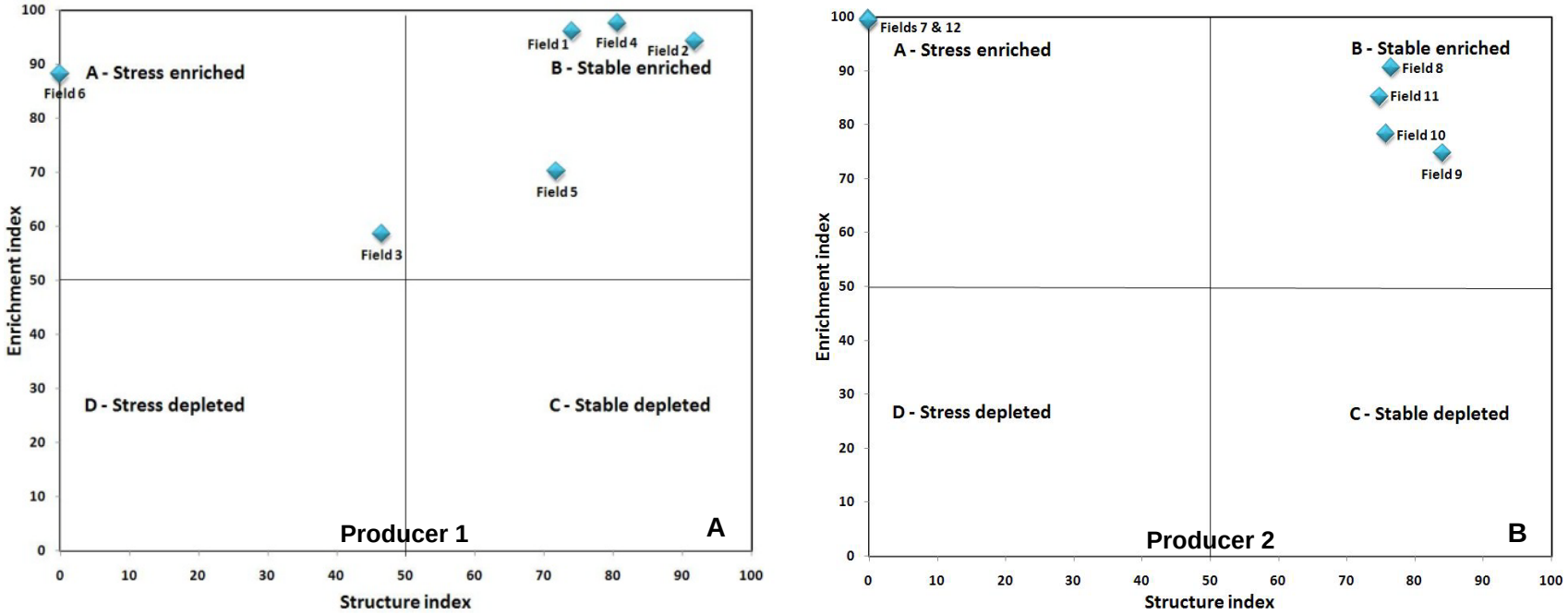


Figure 2.5. (A & B). Enrichment (EI) and structural indices (SI) for non-parasitic nematodes identified from producer 1 & 2's fields in the Christiana area during the 2011 growing season categorised in functional guilds according to their feeding habits and life history characteristics.

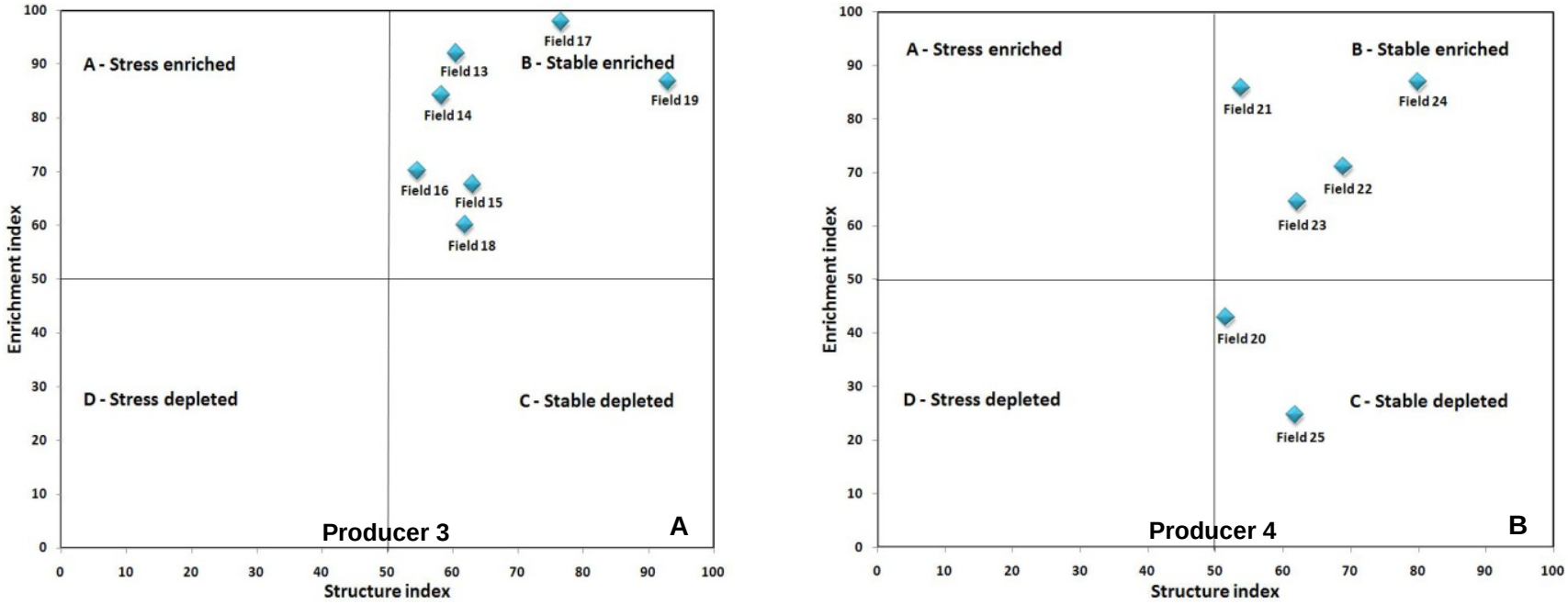


Figure 2.6. (A & B). Enrichment (EI) and structural indices (SI) for non-parasitic nematodes identified from producer 3 & 4's fields in the Christiana area during the 2011 growing season categorised in functional guilds according to their feeding habits and life history characteristics.

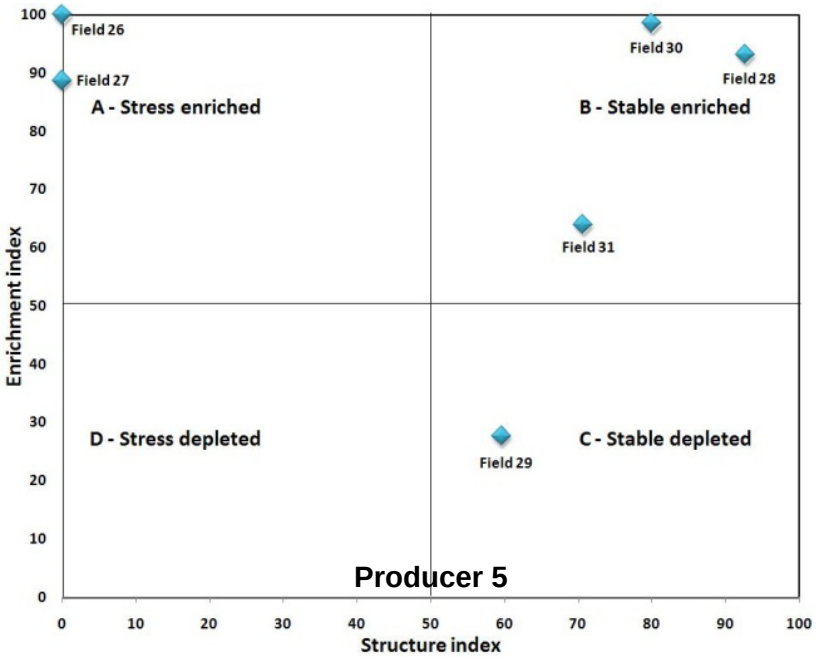


Figure 2.7. Enrichment (EI) and structural indices (SI) for non-parasitic nematodes identified from producer 5's fields in the Christiana area during the 2011 growing season categorised in functional guilds according to their feeding habits and life history characteristics.

Four (1, 2, 4 and 5) of the six fields sampled for producer 1 were plotted in the "Stable Enriched" and two (6 and 3) in the "Stress Enriched" quadrants of the simplified food web representation for NPN (Figure 2.5A). These results reflect that for the "Stable Enriched" quadrant both high EI and SI for fields 1, 2 and 4, but lower SI and EI for field 5. On the Enrichment Trajectory this indicates that soil of both fields 1, 2 and 4 were dominated by enrichment opportunistic bacterivores (cp1), while abundant nematode taxa such as bacterivores and fungivores (cp2) were also present but in lower proportions. For the Structural Trajectory, results showed that bacterivores (cp4-5), carnivores (cp4-5), fungivores (cp4-5) and omnivores (cp 4&5) dominated. However, individuals from $Ba_3$, $Ca_3$ and $Fu_3$ were also present in lower proportions, while none/very low numbers of the $Om_5$ group were present.

In contrast, for the "Stress Enriched" quadrant field 6 had a high EI but a zero SI, indicating the presence of abundant bacterivorous and fungivorous nematode taxa (cp2) in combination with enrichment opportunistic bacterivores (cp1) that dominates. However, no bacterivorous (Ba), carnivorous (Ca) or fungivorous (Fu) nematodes in cp scales 3-5 (reflected on the Structural Trajectory) as well as omnivorous/predator (Om) for cp scales 4 and 5 were present in this field.

For field 3, NPN assemblages present had medium EI and SI levels. For the Enrichment Trajectory the latter indicates that abundant nematode taxa such as bacterivores and fungivores (cp2) dominated, while enrichment opportunistic bacterivores (cp1) also occurred but in low proportions. The SI Trajectory indicated that bacterivores (cp 3&4), carnivores (cp2) and fungivores (cp 3&4) as well as omnivores (cp4) dominated in this field, with very low proportions/if any of the latter guilds for cp 5 were present in this field.

The same general scenario was true for categorisation of fields of producer 2 compared to those for producer 1. Only subtle differences existed for NPN assemblages categorised along EI and SI trajectories of a faunal profile. Four (8, 9, 10 and 11) of the fields were plotted in the "Stable Enriched" quadrant and two (7 and 12) in the "Stress Enriched" quadrant (Figure 2.5B). Refer to explanation of the particular NPN groups grouped according to their cp values as have been done for producer 1 with regard to these two specific faunal quadrants.

All six fields sampled for producer 3 were plotted in the "Stable Enriched" quadrant (Figure 2.6A). Refer to explanations of the status of NPN present in these fields to those supplied for producer 1 above.

For producer 4, four (21, 22, 23 and 24) of the six fields were plotted in the "Stable Enriched" quadrant (see explanation for producer 1) with two (20 and 25) in the "Stable Depleted" quadrant (Figure 2.6B). Fields plotted in the latter quadrant had low (field 25) to medium (field 20) EI and SI. EI for these fields indicated that NPN assemblages represented by $Ba_2$ and $Fu_2$ dominated, while $Ba_1$ also occurred. In terms of the SI, $Ba_4$, $Ca_4$, $Fu_4$ and $Om_4$ dominated. NPN from the latter guilds with cp values of 5 were, generally not present in soil from these fields or in very low numbers.

For producer 5 three (28, 30 and 31) of the six fields were plotted in the "Stable Enriched" (see explanation for producer 1) two (26 and 27) in the "Stress Enriched" (see explanation for producer 1) and one in the "Stable depleted" (see explanation for producer 4) quadrants (Figure 2.7).

None of the fields sampled from the five producers, were plotted in the "Stressed Depleted" quadrant, which indicates the presence of and dominance by bacterivores, carnivores, fungivores and omnivores of cp values 2-4.

## 2.5 Discussion

Thirteen PPN genera and 16 nematode species have been identified from both root and soil samples from the 31 fields in which potato is included as a rotation crop every eight years in the Christiana area. In addition, although not identified to species level, individuals of the *Aphelenchoides* genus of which only *A. ritzemabozi* is an obligate PPN (Hunt, 1993), have been found and preliminary been categorised as fungus feeders until species identification can be done. Of the genera found, only one genus (*Psilenchus*) and six species (*Criconemoides sphaerocephalus*, *Hemicriconemoides brachyurus*, *Paratrichodorus lobatus*, *Rotylenchus caudaphasmidis* and *Tylenchorhynchus ventralis*) have never been associated with maize (Keetch & Buckley, 1984; De Waele & Jordaan, 1988; Kleynhans *et al*., 1996; Riekert, 1996; Riekert & Henshaw, 1998), potato (Kleynhans, 1978; Keetch & Buckley, 1984; Kleynhans *et al*., 1996), sunflower (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Bolton *et al*., 1989), onion (Coetzee, 1968; Keetch & Milne, 1982; Meyer, 1984) or grasses (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Ntidi *et al*., 2012) in cropping systems in SA. In terms of NPN assemblages nine genera (six bacterivorous and three fungivorous) as well as two families (omnivores/predators) have been identified from soil samples from these fields. Also, assessment of NPN populations and classification of fields accordingly in terms of their soil health is reported for the first time in annual crops such as potato-based cropping systems in SA as a result of this study.

Endoparasitic *Meloidogyne* spp. were the predominant PPN group in both soil and root samples collected during this survey from the 31 fields that are part of potato-based rotation systems in the Christiana area. These results support data that RKN are widely distributed in the Christiana potato production areas of SA (Kleynhans *et al*., 1996; Jones *et al*., 2011), where it causes severe damage to the crop (G. Posthumus, pers comm., Maart 2010). Results from this study also coincides with reports from other authors that *Meloidogyne* spp. are considered economically one of the most important PPN genera that are locally associated with crops that are planted in rotation with potato (Kleynhans, 1978; Kleynhans *et al*., 1996; Jones *et al.,* 2011) such as maize (Keetch & Buckley, 1984; De Waele & Jordaan, 1988; Kleynhans *et al*., 1996; Riekert, 1996; Riekert & Henshaw, 1998), sunflower (Keetch & Buckley, 1984; Bolton *et al*., 1989; Kleynhans *et al*., 1996), onion (Coetzee, 1968; Keetch & Milne, 1982; Meyer, 1984) as well as grasses. The latter is also true for weeds (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Ntidi *et al*., 2012) that occur in such crop fields. Also, on a global scale *Meloidogyne* spp. are considered as one of the economically most important and predominant PPN that attack a

wide range of crops (Moens *et al*., 2009) such as potato (Scurrah *et al*., 2005; Castagnone-Sereno, 2006), maize (Mc Donald & Nicol, 2005; Bridge & Starr, 2007), sunflower (Korayem *et al*., 2011), onion (Bridge *et al*., 2005; Mc Donald & Nicol, 2005; Sikora *et al*., 2005) where it causes severe quality and yield losses (Moens *et al*., 2009).

Both *M. incognita* and *M. javanica* were identified during this study but not *M. acronea, M. arenaria*, *M. chitwoodi* and *M. hapla* that are also reported as pests of potato in SA (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Jones *et al*., 2011) and in other countries (Scurrah *et al*., 2005; Bridge & Starr, 2007). *Meloidogyne incognita* and *M. javanica* have also been identified as the predominant RKN in the maize- (Riekert, 1996; Riekert & Henshaw, 1998) and sunflower-producing (Bekker *et al*., 2007) areas of SA. The same scenario applies for the occurrence of these two RKN species in weeds and grasses (Keetch & Buckley, 1984) sampled in commercial as well as small-scale fields of farmers in SA (Ntidi *et al*., 2012). Inclusion of crops such as maize, sunflower, wheat and onion as well as grass cover crops in this potato-producing area on which *Meloidogyne* species survive and reproduce implies that their population levels can build up to reach damaging levels when potato and/or other susceptible crops are planted on such fields. Following *Meloidogyne* spp., other predominant endoparasitic nematode genera and species recorded during this study from both soil and root samples were individuals from the endoparasitic *Pratylenchus* (*P. zeae, P. neglectus and P. scribneri*) and *Ditylenchus* spp. as well as Trichodoridae (*P. lobatus* and *N. minor*) and Hoplolaimidae (*Rotylenchus unisex*, *R. caudaphasmidis*, *Scutellonema brachyurus* and *S. truncatum*).

*Pratylenchus brachyurus*, which is regarded as an economically important parasite of potato in SA (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Jones *et al*., 2011) and other countries (Scurrah *et al*., 2005) in particular, was not found during this study. However, all three *Pratylenchus* spp. that were identified during this study have been associated with potato in SA earlier (Keetch & Buckley, 1984; Kleynhans *et al*., 1996). *Pratylenchus neglectus* and *P. scribneri* are also known to damage potato in temperate, tropical and subtropical regions of the world (Scurrah *et al*., 2005). Important is that *P. neglectus* and *P. zeae* are also associated with maize, grasses, wheat and onions (Kleynhans *et al*., 1996) that are all used as rotation crops with potato in the Christiana area. Not only does *Pratylenchus* spp. cause damage to the potato crop, they are also associated with the fungus (*Verticillium*) that can cause *Verticillium*-wilt in potato (Jones *et al*., 2011).

Although *Ditylenchus destructor* and *D. dipsaci* are regarded as major parasites of potato in countries other than SA (Scurrah *et al*., 2005; Bridge & Starr, 2007), the *Ditylenchus* sp. found in this study could not be identified since only juvenile specimens were encountered in root samples and their population levels were very low. However, in SA only *D. africanus* has been found to be of economical importance only infecting and reducing quality of groundnut kernel consignments (De Waele *et al*., 1989) and therefore, poses no risk to potato (Basson *et al*., 1990). However, high yield losses have been reported from other parts of the world where climatological conditions favour the establishment of *D. destructor* and *D. dipsaci* as a pest of potato (Scurrah *et al*., 2005).

Also members of the Hoplolaimidae family are not regarded as economically important parasites of potato (Scurrah *et al*., 2005; Jones *et al*., 2011) with various species such as *Helicotylenchus cavenessi*, *H. dihystera*, *H. pseudorobustus*, *Rotylenchus brevicaudatus, Scutellonema brachyurum* and *S. cavenessi* being associated with potato in SA (Keetch & Buckley, 1984; Kleynhans, 1991; Kleynhans *et al*., 1996) in particular.

Although J2 of the family Heteroderidae (other than *Meloidogyne* spp.) were also present in samples from two fields during the 2010 survey, no cysts could be isolated. In SA *G. rostochiensis* causes damage to potato in the Bon Accord, Ceres and Sandveld areas, but have not been reported in the Christiana area where this study was conducted (Jones *et al*., 2011). It is, however, important to obtain more soil samples from these specific fields to enable extraction and identification of cysts to make sure of the status of members of the cyst families in this important potato-production area.

Other PPN genera identified during this study such as *Longidorus, Nanidorus, Paratrichodorus*, *Tylenchorhynchus* and *Xiphinema* have also been associated with potato in SA (Keetch & Buckley, 1984; Kleynhans *et al*., 1996; Jones *et al*., 2011) and in other parts of the world (Scurrah *et al*., 2005) but are generally not regarded as important pests of the crop. The presence of *N. minor* and *P. lobatus* in particular in the Christiana potato production area is, however, important since these ectoparasites are vectors of the tobacco rattle virus that causes tuber spraing in potato (Manzanilla-López *et al*., 2004). Also these seemingly lesser important PPN identified in fields from this area have been associated with maize (Keetch & Buckley, 1984; De Waele *et al*., 1988), sunflower (Keetch & Buckley, 1984; Bolton *et al*., 1989), onion (Coetzee, 1968; Keetch & Milne, 1982; Keetch & Buckley, 1984; Kleynhans *et al*., 1996) and grasses (Keetch & Buckley, 1984; Van Biljon, 2004; Ntidi *et al*., 2012) that are planted in rotation with

potato in this area and should be monitored to prevent high build-up of their population levels which may lead to quality and quantity losses.

In terms of NPN assemblages present in the 31 fields sampled in the Christiana area, their total population levels were generally higher during both growing seasons compared to that of the PPN. Bacterivore nematode genera were represented by a wide spectrum with, individuals of the *Rhabditis* ($Ba_1$) genus dominating, followed by *Cephalobus* spp. ($Ba_2$). The latter genera as well as the other bacterivorous genera found during this study are commonly found in soils in SA (Heyns, 1971). Bacterivores are regard as the beneficial nematodes of which the population levels increase substantially and relatively quick after the addition of organic material (Heyns, 1971) or any kind of disturbance (Bongers, 1990; Ferris *et al*., 2001). The fungivore nematode community were represented by a smaller variety of genera, with *Tylenchus* ($Fu_2$) being predominant. In terms of the predator nematodes, only individuals of the Dorylaimidae ($Om_4$) and Mononchidae ($Ca_4$) families were present in relatively low numbers.

Of particular interest is that the majority of the 31 fields were plotted in the 'Stable Enriched" quadrant that indicates that the soils of such fields were relatively healthy, maturing and stable in terms of the beneficial nematodes as indicated by the simplified food web representation (Bongers, 1990; Bongers & Ferris, 1999; Ferris *et al*., 2001). This scenario is surprising and could probably be explained by the inclusion of grass cover crops on these fields for five to six years of the eight-year cropping cycles practiced by these producers. The dominant contribution of particularly bacterivores from the cp 1, 4 and 5 scales, carnivores (Ca 4&5), fungivores (cp 4&5) and omnivores ($Om_4$) contributed substantially to classification of such fields in the "Stable Enriched" quadrant. Generally, the only common factors shared for all fields categorised in the "Stable Enriched" quadrants are the inclusion of grasses (*Eragrostis*, *Digitaria* and/or *Panicum* spp.) and potato. This is, however, also true for the other fields that were classified in either the "Stress Enriched" and/or "Stable Depleted" quadrants. Planting of rotation crops, particularly grass cover crops and potato as well as ploughing generally were the three common factors generally applied to all 31 fields during both years. Also the organic content of these fields were generally low and ranged from 0.15% to 0.48% when determined during 2010 when this study commenced. Therefore, reasons why the majority of these fields were classified as stable and enriched are currently unknown. Although more research is needed to conclude on this scenario, results obtained during this study already represents baseline information for fields in this specific potato-producing area.

Investigation of relationships between both PPN and NPN rendered interesting results but it must be remembered that data for these groups were pooled for all 31 fields thus resulting in low mean population levels for the various parameters, i.e. cultivation practices, nematicides used and crop rotation sequences. Although a few significant relationships between the various PPN as well as NPN nematode genera and cultivation practices, nematicide(s) used as well as cropping sequences were revealed it has to be verified with additional results. Collection of data of this kind for more than one growing season needs to be done and will add substantial value to the science of Nematology as well as to producers and the related crop industries. Of particular interest are findings that application of EDB® in combination with other nematicides rendered significantly lower population levels of some of the endo- and ectoparasitic nematode genera identified during the study. This trend indicates that application of a variety of nematicides rather than one specific product seems to be the best way to reduce population levels of PPN in this area. Concurrently, indications that some NPN, i.e. *Rhabditis* and *Prismatolaimus* spp. had higher population levels in fields where EDB® combined with other nematicides and EDB® alone were applied, respectively, also indicate that interesting and important trends could be revealed should this type of research be extended over more growing seasons. The same applies for crop rotation sequences where RKN population levels were significantly lower in fields where grass, maize and potato were planted compared to those that excluded grass. This may be explained by the poor host status of grasses for RKN and may add substantial value in such cropping systems to manage these parasites. Validation of such information is, however, crucial and can have pertinent implications for designing and planning of management strategies for PPN to fit the needs of not only the Christiana, but also other local producers.

No explanation could at this stage be given for no significant effects being evident found for either PPN or NPN with regard to soil characteristics such as percentages of sand, silt, clay and organic material during this study. It is, however, important to bear in mind that soil from all these fields were all sandy with percentages ranging from 91.6%to 96%. The latter substantiates the predominant occurrence of RKN in sandy soils, such as those occurring in the Christiana area, as been noted by other authors (Greco & Di Vito, 2009).

## 2.6 Conclusions

Although not present in soil and or roots from crops grown in all 31 fields, RKN were identified as the predominant PPN group in the Christiana potato-producing area where it poses a real problem to potato producers. These parasites may also impact adversely on other crops and grass cover crops planted by these producers since they have a wide host range. In terms of NPN assemblages, characterisation of the majority of potato fields in the “Stable Enriched” quadrant was unexpected but is most probably due to the inclusion of grasses during six to seven years of the eight-year cropping cycle. Results obtained during this study are valuable to serve as a baseline study with regard to prevailing community structures, species composition and population levels of both PPN and NPN present in the 31 fields sampled. More such studies should be done in important potato-production areas such as Christiana where life-sustaining grain crops including maize and wheat, oilseed crops such as sunflower and high-cash vegetable crops are produced. This way important correlations between these parasites and factors such as cultivation practices, nematicides used as well as crop rotation sequences can be obtained to assist producers and the industry to minimise the adverse effect of PPN on their crops.

## 2.7 References


Basson, S., De Waele, D. & Meyer, A.J. 1990. An evaluation of crop plants as hosts for *Ditylenchus destructor* isolated from peanut. *Nematropica* 20: 23-29.

Bekker, S., Venter, G.A. & Fourie, H. 2007. Nematode diagnostic service at the ARC-Grain Crops Institute. 18th Symposium of the Nematological Society of Southern Africa. Port Elizabeth, South Africa, 6-10 May 2007. (Poster Presentation).

Black, V. 2008. Potatoes in South Africa. In: GM potatoes in South Africa – a critical analysis. The African Centre for Biosafety, Johannesburg, South Africa. pp. 12-24.

Bolton, C., De Waele, D. & Loots, G.C. 1989. Plant-parasitic nematodes on field crops in South Africa. 3. Sunflower. *Revue de Nématology* 12: 69-76.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83: 14-19.

Bongers, T. & Ferris, H. 1999. Nematode community structure as a bioindicator in environmental monitoring. *Trends in Evolution and Ecology* 14: 224-228.

Bridge, J., Plowright, R.A. & Peng, D. 2005. Nematode parasites of rice. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 87-130.

Bridge, J. & Starr, J.L. 2007. Plant nematodes of agricultural importance A color handbook. Academic Press, Boston, USA. 152 pp.

Castagnone-Sereno, P. 2006. Genetic variability and adaptive evolution in pathogenetic root-knot nematodes. *Heredity* 96: 282-289.

Coetzee, V. 1968. The distribution of the family Heteroderidae (Filipjev, 1934) in South Africa and some host records of *Meloidogyne* species. *South African Journal of Agricultural Sciences* 11: 775-788.

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

De Grisse, A. 1965. A labour-saving method for fixing and transferring eelworms to anhydrous glycerine. Offset, University of Gent, p. 3.

De Waele, D. & Jordaan, E.M. 1988. Plant-parasitic nematodes on field crops in South Africa. 1. Maize. *Revue de Nématology* 11: 65-74.

De Waele, D., Jones, B.L., Bolton, C. & Van den Berg, E. 1989. *Ditylenchus destructor* in hulls and seeds of peanut. *Journal of Nematology* 21: 10-15.

Ferris, H., Bongers, T. & De Goede, R.G.M. 2001. A framework for soil food web diagnostics: extension of nematode faunal analysis concept. *Applied Soil Ecology* 18: 13-29.

Fourie, H., Leswifi, C., Mc Donald, A.H. & De Waele, D. 2007. Host suitability of vetiver grass to *Meloidogyne incognita* and *M. javanica*. *Nematology* 9: 49-52.

Greco, N. & Di Vito, M. 2009. Population dynamics and damage levels. In: Perry, R.N., Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CABI Publishing, Wallingford, UK. pp. 246-274.

Heyns, J. 1971. A guide to the plant and soil nematodes of South Africa. Content Solutions, Pretoria, South Africa.

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

Jones, R.K., Marias, M. & Swart, A. 2011. Aalwurms (Nematodes). In: Denner, F. & Venter, S. (eds) Handleiding vir aartappelproduksie in Suid-Afrika. Landbounavorsingsraad-Roodeplaat Instituut vir Groente en Sierplante. Pretoria, Suid-Afrika. pp. 195-201.

Keetch, D.P. & Buckley, 1984. A check-list of the plant parasitic nematodes of Southern Africa. Technical Communication No. 795. Department of Agricultural Development, South African Government, Republic of South Africa.

Keetch, D.P. & Milne, D.L. 1982. The control of plant-parasitic nematodes. In: Keetch, D.P. & Heyns, J. (eds) Nematology in southern Africa. Science Bulletin Department of Agriculture and Fisheries Republic of South Africa. Science Bulletin No. 400. pp. 113-129.

Kleynhans, K.P.N. 1978. Nematode pests in potatoes. Leaflet of the Department of Agriculture and Technical Services of the Republic of South Africa Potato Series No. G. 13.

Kleynhans, K.P.N., Van den Berg, E., Swart, A., Marais, M. & Buckley, N.H. 1996. Plant nematodes in South Africa. Plant Protection Research Institute Handbook No 8, Pretoria, Business Print. p. 165.

Korayem, A.M., Youssef, M.M.A., Ahmed, M.M. & Mohamed, M.M.M. 2011. Distribution and association of plant parasitic nematodes with some oil crops in Egypt. *Pakistan Journal of Nematology* 29: 79-91.

Manzanilla-López, R.H., Evans, K. & Bridge, J. 2004. Plant diseases caused by nematodes. In: Chen, Z.X., Chen, S.Y. & Dickson, D.W. (eds) Nematology advances and perspectives. Volume 2. Nematode management and utilization. CABI Publishing, Wallingford, UK. pp. 637-716.

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

Meyer, A.J. 1984. Damage to onion by *Nanidorus minor* in the Koue Bokke veld. *Phytophylactica* 16:331.

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

Mucina, L. & Rutherford, M.C. 2006. The vegetation of South Africa, Lesotho and Swaziland. *Strelitzia* 19. South African National Biodiversity Institute, Pretoria, South Africa. p. 516.

Ntidi, K.N., Fourie, H., Mc Donald, A.H., De Waele, D. & Mienie, M.S. 2012. Plant-parasitic nematodes associated with weeds in developing agriculture in South Africa. *Nematology* (in press).

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

Riekert, H.F. 1996. Economic feasibility of nematode control in dryland maize in South Africa. *African Crop Science Journal* 4: 477-481.

Riekert, H.F. & Henshaw, G.E. 1998. Effect of soybean, cowpea and groundnut rotations on RKN build-up and infestation of dryland maize. *African Crop Science Journal* 6: 377-383.

Scurrah, M.I., Niere, B. & Bridge, J. 2005. Nematode parasites of solanum and sweet potatoes. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 193-219.

Seinhorst, J.W. 1962. Modifications of the elutriation method for extracting nematodes from soil. *Nematologica* 8:117-128.

Sellschop, J.P.F. & Du Toit, J.J. 1948. The production of potatoes. *Farming in South Africa* 23: 645-654.

Sikora, R.A., Bridge, J. & Starr, J.L. 2005. Management practices: an overview of integrated nematode management technologies. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 793-824.

Taylor, C.E. & Martin, G.C. 1960. Potato production. 8. Pests. *Rhodesia Agricultural Journal* 57: 60-63.

Van Biljon, E.R. 2004. Crop rotation as part of an integrated pest management programme for the control of plant parasitic nematodes. Proceedings of the Soilborne Plant Diseases Interest Group Symposium, Stellenbosch, 14-16 September 2004.

Yeates, G.W., Ferris, H., Moens, T. & Van der Putten, W.H. 2009. The role of nematodes in ecosystems. In: Wilson, M.J. & Kakouli-Duarte, T. Nematodes as environmental indicators. pp. 1-44.

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

Zijlstra, C., Donkers-Venne, D. H. & Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. *Nematology* 2: 847-853.

# CHAPTER 3

# MOLECULAR IDENTIFICATION OF ROOT-KNOT NEMATODE SPECIES USING SEQUENCE CHARACTERIZED AMPLIFIED REGION BASED POLYMERASE CHAIN REACTION ASSAYS

## 3.1 Introduction

Accurate identification of organisms is fundamental to our understanding and communication about their role in soil ecology (Abebe *et al*., 2011). Knowledge of a pest, in this case, soilborne root-knot nematodes (RKN; *Meloidogyne* spp.), necessitates identification of the species present in a specific field (Moens *et al*., 2009). Because RKN is one of the most economically important pests of a wide variety of agricultural and horticultural crops (Luc *et al*., 2005) correct identification of their juvenile and adult stages is crucial (Powers, 2004) for quarantine and plant protection purposes (Sikora *et al*., 2005). Species identification of these organisms is also essential where more than one crop is included in cropping cycles, such as the Christiana area where this study was conducted. This is also applicable where control of RKN is planned using rotation of susceptible and resistant crops (Manzanilla-López *et al*., 2004). Several new molecular technologies such as sequence characterized amplified region based polymerase chain reaction (SCAR-PCR) assays have been developed and provide tools to assist in accurate and rapid identification of a wide range of plant-parasitic nematodes (PPN), particularly RKN (Moens *et al*., 2009).

An increasing number of species have been described during the last decades within the genus *Meloidogyne*. It is, therefore, important that nematode species descriptions conform to a general standard in order to facilitate accurate comparisons and differential diagnoses of such individuals (Eisenback & Hunt, 2009). Due to the relatively conserved morphology within the RKN group there is an increasing emphasis on using techniques such as molecular sequences to accurately identify these species (Eisenback & Hunt, 2009). On the other hand, due to the nature and morphology of *Meloidogyne* species the differences that exist between them are very small, with certain features being influenced by the host plant as well as a range of environmental factors (Jepson, 1987). Thus, correct identification of RKN is difficult and no single morphological approach or single developmental-stage feature can be used to distinguish among all species belonging to this genus. Therefore, different approaches have been investigated to resolve problems experienced with the identification of RKN species (Hartman & Sasser, 1985; Jepson, 1987), including molecular techniques (Manzanilla-López *et al*., 2004), to supplement morphological identification.

Modern identification methods used to distinguish between RKN species must provide accuracy, speed, reliability, affordability and enable characterisation of specimens new to science (Powers, 2004). A major benefit of using molecular techniques to identify RKN species is that individual J2, which is the most common and infective stage of the RKN found in the soil, can be used. This way, the need to use mature females from roots and/or other parts of host plants, which are not always present during sampling, or establishment of cultures in growth-regulated glasshouses to obtain females, are eliminated. Molecular techniques based on characterisation of the deoxy-ribonucleic acid (DNA) can be used for every stage of the nematode's life cycle (Devran & Söğüt, 2009). This approach, however, requires some expensive equipment such as a PCR thermocycler and a furnished molecular laboratory. None the less, the use of molecular techniques to identify RKN are likely to play an ever increasing role in species identification, especially by those not well trained or experienced in classical morphometrics (Moens *et al*., 2009). Moreover, problems are experienced with morphological identification of RKN such as their sheer abundance and small size (Floyd *et al*., 2002), conservative morphology, the presence of considerable variation within populations of these parasites due to indistinct species boundaries or species complexes and the lack of experts trained as nematode taxonomists (Blok *et al*., 1997; Zijlstra *et al*., 2000; Blok & Powers, 2009). Therefore, it is preferable (although not always possible) to use molecular techniques to complement morphological identification to ensure accurate identification of the RKN species involved.

The main objective for this part of the study was to accurately identify RKN species from fields where various crops and cover crops (i.e. grasses) were included in potato-based cropping systems in the Christiana area of South Africa (SA) both during the 2010 and 2011 growing seasons.

## 3.2 Material and methods

### 3.2.1 Deoxy-ribonucleic acid (DNA) extraction

Soil from each of the 31 fields sampled during 2010 and 2011 was (see Chapter 2, paragraph 2.2.6) transferred to individual 4L pots. One tomato seedling (cv. Rodade) was planted in each pot to enable *in vivo* rearing of the respective RKN species present in soil sampled from each of these fields. Plants were watered three times a week using equal amounts of tap water. Fifty-six days after seedling transplanting the root system of each tomato plant was removed, washed under a gentle stream of tap water to remove excess debris and mature RKN females removed for identification purposes. Ten RKN females, if available, from each of the 31 fields were randomly removed with a scalpel from galls present on each of the respective individual tomato root systems. This procedure was conducted using a stereo microscope at 100x magnification. Single, mature RKN females from each infected tomato root system were placed in an individual microcentrifuge tube (1ml capacity) and crushed with a disposable pipette tip. DNA was isolated following a method by Zijlstra *et al*. (1995) that was adapted for the purpose of this study since mature RKN females were used instead of J2. This was done by extracting DNA from each RKN female by adding 50µl cell lysis buffer (50mM TrisHCl pH 8.0; 100mM NaCl; 10mM EDTA; 1% SDS (v/v)) to each tube as well as 2µl of Proteinase K (10mg/ml). The tube containing the crushed female suspended within the latter solution was incubated at 65ºC for 1 hour. The DNA from each RKN female was extracted using a GeneClean Kit (Bio101) according to the manufacturer's instructions. Three volumes of sodium iodide (NaI) solution (150µl) and 2µl glass milk were added to each tube containing the RKN DNA, mixed and incubated at room temperature (25ºC-27°C) for five minutes. The solution was then centrifuged at 10 000 rpm for five seconds and the supernatant decanted. The pellet, consisting of glass milk with bound RKN DNA remaining at the bottom of the tube was resuspended in 200µl NEW Wash and centrifuged at 10 000rpm for another five seconds. This process was repeated two more times. The liquid supernatant was removed and the pellet containing the RKN DNA dried at room temperature for 12 hours, resuspended in 20µl TE buffer and centrifuged for 30 seconds at 10 000rpm. Two microlitres of RKN DNA was subsequently used for SCAR-amplification reactions.

### 3.2.2 Sequence characterized amplified region (SCAR) amplification

PCR was conducted in a total volume of 20µl, containing 2µl DNA, 1x GoTaq Green Master Mix (Promega) containing GoTaq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer at optimal concentrations and 5pmol each of *M*. *incognita*, *M. javanica*, *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla* forward and reverse primers respectively. DNA amplification for *M. incognita* was done in a ThermoHybaid Thermal Cycler programmed as follows: denaturation at 94ºC for two minutes (one cycle), followed by 40 cycles of denaturation at 94ºC for 30 seconds, annealing at 54ºC for 30 seconds and extension at 72ºC for one minute, followed by a final extension step at 72ºC for five minutes. The same programme was used for *M. javanica* with the exception of the annealing temperature at 64ºC (Zijlstra, 2000; Zijlstra *et al.,* 2000).

Female RKN DNA products from the field sites of which mature RKN were reared successfully, resulting from the amplification processes, were subsequently analysed by electrophoresis in a 3% (m/v) agarose gel with 1x TBE running buffer (89mM Tris-borate, 2.5mM EDTA, pH 8.3). Ethidium bromide (1µg/ml) was added to the gel and samples were visualized under UV-light. One blank reaction (no DNA), one reference sample of *M. incognita* and *M. javanica* monoculture populations that have been reared *in vivo* in separate greenhouses, respectively, and identified by means of morphological characteristics earlier served as the positive control. However, no mature females and subsequently no DNA could be obtained for other RKN species such as *M. arenaria*, *M. hapla*, *M. chitwoodi* or *M. fallax*, which are also economically important species that infects potato in SA (Jones *et al*., 2011). Ultimately, Lambda DNA restricted with HindIII and EcoRI as molecular weight markers were also loaded on both sides of the gel comb to establish the size of the DNA bands for each of the RKN species that were present in the fields sampled. Gels containing the DNA products of the respective RKN females were electrophoresed for 2 hours at 80V and banding patterns were visualised with ultraviolet (UV) illumination. A photograph was taken of each gel containing the RKN female DNA-banding patterns.

### 3.3 Results

Table 3.1. *Meloidogyne* species identified from the 31 fields of the five producers in the Christiana area, indicating their respective deoxy-ribonucleic acid (DNA) fragment sizes (bp).

| Source/Field no. | DNA fragment size(s) (bp) | *Meloidogyne* spp. identified |
|---|---|---|
| **29 (UK1)** | 1 200 and 670 | *M. incognita* and *M. javanica* |
| **3 (CG3)** | 1 200 | *M. incognita* |
| **9 (LH13)** | 1 200 | *M. incognita* |
| **11 (LH20)** | 1 200 | *M. incognita* |
| **14 (A6)** | 1 200 | *M. incognita* |
| **18 (SP11)** | 1 200 | *M. incognita* |
| **19 (S5)** | 1 200 | *M. incognita* |
| **20 (P1)** | 1 200 | *M. incognita* |
| **22 (VH1)** | 1 200 | *M. incognita* |
| **23 (VH2)** | 1 200 | *M. incognita* |
| **25 (VH5)** | 1 200 | *M. incognita* |
| | | |
| **5 (T6T5)** | 670 | *M. javanica* |
| **16 (B2)** | 670 | *M. javanica* |
| **26 (D4)** | 670 | *M. javanica* |
| **28 (GH17)** | 670 | *M. javanica* |
| **Standard RKN populations used** | | |
| **Greenhouse – *in vivo* reared** | 1 200 | *M. incognita* |
| **Greenhouse – *in vivo* reared** | 670 | *M. javanica* |

PCR with the *M. incognita* specific SCAR-primers resulted in amplification of the *M*. *incognita* 1 200bp SCAR-fragment for RKN females reared from 11 of the fields sampled during this study (Table 3.1). DNA from the latter females were similar to those of the standard *M. incognita* race 2 population used, indicating that *M. incognita* were present on 11 (29, 3, 9, 11, 14, 18, 19, 20, 22, 23 and 25) of the 31 fields sampled.

On the other hand, *M. javanica* was identified from RKN females reared *in vivo* from soil of five of the 31 fields sampled (Table 3.1). The 670bp *M. javanica* SCAR fragment was amplified during PCR-reactions with the *M. javanica*-specific SCAR marker with RKN DNA from the five fields (29, 5, 16, 26 and 28) corresponding with that of the standard *M. javanica* population used.

The combined occurrence of *M. incognita* and *M. javanica* in one (29) of the 15 fields were further revealed when both the *M. incognita* (1 200bp) and *M. javanica* (670bp) SCAR fragments were amplified as a result of PCR reactions with both these primers (Table 3.1).

No 420bp, 525bp, 515bp and 610bp SCAR fragments were amplified during any of the PCR reactions when the respective primers for *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla*-specific were added to the DNA products of the mature females from the fields sampled during this study (Table 3.1). As indicated earlier, no mature females of the latter species could be found to enable DNA extraction and use thereof as respective standards during this study.

**3.4 Discussion**

DNA-based identification was successfully used as a tool during this study for verifying and confirming the identity of economically important RKN species that were present in 15 of the 31 fields sampled in the Christiana area (North-West Province of SA). Only *M*. *incognita* and *M*. *javanica* were identified as a result of this study. *Meloidogyne incognita* was identified as the predominant RKN species present on 11 of the fields sampled, while *M*. j*avanica* was identified from five of the fields sampled. In addition results from SCAR-PCR reactions indicated that monospecific populations of *M. incognita* race 2 and *M. javanica*, respectively, were mass-reared *in vivo* and subsequently used as standards to complement the use of primers of these two RKN species during molecular identification done during this study.

Identification and verification of *M*. *incognita* and *M*. *javanica* species in fields of producers in the Christiana area will contribute substantially in terms of crop selection based on resistance of commercially available maize and grass genotypes that could be included in their current cropping systems. Information on the latter crops exists in terms of their host suitability (Van Biljon, 2004; Ngobeni *et al*., 2010) for these RKN respective species. In this way producers can optimise crop production strategies for sustainable production of high-cash crops such as potato. Value adding in

terms of crop production in this particular agricultural area thus emanated as a result of this part of this study.

Although only *M. incognita* and *M. javanica* were identified from fields using the SCAR-PCR technique, it cannot be concluded beyond debate that only these species are present in these fields and in this area. A shortcoming of this particular part of the study is that mature females could not be obtained after *in vivo* rearing of such populations due to the presence of low J2 population levels for some of the fields sampled, i.e. fields 1, 2, 4, 6, 7, 8, 10, 12, 13, 15, 17, 21, 24, 27, 30 and 31. Endeavours will, however, be made to continue with *in vivo* rearing of RKN populations from the soil obtained during the surveys in order to obtain a more complete picture on species identification that represents the majority of the fields sampled in this particular area.

No specimens of other economically important RKN species such as *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M, hapla* could be obtained to ensure that their presence could be detected in DNA samples from the fields included in this study. However, use of primers of the latter species was included as the second best option to try and detect their presence should they be prevalent. It should be noted that endeavours will be made to obtain DNA samples of all RKN species that are regarded as economically important parasites of potato and other rotation crops included in such rotation systems. This way exclusion of RKN species that occurs in fields in potato-producing areas will be prevented and interesting information with regard to the occurrence of these parasites in such areas could be obtained. Such knowledge will benefit producers, the industry and the scientific arena.

### 3.5 Conclusions

Molecular identification of mature RKN females enabled accurate identification of two of the economically most important species in 15 of the 31 fields sampled in the study area. Individual J2 from each field was not used during this study since problems were experienced to isolate such small amounts of DNA. These results will be to the advantage of producers since they can now select poor host cultivars of these respective RKN species identified of crops such as maize as well as grasses to be planted in such fields. This way RKN populations can be reduced to minimise damage to high-value crops such as potato.

The SCAR-PCR method used in this study has proved to be a reliable tool for routine diagnostic identification of RKN and was also used with success by various other researchers (Blok *et al*., 1997; Zijlstra, 2000; Zijlstra *et al*., 2000; Fourie *et al*., 2001; Berry *et al.,* 2008).

### 3.6 References


Abebe, E., Mekete, T. & Thomas, W.K. 2011. A critique of current methods in nematode taxonomy. *African Journal of Biotechnology* 10: 312-323.

Berry, S.D., Fargette, M., Spaull, V.M., Morand, S. & Cadet, P. 2008. Detection and quantification of root-knot nematode (*Meloidogyne javanica*), lesion nematode (*Pratylenchus zeae*) and dagger nematode (*Xiphinema elongatum*) parasites of sugarcane using real-time PCR. *Molecular and Cellular Probes* 22: 168-176.

Blok, V.C., Phillips, M.S. & Fargette, M. 1997. Comparison of sequences from the ribosomal DNA intergenic region of *Meloidogyne mayaguensis* and other major tropical root-knot nematodes. *Journal of Nematology* 29: 16-22.

Blok, V.C. & Powers, T.O. 2009. Biochemical and molecular identification. In: Perry, R.N., Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 98-118.

Devran, Z. & Söğüt, M.A. 2009. Distribution and identification of root-knot nematodes from Turkey. *Journal of Nematology* 41: 128-133.

Eisenback, J.D. & Hunt, D.J. 2009. General morphology. In: Perry, R.N., Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 18-54.

Floyd, R., Abebe, E., Papert, A. & Blaxter, M. 2002. Molecular barcodes for soil nematode identification. *Molecular Ecology* 11: 839-850.

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

Hartman, K.M. & Sasser, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential host treatise and perineal-pattern morphology. In: Barker, K.R., Carter, C.C. & Sasser, J.N. (eds) Advanced treatise on *Meloidogyne*. Volume II. Methodology. North Carolina University Graphics, Raleigh, North Carolina, USA. pp. 69-78.

Jepson, S.B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, UK. 265 p.

Jones, R.K., Marias, M. & Swart, A. 2011. Aalwurms (Nematodes). In: Denner, F. & Venter, S. (eds) Handleiding vir aartappelproduksie in Suid-Afrika. Landbounavorsingsraad-Roodeplaat Instituut vir Groente en Sierplante. Pretoria, Suid-Afrika. pp. 195-201.

Luc, M., Sikora, R.A. & Bridge, J. 2005. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. 871 p.

Manzanilla-López, R.H., Evans, K. & Bridge, J. 2004. Plant diseases caused by nematodes. In: Chen, Z.X., Chen, S.Y. & Dickson, D.W. (eds) Nematology advances and perspectives. Volume 2. Nematode management and utilization. CABI Publishing, Wallingford, UK. pp. 637-716.

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

Ngobeni, L., Fourie, H. & Mc Donald, A.H. 2010. Host suitability of selected South African maize genotypes to the root-knot nematode species *Meloidogyne incognita* race 2 and *Meloidogyne javanica*: A preliminary study. *South African Journal of Plant and Soil* 28: 49-54.

Powers, T. 2004. Nematode molecular diagnostics: from bands to barcodes. *Annual Review of Phytopathology* 42: 367-383.

Sikora, R.A., Bridge, J. & Starr, J.L. 2005. Management practices: an overview of integrated nematode management technologies. In: Luc, M., Sikora, R.A. & Bridge, J. (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, London, UK. pp. 793-824.

Van Biljon, E.R. 2004. Crop rotation as part of an integrated pest management programme for the control of plant parasitic nematodes. Proceedings of the Soilborne Plant Diseases Interest Group Symposium, Stellenbosch, 14-16 September 2004.

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

Zijlstra, C., Donkers-Venne, D.T.H.M. & Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2: 847-853.

Zijlstra, C., Lever, A.E.M., Uenk, B.J. & Van Silfhout, C.H. 1995. Differences between ITS regions of solates of the root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi. Phytopathology* 85: 1231-1237.

# CHAPTER 4

# THE EFFECT OF COVER/GREEN MANURE CROPS ON PLANT-PARASITIC AND NON-PARASITIC NEMATODE ASSEMBLAGES IN A POTATO FIELD

## 4.1 Introduction

Plant-parasitic nematodes (PPN), particularly root-knot nematodes (RKN) are recognized as a serious constraint to intensive crop production systems worldwide (Nyczepir & Thomas, 2009). After decades of high input farming, more sustainable practices based on ecologically sound management techniques are being developed for the agricultural sector. These changes involve reduced pesticide use, integrated pest management (IPM), increased use of biological control products and maintenance and improvement of the quality of ground water and soil (Halbrendt, 1996). However, increased levels of control are likely to be needed in nematode-susceptible, high-value crops such as potato (Nyczepir & Thomas, 2009).

For the last 50 years, potato producers have used methyl bromide, a broad-spectrum fumigant, highly efficient in killing fungi, nematodes, insects and weeds. This fumigant has effectively controlled PPN, particularly in potato-based production systems (Monfort *et al*., 2007). However, methyl bromide was identified as a contributor to the depletion of the stratosphere ozone layer in 1992 and was scheduled for worldwide phase out by 2005 (UNEP, 1992; Noling, 2002; Schneider *et al*., 2003). The same scenario exist for other highly efficient synthetic nematicides, including fumigants (e.g. ethylenedibromide – EDB®) other than methyl bromide, contact as well as systemic products that have been/are registered for use on potato, particularly in South Africa (SA) (Nel *et al*., 2007; CropLife, 2011). Although aldicarb (Temik®) has, for example been phased out for use on potato in SA during 2010 already, it will only be announced in the Government Gazette during June/July 2012 (D. Uys, pers comm., March 2012). Threats about the phasing out of fenamiphos also exist (D. Uys, pers comm., March 2012). With the decreasing number of synthetic nematicides available for use on potato and increasing pressure towards environmental, human and animal safety alternatives for managing these parasites on potato are becoming quintessential.

One potential cultural alternative to synthetic nematicides for management of RKN pests in potato fields is the use of cover/green manure crops with biofumigation characteristics (Monfort *et al*., 2007). Such crops, particularly species from the Brassicaceae family are used as green manure amendments prior to planting (Monfort *et al*., 2007) of crops such as potato. The presence of glucosinolates (GL) in

Brassicaceae plant organs and sufficient activity of the enzyme myrosinase (MYR) to catalyse their hydrolysis have indicated the practical possibility of using such crops for biofumigation of various soilborne organisms (Rosa *et al*., 1997). Incorporating Brassicaceae residues into soil is known to suppress a range of pest and disease-causing organisms, including fungi (Chan & Close, 1987), nematodes (Mojtahedi *et al*., 1991), insects (Brown & Morra, 1997, Mathiessen & Kirkegaard, 1998) bacteria (Akiew *et al*., 1996; Gouws & Mienie, 2000; Gouws & Wehner, 2004) and weeds (Brown & Morra, 1995). When used as a cover/green manure crop, research has demonstrated that many Brassicaceae species have a nematicidal effect against multiple PPN species especially the RKN species *M. incognita* and *M. javanica* (Mojtahedi *et al*., 1991; Lazzeri *et al*., 2004) and the lesion nematode *Pratylenchus neglectus* (Potter *et al*., 1998; Monfort *et al*., 2007).

Glucosinolates, contained by these crops, themselves are not biologically active (McLeod & Steel, 1999). When Brassicaceae tissues are disrupted, however, the GL react with MYR, thioglucosidase and generates biologically active compounds including nitriles, thiocyanates and, most notably isothiocyanates (McLeod & Steel, 1999). To achieve the maximum benefit from utilizing Brassicaceae species as cover and/or green manure crops for the control of soilborne PPN pathogens, a species and cultivar (cv.) with a high potential of GL production and also one that produces large quantities of biomass in the selected geographic location or environment should be selected (Kirkegaard & Sarwar, 1998; Morra & Kirkegaard, 2002; Zasada *et al*., 2003). The objective of this study was to evaluate the effect of selected Brassicaceae species that are commonly available in SA as cover/green manure crops, on a *M. incognita* population as well as soilborne, non-parasitic nematode (NPN) assemblages that are present in a potato field in the Christiana area.

## 4.2 Material and methods

### 4.2.1 Field trial

The field trial was conducted during the 2010/2011 cropping season on a quarter (8ha) of a potato field (included in the survey as field 14; see Chapter 2) on the farm of Mr. Johan Greyling (Figure 4.1), which is situated in the Christiana area (See Figure 2; Chapter 2; North-West Province of SA). This field has a crop rotation history of *Digitaria* grass and maize for the past twenty years. High *M. incognita* population levels (ranging between 1 000-10 000 eggs and J2/20g tubers) prevailed at this particular field during the 2009/2010 growing season when potato was planted there. Although it is not normal practice to plant potato during two consecutive seasons, it has been done for the purpose of this experiment since

it was known that potato planted during the 2009/2010 season suffered quality losses due to high population levels of RKN.

Before planting of the cover/green manure crops, soil samples from all plots were obtained (Sampling interval 1; Table 4.1) for baseline purposes. The cover/green manure crops, all belonging to the family Brassicaceae, planted before the potato follow-up crop in 2011 represented two mustard cultivars *viz*. Nemat (*Brassica juncea*) at a planting density of 6kg seeds/ha and Calienté (*Eruca sativa*) at 11kg seeds/ha. In addition two cultivars of oilseed radishes (*Raphanus sativus*) Terranova and Doublet were planted at a rate of 20kg seeds/ha. These planting densities are recommended for commercial purposes for these particular cultivars (J. Fourie & B. Schoeman pers comm., September 2010). An untreated control as well as a synthetic nematicide ethylenedibromide (EDB®) at 40ℓ/ha (a.s. 1 800g/ℓ) treatment were also included in the trial, but were left fallow during planting of the Brassicaceae crops.

Figure 4.1. The trial site where four Brassicaceae cultivars and a follow-up potato crop were planted at Mr. Johan Greyling's farm in the Christiana area during the 2010/2011 growing season.

The concentration of GL in the leaves and shoots of Brassicaceae crops decreases as the plant matures (Uppstrom, 1983). Since it is suggested that the breakdown products of GL are responsible for nematicidal activities, the age of incorporation of its aerial parts may significantly influence the suppression of nematode populations (Mojtahedi *et al*., 1991). Therefore, at the 50% flowering stage, which was eight weeks after planting of the Brassicaceae crops, soil and root samples were obtained from all Brassicaceae crops while only soil samples were obtained from plots that were designated for the untreated control and EDB® treatment. This represented sampling interval 2. Subsequently, the

aerial parts of the four Brassicaceae cultivars were slashed, chopped and ploughed into the soil using commercial implements. After being left in the soil for seven weeks to enable biofumigation and decomposition of organic material, potato tubers of cv. Mondial were planted on all plots including the untreated control and EDB®-fumigated ones. Before planting of the potato crop, twenty nematode samples were taken randomly per replicate for each treatment and combined. Subsequently one representative sub-sample for each treatment was taken for nematode extraction purposes. Nematode sampling dates and types of samples taken are indicated in Table 4.1.

Two rows of potato (cv. Mondial) were planted in each plot (including those designated for the untreated control and EDB® treatments) after the cover/green manure crops were incorporated and left for seven weeks to enable biofumigation. Before planting of the potato crop, soil samples were obtained (Sampling Interval 3) from all plots for nematode extractions. The trial site was then ploughed and 800kg/ha fertiliser [N:P:K (3:1:5)] was incorporated across the site. Seven weeks after planting, at potato tuber initiation (Sampling Interval 4), root and tuber as well as soil samples were obtained for nematode extractions.

The prevailing air temperatures at the trial site ranged between 15ºC–30ºC for the duration of the trial, while rainfall figures between 100mm–300mm were measured for the same period. The trial layout was a randomised complete block design (RCBD; Figure 4.2) with five replicates and six treatments (see paragraph above). Each treatment represented a 5m wide plot with a buffer zone of 200mm on each side. Furthermore, each plot differed in length ranging from 300m-150m. Therefore, samples were only taken inside a 100m long sampling site that stretched across the trial site (Figure 4.3) for the purpose of this study. Soil at this trial site consisted of 91.8% sand, 1.2% silt, 0.31% organic C and had a $pH_{(H_2O)}$ of 6.55.

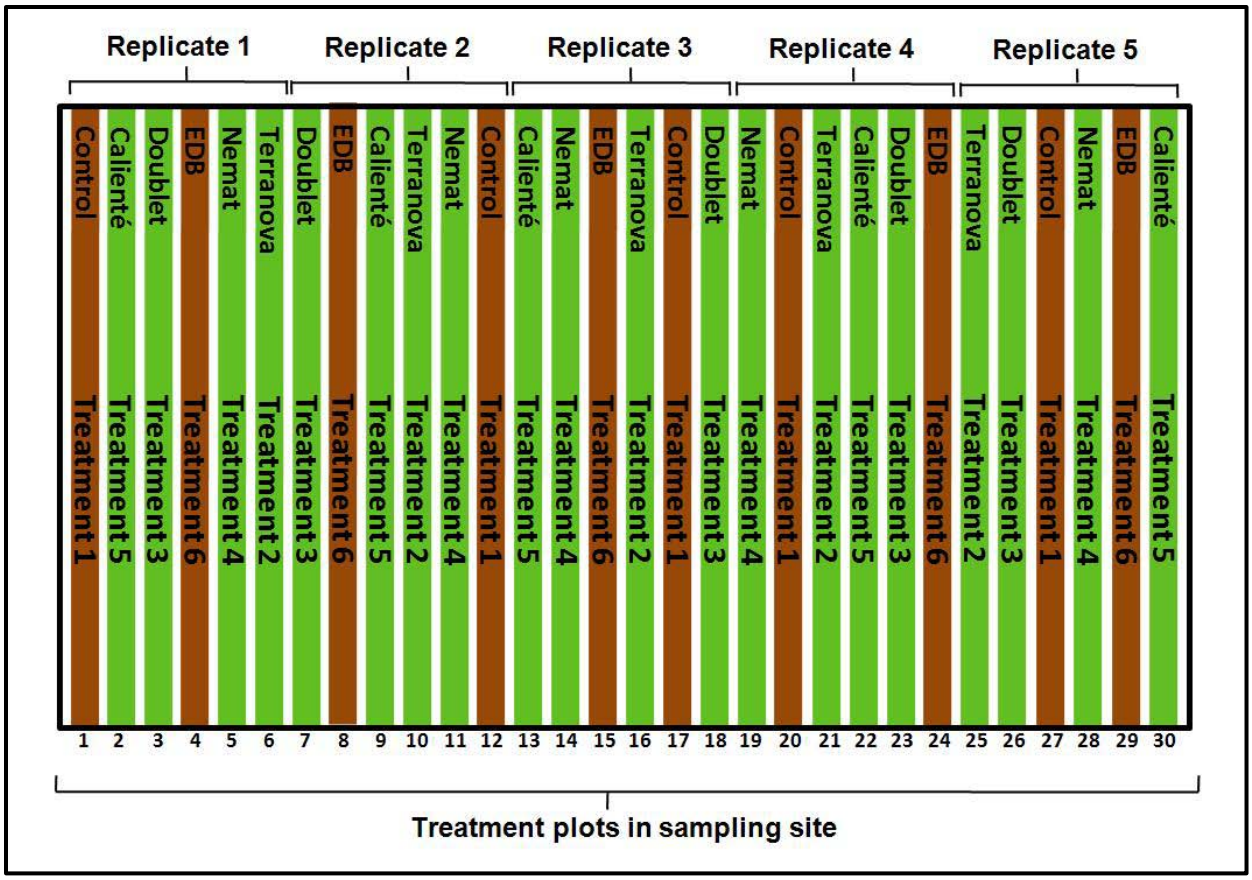


Figure 4.2. The trial layout where the Brassicaceae and potato crops were planted in the Christiana area during the 2010/2011 growing season represented a randomised complete block design (RCBD).

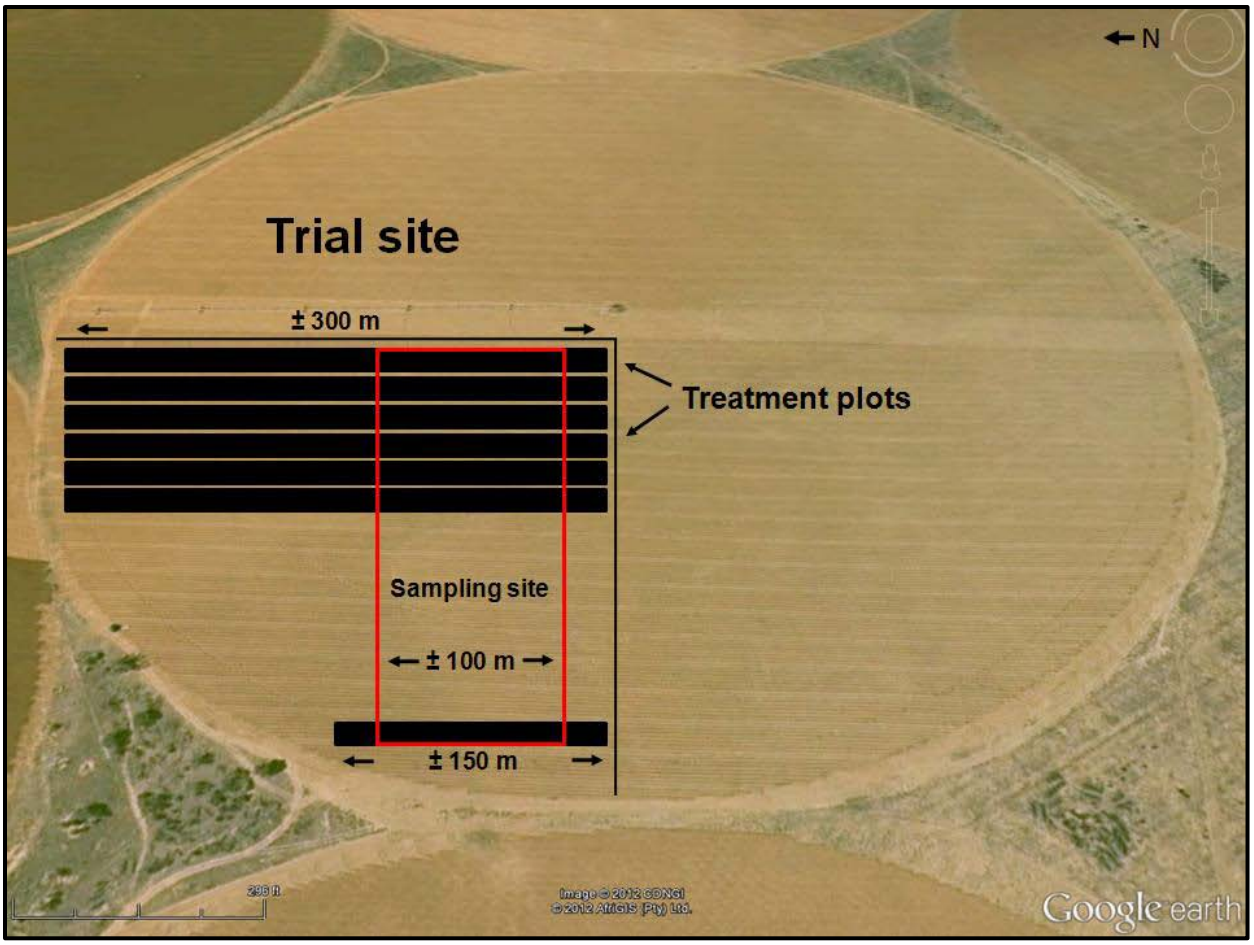


Figure 4.3. An aerial illustration (Google Earth, 2012) of the field where the Brassicaceae and potato crop trial was planted during the 2010/2011 growing season in the Christiana area.

Table 4.1. Nematode sampling dates for four sampling intervals, dates of planting of Brassicaceae and potato crops as well as type of samples collected during the 2010/2011 growing season for the trial planted in the Christiana area.

| Date when general production practices were conducted | General practices conducted for trial purposes | Date of nematode sampling | Type of nematode sample(s) collected |
|---|---|---|---|
| 15 October 2010 | Planting of cover/green manure crops | 15 October 2010 (Sampling interval 1) | Soil samples only |
| 15 November 2010 | Application of fertiliser N:P:K | | |
| | 50% flowering of cover/green manure crops (8 weeks after planting) | 14 December 2010 (Sampling interval 2) | Soil and root/tuber samples |
| 15 December 2010 | Incorporation of aerial parts of cover/green manure crops | | |
| | Six weeks after incorporation of cover/green manure crops | 24 January 2011 (Sampling interval 3) | Soil samples only |
| 29 January 2011 | Application of EDB® on plots reserved for fumigation treatment | | |
| 2 February 2011 | Potato crop planted on all plots of trial site (7 weeks after incorporation of aerial parts of Brassicaceae crops) | | |
| | Tuber initiation of potato crop (seven weeks after planting) | 14 March 2011 (Sampling interval 4) | Soil, roots and tuber samples |

### 4.2.2 Nematode extractions

#### 4.2.2.1 Root (50g and 5g) and tuber (20g) samples

The adapted NaOCl method was used for the extraction of RKN eggs and females from the 50g root sub-samples (see Chapter 2; Paragraph 2.2.3.2).

Nematodes were extracted from 5g root as well as 20g potato tuber sub-samples of each treatment and replicate of both the Brassicaceae and potato crops using the adapted sugar-flotation method (Hooper *et al*., 2005), which has originally been described by (Coolen & D'Herde, 1972) (see Chapter 2; Paragraph 2.2.3.2).

#### 4.2.2.2 Soil samples (200g)

Extraction of nematodes, both PPN and NPN, from 200g of each soil sub-sample collected for each treatment and replicate was done using the adapted decanting and sieving method (Hooper *et al*., 2005), which has originally been described by Cobb, 1918. The latter was followed by the sugar centrifugal-flotation method (Caveness & Jensen, 1955).

- **Decanting and sieving method**

**Principle**

This method is based upon the density and size of nematodes. Soil samples were mixed with water in a 5L bucket, stirred manually using a kitchen spoon and allowed to settle for 30 seconds. During this time about 90% of the nematodes stay in suspension. About 20% of the nematodes originally present in the soil sample will be lost using this method (Cobb, 1918).

**Procedure**

A 200g soil sample was soaked in tap water where soil particles and debris with a diameter of more than 1mm were removed by passing the sample through a 710µm-mesh sieve, which was nested on a 5L plastic bucket. The soil residue on the sieve was washed for about 2 minutes, discarded and the bucket filled up to 5 litres with tap water. The soil within the bucket was then thoroughly mixed with the water and the mixture allowed to settle down for about 30 seconds. The sediment that had settled to the bottom of the bucket was discarded when the dissolved soil-water mixture was decanted through a 25µm-mesh sieve. The nematodes, however, that were contained within the soil-water mixture were left behind on the latter sieve. The entire procedure was repeated again by adding tap water to the sediment present at the bottom of the 5L bucket. The process was repeated as described above until the nematodes and fine soil particles retained on the 25µm-mesh sieve were washed into 50ml centrifuge tubes.

- **Water centrifugation**

The soil within the centrifuge tubes for each sample was centrifuged for 5 minutes with a Relative Centrifugal Force (RCF) of 3000rpm using a Hettich Zentrifugen Roto fix 32 A centrifuge. After centrifugation, the supernatant that contained debris was carefully decanted and discarded. Nematodes were at this stage present at the bottom of the centrifuge tubes due to the centrifugation process. From

this step onwards, the nematodes were subjected to the sugar centrifugal-flotation method for final extraction from each of the soil samples.

- **Sugar centrifugal-flotation method**

**Principle**

This method is based on the specific gravity of nematodes. Terrestrial nematodes have a specific gravity of about 1.08, while that of marine nematodes are approximately 1.13. After the centrifugation process in water that was done during the decanting and sieving method, only organic material with a specific gravity lower than 1 remains in suspension and is discarded from each of the centrifuge tubes. When centrifuged in a sugar solution with a higher specific gravity (1.15) than that of the nematodes, these parasites will remain in suspension and this way being separated from soil particles with a specific gravity larger than 1.15.

**Procedure**

a) A sucrose solution (specific gravity of 1.15) was added to each of the centrifuge tubes containing the nematodes. The sucrose solution was prepared by adding 624g sugar to 1L of tap water.
b) The sugar solution and the sediment containing the nematodes in each of the centrifuge tubes were thoroughly stirred with a spatula and centrifuged for 1 minute at a RCF of 4000rpm. The spatula was rinsed in clean tap water every time after it was used to stir the solution in the separate centrifuge tubes to prevent transfer of nematodes from one sample to the next.
c) After the sugar centrifugation process was completed, the supernatant that contained the nematodes was decanted on a 25µm-mesh sieve and immediately rinsed with tap water. This was done to limit the time that the nematodes were exposed to the sugar solution to less than four minutes. This way plasmolysis of the nematodes was prevented.
d) The nematodes were finally washed into a 100ml plastic sample bottle for counting of RKN J2. For NPN counting and identification to family/genus level were done.

### 4.2.3 Identification of *Meloidogyne* species from the Christiana trial site

#### 4.2.3.1 Morphological identification

- **Identification of *Meloidogyne* females from infected tomato roots**

The *Meloidogyne* sp. present at the trial site was reared *in vivo* to obtain mature females both for morphological and molecular identification (see Chapter 2, paragraph 2.2.6). For morphological identification, galls containing mature females were selected on root fragments of tomato (cv. Rodade) plants. Root tissue was gently forced open with a forceps and scalpel to remove adult females. Each individual female was transversely dissected behind the region where the body was swollen and the body tissues pushed out. The head of each female was transferred to a drop of glycerin/lactophenol on a glass microscope slide. The area on the cuticle containing the perineal pattern was removed and placed on a perspex cutting block in a drop of glycerin/lactophenol with the dome-like posterior end facing upwards. The perineal pattern was trimmed to a flat square and transferred next to the female's head on the microscope slide, exterior side facing upwards (Hartman & Sasser, 1985). Twenty-one heads and perineal patterns of mature females (Kleynhans, 1991) were cut and transferred, next to each other, on a single microscope slide that was sealed with a cover slip and colourless Cutex. This enabled an accurate indication of the sp. or spp. present for this trial site. The slide was properly labelled and sp. identification was done under a light microscope

#### 4.2.3.2 Molecular identification

The same procedure was followed for identification of RKN using molecular techniques as described in Chapter 3, paragraph 3.2.

### 4.2.4 Data analysis

Data for both PPN and NPN were subjected to an analysis of variance (ANOVA) using Statistica Version 10 for Windows. Means was separated using the Tukey Test where $P \leq 0.05$. Nematode data was transformed to $\log_{10}(x+1)$ to remove the inherent variability before analyses. In addition, nematode faunal analyses were performed for NPN as indicators of the food web structure, status and functionality, as well as resource availability (Ferris *et al*., 2001; Ferris & Matute, 2003) at this particular trial site as described in Chapter 2, paragraph 2.4.3.

## 4.3 Results

### 4.3.1 Identification of root-knot nematode (RKN) species

The RKN species present at the Christiana trial site was identified as *M. incognita* using molecular techniques (Zijlstra *et al*., 2000) as well as by using morphological characteristics (Kleynhans, 1991). Morphological identification of this species was also confirmed by Dr. Mariette Marais (Nematode Taxonomist: ARC-PPRI, Pretoria).

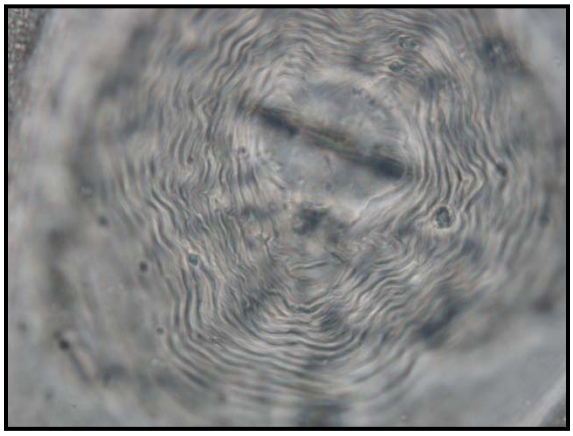

Figure 4.4. A perennial pattern of a mature *M. incognita* female reared *in vivo* from J2 present in soil samples obtained from the site at Christiana where the Brassicaceae and potato trial was planted during the 2010/2011 growing season.

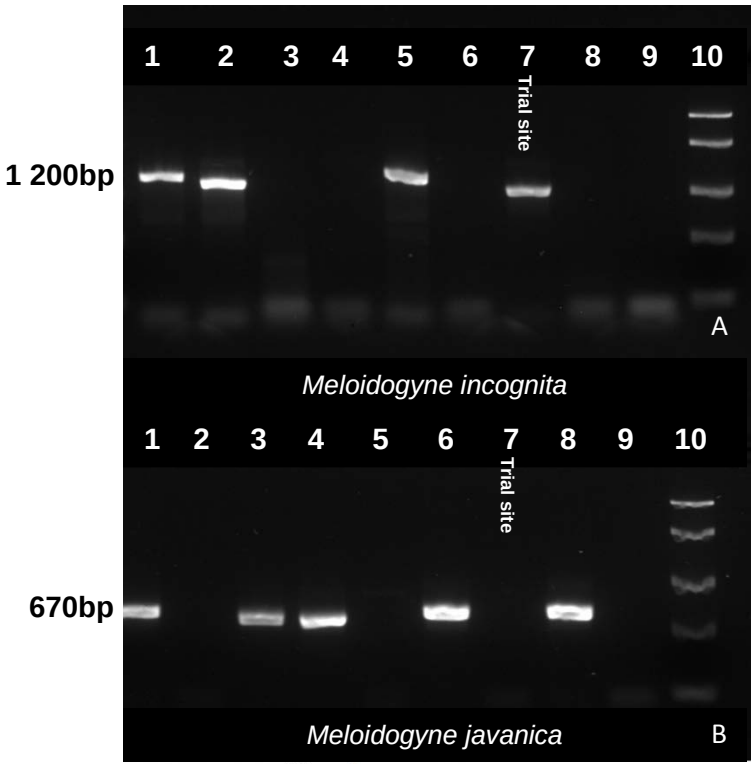


Figure 4.5 (A & B). Amplification products (1 200bp) of mature root-knot nematode females (lanes 2-7), present in fields in the Christiana area where the Brassicaceae trial was conducted during the 2010/2011 growing season, identified by means of the sequence characterised amplified region-polymerase chain reactions (SCAR-PCR) method. [lane 1 (A) = positive standard from *in vivo* reared *Meloidogyne incognita* populations earlier positively identified; lane 1 (B) = positive standard from *in vivo* reared *Meloidogyne javanica* populations earlier positively identified; lane 2 = field 13 (A1), lane 3 = field 16 (B2); lane 4 = field 28 (GH17); lane 5 = field 19 (S5); lane 6 = field 26 (D4); lane 7 = field 14 (A6 – trial site where Brassicaceae trial was conducted); lane 8 = field 5 (T6T5); lane 9 = blank tap water control and lane 10 = 1kb DNA marker].

PCR with the *M. incognita* specific SCAR-primer resulted in amplification of the *M. incognita* 1 200bp SCAR-fragment for the ten mature RKN females that were pooled after they have been obtained from *in vivo*-reared tomato roots (cv. Rodade) that were planted in soil containing RKN eggs and J2 from the site (represented by lane 7; Figure 4.5A) where the trial was conducted. These results agreed with and were substantiated by those for the positive standard used during this part of the study which represented *M. incognita* from a monoculture, *in vivo* reared population.

### 4.3.2 Root and tuber data for Brassicaceae and potato crops

Root (Calienté and Nemat) and tuber (Doublet and Terranova) samples were obtained during 50% flowering of these cover/green manure crops (Sampling interval 2), while root and tuber samples of potato were obtained during tuber initiation of the potato crop (Sampling interval 4). During the other two sampling intervals (1 and 3) no crops were present and only soil samples were collected.

#### 4.3.2.1 Roots and tubers (50g and 5g): Brassicaceae crops

Table 4.2. *Meloidogyne incognita* egg and J2 numbers [$\log_{10}(x+1)$] in root/tuber samples of Brassicaceae crops planted before potato at a trial site in the Christiana area during the 2010/2011 growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Sampling interval | Untreated control (T 1) | Terranova (T 2) | Doublet (T 3) | Nemat (T 4) | Calienté (T 5) | EDB® (T 6) | *P* value | F ratio |
|---|---|---|---|---|---|---|---|---|
| **50g roots/tubers (Brassicaceae crops)** | | | | | | | | |
| **2** | Left fallow | 3.9 (48) | 4.7 (109) | 3.4 (29) | 5.2 (180) | Left fallow | 0.8184 | 0.31 |
| **5g roots/tubers (Brassicaceae crops)** | | | | | | | | |
| **2** | Left fallow | 1.8 (5) | 1.1 (2) | 1.5 (3) | 2.7 (14) | Left fallow | 0.3114 | 1.34 |

Only *M. incognita* J2, were present in root (Calienté and Nemat) and tuber (Doublet and Terranova) samples collected from the various cover/green manure cultivars (Sampling interval 2) planted before a potato crop at this trial site.

For *M. incognita* eggs and J2 numbers/50g roots/tubers collected during sampling interval 2 (50% flowering stage), no significant differences ($P \leq 0.05$) existed between the four cover/green manure crops (Table 4.2). *Meloidogyne incognita* population levels in 50g roots/tubers ranged from 29 (Nemat) to 180 (Calienté). Both the untreated control and EDB® plots were left fallow during this stage.

No significant difference ($P \leq 0.05$) existed among the different Brassicaceae treatments with regard to *M. incognita* J2 numbers/5g roots/tubers for sampling interval 2 (Table 4.2). J2 population levels were,

however, very low and ranged from 2 (Doublet) to 14 (Calienté). Both the control and EDB® plots were left fallow and, therefore, no root/tuber data were obtained during sampling interval 2.

#### 4.3.2.2 Roots (50g and 5g) and tubers (20g): Potato crop

Table 4.3. *Meloidogyne incognita* egg and J2 numbers [$\log_{10}(x+1)$] in root and tuber samples of potato crop planted at a trial site in the Christiana area during the 2010/2011 growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Sampling interval | Untreated control (T 1) | Terranova (T 2) | Doublet (T 3) | Nemat (T 4) | Calienté (T 5) | EDB® (T 6) | *P* value | F ratio |
|---|---|---|---|---|---|---|---|---|
| **50g roots (Potato crop)** | | | | | | | | |
| **4** | 10.2a (26902) | 9.8ab (18032) | 10a (22025) | 10.2a (26902) | 11a (59873) | 8.2b (3639) | 0.0140 | 6.27 |
| **5g roots (Potato crop)** | | | | | | | | |
| **4** | 7a (1095) | 6.2ab (492) | 5.5ab (244) | 5.6ab (269) | 7.7a (2207) | 4.2b (66) | 0.0025 | 5.58 |
| **20g tubers (Potato crop)** | | | | | | | | |
| **4** | 1.7 (4) | 1.5 (3) | 0.9 (1) | 1 (2) | 1 (2) | 2.4 (10) | 0.0600 | 2.59 |

*Meloidogyne incognita* was the only PPN present in root and potato tuber samples obtained during sampling interval 4.

During tuber initiation (Sampling interval 4) of the potato crop *M. incognita* population levels were substantially higher than during the 50% flowering stage (Sampling interval 2) of the Brassicaceae crops for both the 50g and 5g roots (Tables 4.2 & 4.3). No significant difference ($P \leq 0.05$) in egg and J2 numbers/50g roots was, however, evident among the plots where the four cover/green manure crop treatments were planted before the potato crop and the untreated control contained the lowest egg and J2 population levels. EDB® treated plots, however, maintained significantly ($P \leq 0.05$) higher *M. incognita* egg and J2 numbers/50g roots compared to the three Brassicaceae treatments Doublet, Nemat and Calienté as well as the untreated control plots, but not compared to Terranova. Population levels of this RKN species were, however, still high (3 639 eggs and J2/50g roots) for the EDB® treatment. Plots

planted with Calienté had the highest number of eggs and J2 numbers/50g roots (59 873 eggs and J2/50g roots), followed by Nemat (26 902), Doublet (22 025) and Terranova (18 032). Untreated control plots had similar egg and J2 population levels than plots planted with Nemat.

For the 5g root samples, EDB® treated plots maintained significantly ($P \leq 0.05$) less J2/5g roots than the untreated control and Calienté treated plots (Table 4.3). Population levels of *M. incognita* J2 ranged from 66/5g roots (EDB®) to 2 207/5g roots (Calienté).

No significant ($P \leq 0.05$) difference was obtained among the six treatments in terms of *M. incognita* eggs and J2 numbers/20g tubers for sampling interval 4 (Table 4.3). Plots that were treated with EDB® had the highest number of J2/20g tubers (10), Nemat and Calienté (2) and plots that were planted with Doublet (1) had the lowest number of J2/20g tubers.

## 4.4 Soil data

### 4.4.1 Plant-parasitic nematodes (PPN)

PPN that were present in 200g soil samples at this trial site included the endoparasitic *M*. *incognita* as well as *Pratylenchus* spp., semi-endo/ectoparasitic *Helicotylenchus* spp., *Rotylenchus* spp. and *Scutellonema* spp. and ectoparasitic *Criconema* and *Tylenchorhynchus* spp. Since population levels of all these PPN, except those for *M. incognita*, were very low throughout the duration of the trial (Table 1; Appendix 1), only those for *M. incognita* will be discussed further.

Table 4.4. *Meloidogyne incognita* J2 numbers/200g soil samples [$\log_{10}(x+1)$] for four sampling intervals from a trial site in the Christiana area where Brassicaceae crops were planted before potato during the 2010/2011 growing season (means in parenthesis represents the values that were back transformed and not the real means).

| Sampling interval | Untreated control (T 1) | Terranova (T 2) | Doublet (T 3) | Nemat (T 4) | Calienté (T 5) | EDB® (T 6) | *P* value | F ratio |
|---|---|---|---|---|---|---|---|---|
| **1** | 0.7 (2) | 0.3 (1) | 0.3 (1) | 0.3 (1) | 2 (9) | 1.5 (11) | 0.1437 | 1.88 |
| **2** | 1.2a (3) | 0.3ab (0.6) | 0.3ab (0.4) | 0.2b (0.4) | 0.3ab (0.4) | 0.3ab (0.4) | 0.0324 | 3.07 |
| **3** | 1.5a (4) | 0.2b (0.4) | 0.3b (0.6) | 0b | 0.1b (0.2) | 0.4b (0.6) | 0.0001 | 9.01 |
| **4** | 3 (55) | 3 (92) | 1.7 (22) | 1 (5)a | 5 (300)b | 2.4 (20) | 0.0521 | 2.68 |

Although *M*. *incognita* J2 numbers were generally very low in soil samples at this trial site throughout the duration of the trial, it increased substantially in plots of all treatments included from sampling intervals 1 to 4 (Table 4.4).

Significant ($P \leq 0.05$) differences were only evident among the various treatments during sampling intervals 2 and 3, with the untreated control containing significantly ($P \leq 0.05$) lower J2 numbers/200g soil than those for Nemat and the other treatments included (the three Brassicaceae cultivars as well as EDB®).

### 4.4.2 Non-parasitic nematodes (NPN)

Table 4.5. Functional guilds, to which non-parasitic nematode genera/families (identified in soil samples from the Christiana trial site where Brassicaceae and potato crops were planted), were assigned according to their feeding habits and life stage characteristics (Bongers, 1990).

| Genus | Guild |
|---|---|
| ***Cephalobus*** | $Ba_1$ |
| ***Cruznema*** | $Ba_1$ |
| ***Diploscapter*** | $Ba_1$ |
| ***Mesorhabditis*** | $Ba_1$ |
| ***Panagrolaimus*** | $Ba_1$ |
| ***Rhabditis*** | $Ba_1$ |
| ***Acrobeles*** | $Ba_2$ |
| ***Acrobeloides*** | $Ba_2$ |
| ***Chiloplacus*** | $Ba_2$ |
| ***Eucephalobes*** | $Ba_2$ |
| ***Aphelenchus*** | $Fu_2$ |
| ***Aphelenchoides*** | $Fu_2$ |
| ***Tylenchus*** | $Fu_2$ |
| **Doryliamidae** | $Om_4$ |

ba: bacterivores, fu: fungivores, om: predator (omnivore/carnivore). Suffix numbers represent cp values for the various taxa.

NPN present at this trial site were categorised into different non-parasitic nematode guilds, representing bacterivores (cp 1&2), fungivores (cp2) and omnivores [cp4 (Table 4.5)].

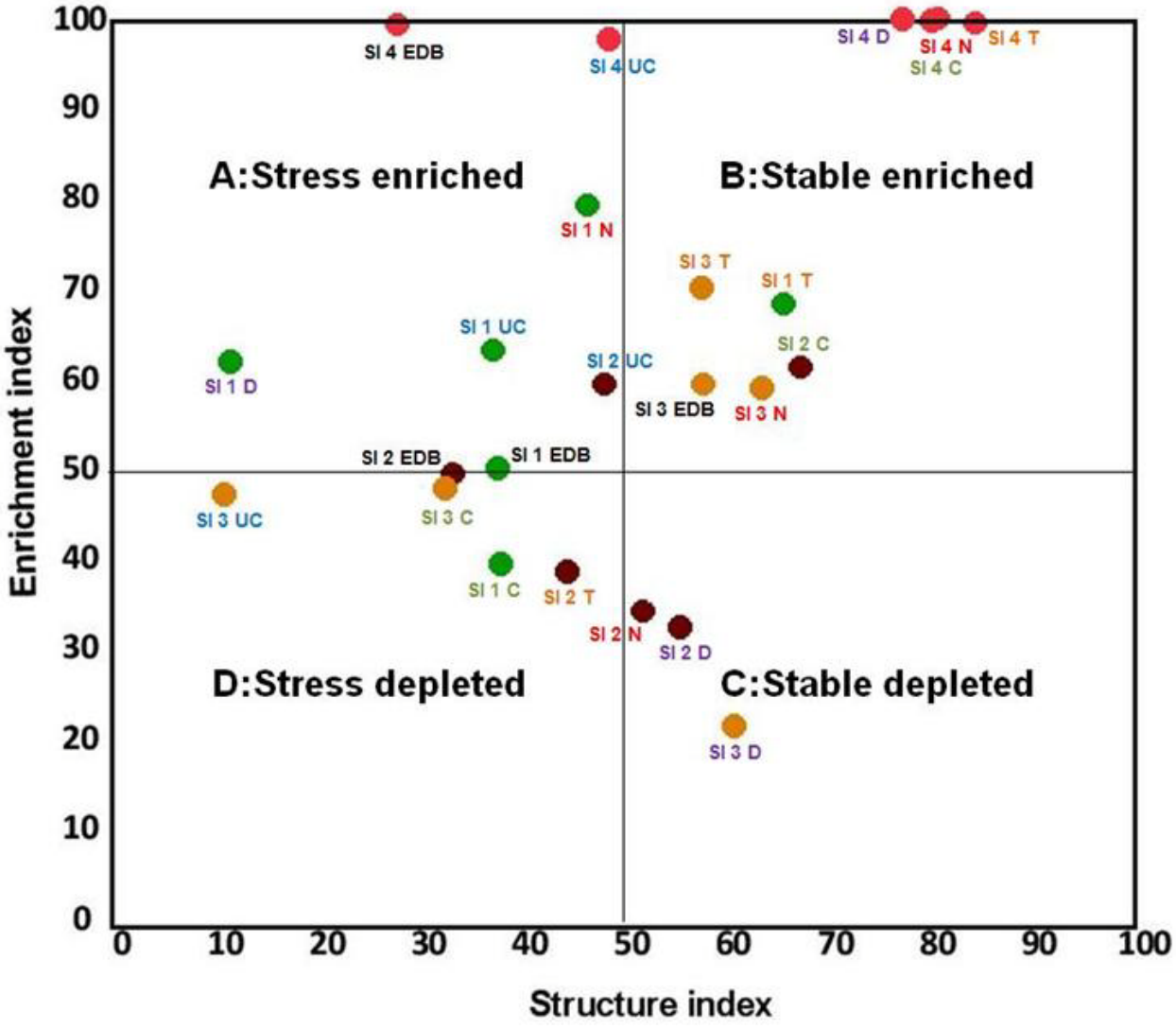


Figure 4.6. Enrichment (EI) and structural indices (SI) of non-parasitic nematode (NPN) assemblages for four sampling intervals for the various treatments applied at a trial where Brassicaceae and potato crops were planted in the Christiana area during the 2010/2011 growing season.

Categorisation of both the different treatments and sampling intervals included in the Brassicaceae and potato trial in the Christiana area differed substantially with regard to its placement in the simplified food web (Figure 4.6). These results substantiate the typical spot occurrence of nematodes in soils (Been & Schomaker, 2006) and represent the inherent variation in nematode communities in terms of their distribution before the trial commenced.

- **Sampling interval 1 (before commencement of the trial)**

NPN assemblages in soil samples obtained from plots designated for Doublet, Nemat and, untreated control treatments obtained during the first sampling interval 1 (indicated by green dots) were generally categorised within the "Stress Enriched" quadrant (Figure 4.6), which represents a disturbed food web condition (Ferris *et al*., 2001). Those obtained for plots designated for Terranova were plotted in the "Stable Enriched" quadrant, indicating a maturing food web while those designated for Calienté were plotted in the "Stress Depleted" quadrant that represents a degraded food web. Plots designated for EDB® applications were shared between the "Stress Enriched" and "Stress Depleted" quadrants and are thus classified as disturbed and/or degraded.

Soil, NPN communities for plots designated for planting of Doublet, untreated control and Nemat were dominated by $Ba_1$ on the Enrichment Trajectory (medium-high EI), while those for Nemat plots were dominated by $Fu_2$ (medium EI) (Figure 4.6). On the Structure Trajectory plots designated for Doublet treatments were dominated by $Ca_2$, $Ca_3$, $Fu_3$ and $Ba_3$ (low SI). Plots designated for Nemat, untreated control and EDB® plots were, however, dominated by $Om_4$, $Ca_4$, $Fu_4$ and $Ba_4$ (medium SI) in terms of the Structure Trajectory.

Soil, NPN communities for plots designated for planting Terranova were dominated by $Ba_1$ (medium-high EI) and $Om_4$, $Ca_4$, $Fu_4$ and $Ba_4$ (medium-high SI) in terms of the Structure Trajectory (Figure 4.6). Plots designated for planting Calienté were, however, dominated by $Fu_2$ (low-medium EI) on the Enrichment Trajectory and $Om_4$, $Ca_4$, $Fu_4$ and $Ba_4$ (low-medium SI) on the Structure Trajectory.

- **Sampling Interval 2 (50% flowering of the Brassicaceae crops)**

During the second sampling interval (indicated by brown dots) changes were evident in terms of categorisation of the various treatments in terms of their NPN assemblages regarding their food web status (Figure 4.6). Food web conditions for EDB® (medium EI and low-medium SI) and untreated control (medium-high EI and medium SI) plots generally remained disturbed ("Stress Enriched"; quadrant A), while those for Doublet improved from disturbed ("Stress Depleted"; quadrant A) with high EI but low SI to structured ("Stable Depleted"; quadrant C) with low-medium EI but medium-high SI. Food web conditions for plots planted with Calienté improved from degraded ("Stress Depleted"; quadrant D) with low-medium EI and low SI to maturing ('Stable Enriched"; quadrant B) with medium-high EI and SI. However, plots planted with Terranova changed from maturing food web conditions

("Stable Enriched"; quadrant B) with high EI and medium-high SI to degraded ("Stress Depleted; quadrant D) with low-medium EI and SI. Sampling interval 3 (eight weeks after incorporation of aerial parts of Brassicaceae cultivars – indicated by orange dots)

- **Sampling interval 3 (incorporation of cover/green manure crops)**

During the third sampling interval (indicated by orange dots) changes in terms of food web conditions were again observed for the six treatments (Figure 4.6). Plots designated for EDB® treatments changed from disturbed (medium EI and low-medium SI)] to maturing (medium-high EI and SI) food web conditions, while those for the untreated control changed from disturbed (medium to high EI and medium SI) to degraded (medium EI and low SI). Food web conditions for Doublet remained structured but decreased in terms of its EI and increased in terms of its SI. Those for Calienté changed from matured (medium-high EI and SI) to degraded (medium EI and low-medium SI). For Nemat, soil food web conditions changed from structured (low-medium EI and medium SI) to mature (medium-high EI and SI) and for Terranova from degraded (low-medium EI and SI) to matured (medium-high EI and SI).

- **Sampling interval 4 (tuber initiation of potato crop)**

For the fourth sampling interval (indicated by red dots), soil food web conditions for all four Brassicaceae treatments were indicated as maturing (high EI and medium-high SI), while those for EDB® (high EI and low-medium SI) and untreated control (high EI and medium SI) plots were indicated as disturbed (Figure 4.6).

In general a substantial improvement in terms of soil food web conditions for three of the four Brassicaceae cultivars from disturbed (for Doublet and Nemat) and degraded (for Calienté) were evident from Sampling Intervals 1-4. In contrast, soil food web conditions for the EDB® and untreated control plots, except that the EI of both the latter treatments increased during the duration of the experiment, generally remained in disturbed quadrant. While an increase in $Ba_1$ was the main trend visible for both the latter treatments during the duration of this trial, the same were evident for the four Brassicaceae treatments as well as in increase in $Om_4$, $Ca_5$, $Fu_5$ and $Ba_5$.

## 4.5 Discussion

Second-stage juveniles (J2) of the predominant endoparasitic species *Meloidogyne incognita* present in soil at the Christiana trial site were able to infect and develop to mature, egg-producing females in roots/tubers of all four of the Brassicaceae cultivars evaluated during this study. The build-up of relatively high population levels of this local *M. incognita* population in tubers of the two oilseed radish (Doublet and Terranova) and roots of the two mustard (Calienté and Nemat) cultivars at this trial site was not expected. Various reports (Mojtahedi *et al*., 1993; Kirkegaard & Sarwar, 1998; Morra & Kirkegaard, 2002; Zasada *et al*., 2003; Lazzeri *et al*., 2004; Curto *et al*., 2005) suggested that Brassicaceae crops generally are poor hosts to PPN, particularly *Meloidogyne* spp., which was not the case in this study. Furthermore, the four Brassicaceae cultivars used in this study were unable to reduce *M. incognita* population densities at the Christiana trial site by means of their biofumigation effects. The latter trend was reflected in the high population levels of this RKN sp. in roots of a follow-up potato crop sampled during tuber initiation. At this stage the approach towards the objective of the Brassicaceae trial had to be changed and a registered synthetic nematicide was applied to limit potential RKN damage to potato tubers and this way minimise quality losses. Although it is not normal practice for producers to plant potato during successive growing seasons on the same field, it was only done for the purpose of this study, as mentioned earlier.

In addition to data obtained on the plant-parasitic species *M. incognita* during this study, novel information about the NPN assemblages and their substantial changes in terms of community structures as a result of Brassicaceae amendments were gathered for annual crops, in this case potato. Previous studies in SA in this regard for PPN and NPN assemblages have only been done on perennial crops such as sugar cane and vineyard (Berry & Wiseman, 2003; Berry & Rhodes, 2006; Kruger *et al.*, 2011) as well as for common scab on seed potatoes (Gouws & Wehner, 2004). Valuable information, indicating pronounced shifts of NPN assemblages in plots where the four Brassicaceae cultivars were planted from the “Stress Depleted” (degraded) and/or “Stressed Enriched” (disturbed) to the “Stable Enrich” (maturing) quadrants obtained during this study indicate the positive effect of these crops in terms of soil health.

In terms of the maintenance of *M. incognita* in roots/tubers of Brassicaceae spp. used all four cultivars generally maintained relatively high RKN eggs and J2. Although cv. Nemat maintained the lowest egg and J2 numbers, it did not differ significantly from the other three cultivars. In addition, crop roots of the follow-up potato crop planted in plots seven weeks after aerial plant parts of cv. Nemat were

incorporated maintained similar, very high *M. incognita* population levels compared to those in plots where the other three Brassicaceae cultivars were planted and their aerial parts incorporated. Lazerri *et al*. (2004) and Curto *et al.* (2005), however, reported that cv. Nemat was a poor host to the *M. incognita* populations used in their studies.

Results obtained in terms of the host suitability and poor biofumigation effects of Brassicaceae species evaluated during this study with regard to the local *M. incognita* population is in contradiction with those by Kirkegaard and Sarwar (1998), Morra and Kirkegaard (2002), Zasada *et al*. (2003), Lazzeri *et al*. (2004) and Curto *et al.* (2005). The latter authors evaluated different Brassicaceae species, namely *E. sativa*, *B. juncea* and *R. sativus* for their high active GL content and biocidal activity against *M. incognita* J2. All varieties screened during the latter studies were recommended for use in pest management programmes based on their ability to reduce *M. incognita* infestation levels in the soil due to their high GL content and subsequent good nematicidal activity. Mojtahedi *et al*. (1993) also reported that rapeseed of cultivar Jupiter (*Brassica napus*) was effective in reducing *M. chitwoodi* population densities on field-grown potato.

However, other studies in which the effects of Brassicaceae species on *M. incognita* were evaluated were similar to results obtained during this study. For example, Monfort *et al*. (2007) found that *M. incognita* was capable of reproducing on a majority of the Brassicaceae species they screened. Liébanas and Castillo (2004) further reported that all the Brassicaceae varieties they tested were hosts to *M. arenaria, M. incognita* and *M. javanica*. Also, Johnson *et al.* (1992) found no significant reduction of nematode numbers after incorporation of rapeseed green manures to control *M. incognita* and *M. javanica* on squash (*Cucurbita pepo*) in Georgia, North America.

Furthermore, studies with *M. javanica* with regard to various Brassicaceae species suggested that although Brassicaceae crops are hosts to these RKN, mechanism(s) responsible for their poor host status become effective only after invasion by J2 (McLeod *et al*., 2001). Although Brassicaceae crops are hosts to RKN, they are poorly invaded by J2 and suppress their concurrent development, reducing their risk to increase in numbers in roots/tubers of Brassicaceae crops before their incorporation into soil as green manures (McLeod *et al*., 2001). The latter scenario did not seems to be true for the four Brassicaceae cultivars used during this study since relative high populations levels, except for cv. Nemat, were recorded for the *M. incognita* population in roots/tubers at 50% flowering of these crops. In addition, the high *M. incognita* population pressure that existed at the Christiana trial site is expected to have contributed to the poor performance of these cultivars in terms of their inability to reduce

population levels of these parasites during this study in such a short period of time. Another possible hypothesis is that the persistence and the activity of some soil micro-organisms, of which no information was obtained during this study, could have played an additional role in isothiocynate loss or degradation of the Brassicaceae cultivars (Rumberger & Marschner, 2003). Also, green manure cultivars that have high leaf concentrations of GL are needed before the potential offered by Brassicaceae crops in terms of reducing economically important PPN are fully realized (McLeod & Steel, 1999). GL concentrations for the four cultivars used in this study as well as that of other Brassicaceae cultivars that have potential as cover/green manure crops to be used under local conditions should be determined to indentify superior ones in this regard. It should also be noted that for future research, more than one incorporation of aerial amendments of Brassicaceae crops should be considered and that trials should be conducted over longer periods of time and over more than one growing season using different planting densities of different Brassicaceae crops, in order to better conclude about their efficacy in terms of biofumigation. The latter reasons may be responsible for the lack/reduced nematicidal activity of the four Brassicaceae cultivars used against the local *M. incognita* during this study. These recommendations are supported by Zasada and Ferris (2004) who reported that suppression of different PPN species is dependent on the rate of green manure application in soil as well as Brassicaceae GL concentrations within aerial plant parts.

In terms of the NPN assemblages, pronounced changes in terms of their categorisation within the simplified soil food web (Bongers & Ferris, 1999; Ferris *et al*., 2001) were evident for plots where the Brassicaceae cultivars were planted compared to those treated with EDB® as well as the untreated control ones. However, although the diversity of NPN increased at this trial site as a result of the amendment of Brassicaceae crops, population levels of *M. incognita* in roots of potato at tuber initiation were still high in all plots including Brassicaceae-amended ones. In general results for soil, NPN assemblages gained during this study showed that substantial increases in the diversity (genera and/or families) and population levels of particularly bacterial- and fungus-feeding NPN were evident for all six treatments during the duration of the trial. The presence of enrichment and general opportunistic NPN guilds, representing bacterivores, carnivores and fungivores with cp values of 1 & 2 thus resulted in high EI for all treatments at the end of the Brassicaceae trial (Sampling Interval 4). However, higher NPN diversity represented by guilds with cp values (3-5) higher than 2 was also present in Brassicaceae-amended plots.

The predominant bacterivores ($Ba_1$) that were present in EDB® as well as the untreated control plots are regarded as entry-level indicators of enrichment, multiplying rapidly by feeding on other soilborne

micro-organisms that decompose organic matter (Ferris *et al*., 2001). Predominance by $Ba_1$ communities in particular resulted in the increased and high EI for the latter treatments during fourth sampling interval. The presence of enrichment and general opportunistic NPN guilds, representing bacterivores, carnivores and fungivores with cp values of 1 & 2 thus resulted in high EI for all treatments at the end of the Brassicaceae trial (Sampling Interval 4). However, higher NPN diversity represented by guilds with cp values (3-5) higher than 2 was also present in Brassicaceae-amended plots, indicating the extent to which decomposition of organic material present in soil has occurred (Ferris & Bongers, 2006; Ferris & Bongers, 2009). Disruption of the soil as a result of cultivation practices could have contributed to the latter scenario for these treatments. The absence of NPN guilds with cp values ranging between 3 and 5 in EDB® and untreated control plots, however, indicated that soil food webs in these plots were disturbed and unstable (Ferris *et al*., 2001; Ferris & Bongers, 2009).

The concurrent occurrence of bacterivores, fungivores, carnivores as well as omnivores with higher cp values (4-5) in Brassicaceae-amended plots indicates that the NPN communities in the latter plots benefited from and responded positively to such amendments. Although enrichment opportunists ($Ba_1$) built up and had high EI values for all Brassicaceae-amended plots during sampling interval 4, general opportunists ($Fu_2$) that are not necessarily dependent on the same resources were replenished by persisters during further succession. The latter represented bacterivores, fungivores, carnivores and omnivores with cp values ranging between 3-5. Amendment using the four Brassicaceae crops thus resulted in an increase in the diversity of the latter NPN guilds, resulting in soil food webs being stable, complex, structured and mature (Ferris *et al*., 2001; Ferris & Bongers, 2009). The pronounced shifts in NPN community structures in Brassicaceae-amended plots obtained during this study coincide with those from other studies where the effects of Brassicaceae amendments were evaluated on NPN assemblages in soil environments (Osler *et al*., 2000; Stirling & Stirling, 2003).

NPN nematodes are a food source for natural enemies, representing other soilborne micro-organisms such as bacteria, fungi, mites, protozoa and others, of nematodes (Stirling, 1991). These NPN assemblages play an important role in nutrient cycling and plant nutrition (Ingham *et al*., 1985; Griffiths, 1994). Therefore, information gained on improvement of soil health using Brassicaceae amendments during this study is important, particularly in SA where no official reports on this topic has been published, from a soil health perspective. It also serve as a reminder that as we strive to increase the efficacy of biofumigation, we need to conserve beneficial organisms, which is an achievable goal (Stirling & Stirling, 2003). Since NPN nematodes are less sensitive than PPN to the toxins produced by Brassicaceae crops (Halbrendt & Jing, 1996) and because their generation times are relatively short,

populations tend to recover quickly following treatment with crop residues of Brassicaceae species that have nematicidal characteristics (Sturz & Kimpinski, 1999). In addition, proof that organisms such as PPN differ markedly in their sensitivity to ITC's (Sarwar *et al*., 1998) and that GL profiles vary between Brassicaceae species and are influenced by the environment (Kirkegaard & Sarwar, 1998; Sarwar & Kirkegaard, 1998), suggests that it may eventually be possible to maximise the effect of Brassicaceae amendments on key pathogens such as RKN, while minimising their impact on non-target, beneficial species (Stirling & Stirling, 2003).

### 4.6 Conclusions

According to the authors' knowledge, this is the first study on the effects of Brassicaceae crops both for PPN (*M. incognita* in this case) and NPN on potato in SA. Although none of the Brassicaceae crops used in this study resulted in a significant reduction of population levels of *M. incognita* (predominant PPN) in roots and/or tubers of Brassicaceae and potato crops, pronounced improvement in terms of soil health has been demonstrated using categorisation of NPN assemblages according to a simplified food web. To optimise the effect of Brassicaceae crops in potato-based cropping systems in SA, the following recommendations are proposed:

- Prevent the build-up of RKN to very high population levels in potato fields before planting of a green manure crops such as Brassicaceae.
- Screening of all Brassicaceae cultivars available in SA for their host status to various economically important RKN that occur in local potato-producing areas.
- Experimenting with higher cover crop planting densities.
- Slashing and incorporation of aerial parts should be done more than once during the growth period.
- Similar trials should be conducted over a longer period during which cover crops are planted on a more frequent basis.
- Glucosinolate content should be determined for local Brassicaceae crops.
- Combination of biofumigation with other environmentally-friendly nematode control strategies, such as soil solarisation, steam sterilisation, natural nematicidal products.

It is the ultimate goal not to completely eliminate chemicals from nematode management programs, but rather to move towards "softer", more environmentally friendly or even perhaps biological products to ensure that farmers would have alternative management strategies in place as the more toxic, synthetic chemicals are removed from markets.

## 4.7 References


Akiew, S., Trevorrow, P.R. & Kirkegaard, J.A. 1996. Mustard green manure reduces bacterial wilt. *Bacterial Wilt Newsletter* 13: 5-6.

Been, T.H. & Schomaker, C. H. 2006. Distribution patterns and sampling. In: Perry, R.N. & Moens, M. (eds) Plant nematology. CABI Publishing, Wallingford, UK. pp. 302-326.

Berry, S.D. & Rhodes, R. 2006. Green manure crops: agronomic characteristics and effect on nematodes. *Proceedings of the South African Sugar Technologists Association* 80: 269-273.

Berry, S.D. & Wiseman, E.J. 2003. The effect of green manure crops on plant parasitic nematodes in the South African sugar industry. *Proceedings of the South African Sugar Technologists Association* 77: 114-117.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83: 14-19.

Bongers, T. & Ferris, H. 1999. Nematode community structure as a bio-indicator in environmental monitoring. *Trends in Ecology and Evolution* 14: 224-228.

Brown, P.D. & Morra, M.J. 1995. Glucosinolate-containing plant tissues as bioherbicides. *Journal of Agricultural Food Chemistry* 43: 3070-3074.

Brown, P.D. & Morra, M.J. 1997. Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy* 61: 167-231.

Caveness, F.E. & Jensen, H.J. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs in soil. *Proceedings of the Helminthological Society of Washington* 22: 87-89.

Chan, M.K.Y. & Close, R.C. 1987. *Aphanomyces* root rot of peas. 3. Control by the use of cruciferous amendments. *New Zealand Journal of Agricultural Research* 30: 225-233.

Cobb, N.A. 1918. Estimating the nema population of the soil. Agricultural Technology Circular Bureau Plant Industry, United States Department of Agriculture. No.1. 48 pp.

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

CropLife, 2011. Agricultural remedies database. http://www.croplife.co.za/CroplifeSAInitiatives.aspx?AgriculturalRemediesDatabase=true (Accessed on: 20 February 2012).

Curto, G., Dallavalle, E. & Lazzeri, L. 2005. Life cycle duration of *Meloidogyne incognita* and host status of Brassicaceae and Capparaceae selected for glucosinate content. *Nematology*, 7: 203-212.

Ferris, H. & Bongers, T. 2006. Nematode indicators of organic enrichment. *Journal of Nematology* 38: 3-12.

Ferris, H. & Bongers, T. 2009. Indices developed specifically for analysis of nematode assemblages. In: Wilson, M.J. & Kakouli-Duarte, T. (eds) Nematodes as environmental indicators. pp. 124-145.

Ferris, H., Bongers, T. & De Goede, R.G.M. 2001. A framework for soil food web diagnostics: extension of nematode faunal analysis concept. *Applied Soil Ecology* 18: 13-29.

Ferris, H. & Matute, M.M. 2003. Structural and functional succession in the nematode fauna of a soil food web. *Applied Soil Ecology* 23: 93-110.

Gouws, R. & Mienie, N.J.J. 2000. Biofumigation of common scab of potatoes in the Republic of South Africa. In: Williams, C.M. & Walters, L.J. (eds) Potatoes 2000 "Linking research to practice". Australian Potato Research, Development and Technology Transfer Conference, 31 July – 3 August 2000, Adelaide, South Australia. pp. 261-263.

Gouws, R. & Wehner, F. C. 2004. Biofumigation as alternative control measure for common scab on seed potatoes in South Africa. *Agroindustria* 3: 5-8.

Griffiths, B.S. 1994. Microbial-feeding nematodes and protozoa in soil: their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. *Plant and Soil* 164: 25-33.

Halbrendt, J.M. 1996. Allelopathy in the management of plant-parasitic nematodes. *Journal of Nematology* 28: 8-14.

Halbrendt, J.M. & Jing, G.N. 1996. Cruciferous green manure as an alternative to nematicide: the effect of glucosinolate content. In: Princen, L.H. & Rossi, C. (eds) Proceedings of the Ninth international Conference on Jojoba and its Uses and the third International Conference on New Industrial Crops and Products. pp. 458-465.

Hartman, K.M. & Sasser, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential host treatise and perineal-pattern morphology. In: Barker, K.R., Carter, C.C. & Sasser, J.N. (eds) Advanced treatise on *Meloidogyne*. Volume II. Methodology. North Carolina University Graphics, Raleigh, North Carolina, USA. pp. 69-78.

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

Ingham, R.E., Trofymow, J.A., Ingham, E.R. & Coleman, D.C. 1985. Interactions of bacteria, fungi, and their nematode grazers on nutrient cycling and plant growth. *Ecological Monographs* 55: 119-140.

Johnson, A.W., Golden, A.M., Auld, D.L. & Sumner, D.R. 1992. Effects of rapeseed and vetch as green manure crops and fallow on nematodes and soil-borne pathogens. *Journal of Nematology* 24: 117–126.

Kirkegaard, J.A. & Sarwar, M. 1998. Biofumigation potential of brassicas. I. Variation in glucosinolate profiles of diverse field-grown brassicas. *Plant and Soil* 201: 71-89.

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

Kruger, N., Fourie, J. & Malan, A. 2011. The role of cover crops in suppressing plant-parasitic nematodes in vineyards. 20th Symposium of the Nematological Society of Southern Africa. Stellenbosch, 15-18 May 2011. (Oral presentation).

Lazzeri, L., Leoni, O., Bernardi, R., Malaguti, L. & Cinti, S. 2004. Plants, techniques and products for optimising biofumigation in full field. *Agroindustria* 3: 281-288.

Liébanas, G. & Castillo, P. 2004. Host suitability of some crucifers for root-knot nematodes in southern Spain. *Nematology* 6: 125-128.

Mathiessen, J.N. & Kirkegaard, J.A. 1998. Biofumigation for managing soil-borne pests: Progress, pitfalls and prospects. In: Zalucki, M.P., Drew, R. & White G.G. (eds) Proceedings of the 6th Australasian Applied Entomological Research Conference, Brisbane, Australia. pp. 364-372.

McLeod, R.W., Kirkegaard, J.A. & Steel, C.C. 2001. Invasion, development, growth and egg laying by *Meloidogyne javanica* in Brassicaceae crops. *Nematology* 3: 463-472.

McLeod, R.W. & Steel, C.G. 1999. Effects of brassica-leaf manures and crops on activity and reproduction of *Meloidogyne javanica*. *Nematology* 1: 613-624.

Mojtahedi, H., Santo, G.S., Hang, A.N. & Wilson, J.H. 1991. Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. *Journal of Nematology* 23: 170-174.

Mojtahedi, H., Santo, G.S., Wilson, J.H. & Hang, A.N. 1993. Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. *Plant Disease* 77: 42-46.

Monfort, W.S., Csinos, J., Desaeger, K., Seebold, K., Webster, T.M. & Diaz-Perez, J.C. 2007. Evaluating *Brassica* species as an alternative control measure for root-knot nematode (*M. incognita*) in Georgia vegetable plasticulture. *Crop Protection* 26: 1359-1368.

Morra, M.J. & Kirkegaard, J.A. 2002. Isothiocyanate release from soil-incorporated Brassica tissues. *Soil Biology and Biochemistry*. 34: 1683-1690.

Nel, A, Krause, M. & Khelawanlall, N. 2007. A guide for the control of plant pests. 39th Edition. Department of Agriculture, Republic of South Africa. Government Printer, Pretoria. 116 p.

Noling, J.W. 2002. The practical realities of alternatives to methyl bromide: concluding remarks. *Phytopathology* 92: 1373-1375.

Nyczepir, A.P. & Thomas, S.H. 2009. Current and future management strategies in intensive crop production systems. In: Perry, R.N. Moens, M. & Starr, J.L. (eds) Root-knot nematodes. CAB International, Wallingford, UK. pp. 412-443.

Osler, G.H.R., Van Vliet, P.C.J., Gauci, C.S. & Abbott, L.K. 2000. Changes in free living soil nematode and micro-arthropod communities under a canola-wheat-lupin rotation in Western Australia. *Australian Journal of Soil Research* 38: 47-59.

Potter, M.J., Davies, K. & Rathjen, A. 1998. Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematodes (*Pratylenchus neglectus*). *Journal of Chemical Ecology* 24: 67-80.

Rosa, E.A.S., Heaney, R.K., Fenwick, G.R. & Portas, C.A.M. 1997. Glucosinolates in crop plants. *Horticultural Reviews* 19: 99-215.

Rumberger, A. & Marschner, P. 2003. 2-Phenylethylisothiocianate concentration and microbial community composition in the rhizosphere of canola. *Soil Biology and Biochemistry* 35: 445-452.

Sarwar, M. & Kirkegaard, J.A. 1998. Biofumigation potential of brassicas. II. Effect of environment and ontogeny on glucosinolate production and implications for screening. *Plant and Soil* 201: 91-101.

Sarwar, M., Kirkegaard, J.A., Wong, P.T.W. & Desmarchelier, J.M. 1998. Biofumigation potential of brassicas. III. *In vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant and Soil* 201: 103-112.

Schneider, S.M., Rosskopf, E.N., Leesch, J.G., Chellemi, D.O., Bull, C.T. & Mazzola, M. 2003. United States Department of Agriculture-Agricultural Research Service research on alternatives to methyl bromide: pre-plant and post-harvest. *Pest Management Science* 59: 814-826.

Stirling, G.R. 1991. Biological control of plant-parasitic nematodes. CAB International, Wallingford, UK. 282 p.

Stirling, G.R. & Stirling, A.M. 2003. The potential of *Brassica* green manure crops for controlling root-knot nematode (*Meloidogyne javanica*) on horticultural crops in a subtropical environment. *Australian Journal of Experimental Agriculture* 43: 623-630.

Sturz, A.V. & Kimpinski, J. 1999. Effects of fosthiazate and aldicarb on populations of plant-growth-promoting bacteria, root-lesion nematodes and bacteria-feeding nematodes in the root zone of potatoes. *Plant Pathology* 48: 26-32.

UNEP (United Nations Environment Programme). 1992. Montreal Protocol Assessment. Methyl Bromide: its atmospheric science, technology and economics; Synthesis report of the methyl bromide interim scientific assessment and methyl bromide interim technology and economic assessment; United Nations: New York, p. 41.

Uppstrom, B. 1983. Glucosinolate pattern in different growth stages of high and low glucosinolate varieties of *Brassica napus. Sveriges tsadesforenings Tidskrift* 93: 331-336.

Zasada, I.A. & Ferris, H. 2004. Nematode suppression with brassicaceous amendments: Application based upon glucosinolate profiles. *Soil Biology and Biochemistry* 36: 1017-1024.

Zasada, I.A., Ferris, H., Elmore, C.L., Roncoroni, J.A., MacDonald, J.D., Bolkin, L.R. & Yakabe, L.E. 2003. Field applications of brassicaceous amendments for control of soilborne pest and pathogens. Plant Health Progress doi: 10.1094/PHP-2003-1120-01-RS.

Zijlstra, C., Donkers-Venne, D.T.H.M. & Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2: 847-853.

# CHAPTER 5

# GENERAL CONCLUSIONS AND FUTURE PROSPECTS

## 5.1 Conclusions

The two nematode surveys conducted during the 2010 and 2011 growing seasons resulted in one plant-parasitic nematode (PPN) family, 13 genera and 16 nematode species being identified from both root and soil samples from the 31 fields in which potato is included as a rotation crop every eight years in the Christiana area of South Africa (SA). Other rotation crops included in these fields are maize, sunflower, onion, as well as grass cover crops. Root-knot nematode (RKN) species were the predominant genera with two species being identified using the molecular SCAR-PCR method, namely *M. incognita* and *M. javanica*. These two species generally also are the predominant ones, which cause problems to other rotation crops included in potato fields in the Christiana area. Results obtained during this study substantiated data that RKN are widely distributed in the Christiana potato-production area of SA where it causes severe damage to the crop. Furthermore, it is also in agreement with reports that RKN have a wide host range and are considered economically one of the most important PPN genera that are locally associated with crops planted in rotation with potato such as maize, sunflower, onion, as well as grasses and weeds.

In terms of the other economically important endo- and semi endo-/ecto- as well as ectoparasitic nematodes present in the 31 fields sampled, *Pratylenchus* spp. generally ranked second with individuals of the Hoplolaimidae and Trichodoridae families following. *Ditylenchus* spp. and J2 individuals of the Heteroderidae family (other than *Meloidogyne* spp.) were also present, but in low numbers. Also of significance is that one genus (*Psilenchus*) and six species (*Criconemoides sphaerocephalus*, *Hemicriconemoides brachyurus*, *Paratrichodorus lobatus*, *Rotylenchus caudaphasmidis* and *Tylenchorhynchus ventralis*) have never before been associated with maize, potato, sunflower, onion and grasses in cropping systems in SA and are thus new records.

Concerning non-parasitic nematode (NPN) assemblages, this is the first official report for SA on categorisation of fields planted to annual crops in terms of their soil health status and thus represents baseline information in this regard. Nine NPN genera of which six were bacterivores and three fungivores as well as two predator families have been identified from soil samples obtained from the 31 fields. Bacterivore nematode genera were represented by a wide spectrum with individuals of the *Rhabditis* genus belonging to the $Ba_1$ guild that dominated and resulted in high EI values, followed by

*Cephalobus* spp. ($Ba_2$). The latter genera as well as the other bacterivorous genera identified from these fields commonly occur in soils in SA. These NPN are regarded as the beneficial nematodes of which population levels increase substantially and relatively quickly after the addition of organic material or any kind of disturbance such as cultivation practices. The fungivore nematode communities of the 31 fields sampled were represented by a smaller variety of genera, with *Tylenchus* spp. ($Fu_2$) being predominant. In terms of the omnivore/predator and carnivore nematodes, individuals of the Dorylaimidae ($Om_{2-5}$) and Mononchidae ($Ca_{2-4}$) families were respectively present and are responsible for the high SI values obtained for these fields.

In terms of soil food web conditions, the majority of the 31 fields were plotted in the "Stable Enriched" quadrant that indicates that the soils of such fields were maturing and thus relatively healthy in terms of the beneficial nematodes as indicated by the simplified food web. This scenario is surprising and could probably be explained by the inclusion of grass cover crops on these fields for five to six years of the eight-year cropping cycles practised by these producers. The dominant contribution of bacterivore nematodes ($Ba_1$) contributed substantially to high EI values, while the presence of bacterivore, fungivore, omnivore/predator as well as carnivore guilds with higher cp values ranging, between 3-5, contributed to the medium to higher SI values for such fields.

The presence of a few significant relationships between the various PPN as well as NPN nematode genera with regard to cultivation practices, nematicide use as well as cropping sequences revealed interesting trends, but have to be verified by additional results before any conclusions can be made in this regard. Only then will it add substantial value to producers, the related crop industries and the science of Nematology. However, of particular interest are findings that application of EDB® in combination with other nematicides rendered significantly lower population levels of some of the predominant PPN genera such as *Meloidogyne* and *Pratylenchus* spp. identified. Concurrently, indications that some NPN, i.e. *Rhabditis* and *Prismatolaimus* spp., had higher population levels where EDB® combined with other nematicides than EDB® alone, respectively, were applied also indicate that interesting and important trends could be revealed should this type of research be extended over more growing seasons. The same applies for some of the PPN and NPN that reacted significantly with regard to specific crop rotation sequences as well as cultivation practices. Validation of such information can have important implications for designing and planning of nematode management strategies. Unfortunately no significant effects were, however, found for either PPN as well as NPN with regard to soil characteristics such as percentages of sand, silt, clay and organic material.

Concerning the use of Brassicaceae amendments as an environmentally-safe alternative to synthetic nematicides, *M. incognita* were identified as the predominant PPN present at the field site where the trial was conducted during the 2010/2011 growing seasons. J2 of the latter RKN species were able to infect and develop to mature, egg-producing females in roots/tubers of all four the Brassicaceae cultivars evaluated. The presence of relatively high population levels of this local *M. incognita* population in tubers of the two oilseed radish (Doublet and Terranova) and roots of the two mustard (Calienté and Nemat) cultivars at this trial site were, however, not expected and is not in agreement with the majority of reports related to this topic. The subsequent build-up of high population levels of this RKN species in roots of a follow-up potato crop sampled during tuber initiation, further substantiated results that the Brassicaceae cultivars used was not successful in reducing numbers of this parasite effectively at this specific trial site. As a result, the approach had to be reconsidered during tuber initiation and a registered synthetic nematicide was applied to limit RKN damage to potato tubers and this way minimise quality losses. Although results in terms of the host suitability showed that Nemat was the poorest host of the four Brassicaceae cultivars used, it still supported development of individuals of this RKN population. Also, no significant differences in terms of RKN numbers in roots of the follow-up potato crop in plots amended with Nemat compared to those for the other three Brassicaceae cultivars were recorded. Although it is not normal practice for producers to plant potato during successive growing seasons, it was only done for the purpose of this study.

In addition to data obtained on the plant-parasitic species *M. incognita* during this study, novel information about the NPN assemblages and their substantial changes in terms of community structures as a result of Brassicaceae amendments were gathered for annual crops, in this case potato. Previous studies in SA in this regard have only been done for perennial crops such as sugar cane and vineyard as well as for common scab on seed potatoes. Valuable information, indicating pronounced shifts of NPN assemblages in plots where the four Brassicaceae cultivars were planted from mainly the “Stress Depleted” (degraded) and/or “Stressed Enriched” (disturbed) to the “Stable Enriched” (maturing) quadrants indicate the positive effect of these crops in terms of soil health. In general substantial increases in the diversity (genera and/or families) and population levels of particularly bacterial- and fungus-feeding NPN were evident for all six treatments during the duration of the trial. The presence of enrichment and general opportunistic NPN guilds, representing bacterivores and fungivores with cp values of 1 and 2 thus resulted in high EI values for all treatments at the end of the Brassicaceae trial (Sampling interval 4). However, higher NPN diversity represented by guilds with cp values (3-5) higher than 2 that was also present in Brassicaceae-amended plots and resulted in the higher SI values. The latter indicate that such fields were maturing since it represents stability, food

web complexity and connectance in terms of the prevailing nematode assemblages. High EI values were most probably mainly due to disruption of the soil as a result of cultivation practices before and during planting of the crops, while the presence of Brassicaceae amendments most probably contributed predominantly to the presence of nematode diversity in terms of the higher guilds present in soil of such plots.

The outcome of this study, which included the two nematode surveys as well as the evaluation of the effect of Brassicaceae cultivars in terms of their host suitability to and biofumigation effects on a prevailing *M. incognita* population, provided valuable information to producers in the Christiana area. Such information can add value since it forms baseline studies that can already be build upon in efforts to manage RKN in particular but also NPN assemblages in their potato-based cropping systems.

### 5.2 Future prospects

The current study can be improved and value added to the results obtained by addressing the following issues:

- ✓ Obtaining of more soil and crop root/tuber samples from the 31 fields in the Christiana area to i) identify PPN present in each of the fields to species level and ii) increase the probability of discovering significant effects of various cultivation practices, nematicides as well as crop rotation sequences both for PPN and NPN.
- ✓ Screening of all Brassicaceae cultivars available in SA to determine i) glucosinolate content and ii) their host status to economically important RKN species that occur in potato-producing areas.
- ✓ Experimenting with Brassicaceae cultivars by planting higher densities to optimise their use in terms of amendment and biofumigation.
- ✓ Slashing and incorporation of aerial parts of Brassicaceae cultivars more than once during the growth period to determine the minimum application(s) in this regard.
- ✓ Conducting similar Brassicaceae trials over a longer period during which such crops are planted on a more frequent basis in potato-based cropping systems.
- ✓ Combination of biofumigation using Brassicaceae cultivars with other environmentally-friendly nematode control strategies, such as soil solarisation, steam sterilisation, natural products with nematicidal activity.
- ✓ Recording of pH and rainfall data to determine whether there could be correlations between the pH or amount of rainfall during the trial and degrading of parts of Brassicaceae crops in soil.

# Appendix A

**PPN (excluding *M. incognita*) and NPN J2 numbers/200g soil**

## Legend

**Treatments:**

T1 = Treatment 1 (Untreated control)

T2 = Treatment 2 (Terranova)

T3 = Treatment 3 (Doublet)

T4 = Treatment 4 (Nemat)

T5 = Treatment 5 (Calienté)

T6 = Treatment 6 (EDB® treatment)

Table 1. PPN (excluding *M. incognita*) J2 numbers/200g soil samples [$\log_{10}(x+1)$] for four sampling intervals from a trial site in the Christiana area where Brassicaceae crops were planted before potato during the 2010/2011 growing season (means in parenthesis represents the values that were back transformed and not the real means).

| **Sampling interval 1** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nematode genera/family/spp.** | **Untreated control (T 1)** | **Terranova (T 2)** | **Doublet (T 3)** | **Nemat (T 4)** | **Calienté (T 5)** | **EDB®[a] (T6)** | ***P* value** | **F ratio** |
| ***Criconema* spp.** | 1.1 (2) | 0.8 (1) | 0.8 (1) | 0.3 (0.3) | 0.7 (1) | 1.3 (2) | 0.8518 | 0.39 |
| **Hoplolaimidae spp.** | 0.3 (0.3) | 0 | 0 | 0.3 (0.3) | 0 | 0 | 0.5878 | 0.76 |
| **Trichodoridae** | 0.3 (0.3) | 0 | 0 | 0 | 0 | 0 | 0.4430 | 1 |
| ***Pratylenchus* spp.** | 0.3 (0.3) | 0.3 (0.3) | 0 | 0 | 0.5 (0.6) | 0 | 0.6543 | 0.66 |
| ***Tylenchorhynchus* spp.** | 0 | 0 | 0 | 0 | 0 | 0.3 (1) | 0.4430 | 1 |
| **Total PPN** | 1.7 (1) | 1 (2) | 1.2 (2) | 0.8 (1) | 2.2 (8) | 2.1 (7) | 0.5585 | 0.81 |
| **Sampling interval 2** | | | | | | | | |
| ***Criconema* spp.** | 0 | 0.2 (0.2) | 0 | 0 | 0 | 0.1 (0.1) | 0.4430 | 1 |
| **Hoplolaimidae spp.** | 0 | 0.1 (0.1) | 0 | 0.1 (0.1) | 0.3 (0.3) | 0 | 0.2057 | 1.6 |
| **Trichodoridae** | 0 | 0 | 0 | 0.1 (0.1) | 0.3 (0.3) | 0.1 (0.1) | 0.6111 | 0.73 |
| ***Tylenchorhynchus* spp.** | 0 | 0 | 0 | 0 | 0 (0) | 0.3 (0.3) | 0.0528 | 2.67 |
| **Total PPN** | 1.2 (2) | 0.6 (1) | 0.3 (0.3) | 0.5 (1) | 0.8 (1) | 0.6 (1) | 0.2249 | 1.53 |

| Sampling interval 3 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nematode genera/family/spp.** | **Untreated control (T 1)** | **Terranova (T 2)** | **Doublet (T 3)** | **Nemat (T 4)** | **Calienté (T 5)** | **EDB®[a] (T6)** | ***P* value** | **F ratio** |
| ***Criconema* spp.** | 0 | 0.3 (0.3) | 0 | 0.1 (0.1) | 0.1 (0.1) | 0 | 0.2057 | 1.6 |
| **Hoplolaimidae spp.** | 0.1 (0.1) | 0.3 (0.3) | 0.1 (0.1) | 0.1 (0.1) | 0 | 0.1 (0.1) | 0.9154 | 0.29 |
| **Trichodoridae** | 0 | 0 | 0.2 (0.2) | 0 | 0 | 0.1 (0.1) | 0.4430 | 1 |
| ***Tylenchorhynchus* spp.** | 0.3 (0.3) | 0 | 0 | 0.1 (0.1) | 0 | 0.3 (0.3) | 0.4633 | 0.96 |
| **Total PPN** | 1.6b (2) | 0.7ab (1) | 0.5ab (1) | 0.3a (0.3) | 0.3a (0.3) | 0.8ab (1) | 0.0334 | 3.04 |
| **Sampling interval 4** | | | | | | | | |
| ***Criconema* sp.** | 0.4 (0.5) | 0.5 (0.6) | 0 | 0 | 0 | 0 | 0.5856 | 0.77 |
| **Hoplolaimidae sp.** | 0 | 0.7 (1) | 0.4 (0.5) | 0 | 0 | 0 | 0.5697 | 0.79 |
| **Total PPN** | 3ab (19) | 3.6ab (35) | 1.8ab (5) | 1a (2) | 5b (147) | 2.4ab (10) | 0.0364 | 2.97 |

[a]EDB®= ethylenedibromide

Table 2. Numbers [$\log_{10}(x+1)$] of non-parasitic nematodes J2 numbers/200g soil samples for four sampling intervals from a trial site in the Christiana area where Brassicaceae crops were planted before potato during the 2010/2011 growing season (means in parenthesis represents the values that were back transformed and not the real means).

| **Sampling interval 1** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nematode genera/family** | **Untreated control (T 1)** | **Terranova (T 2)** | **Doublet (T 3)** | **Nemat (T 4)** | **Calienté (T 5)** | **EDB®[a] (T 6)** | ***P* value** | **F ratio** |
| ***Acrobeles* spp.** | 2.4 (10) | 2.3 (9) | 2.7 (14) | 1.5 (3) | 2.5 (11) a | 2 (6) | 0.5390 | 0.84 |
| ***Acrobeloides* spp.** | 0 | 0.3 (0.3) | 0 | 0 | 0 | 0.3 (0.3) | 0.5878 | 0.76 |
| ***Aphelenchus* spp.** | 0.7 (1) | 0.5 (0.6) | 0.8 (1) | 0.3 (0.3) | 0 | 1.2 (2) | 0.6559 | 0.66 |
| ***Aphelenchoides* spp.** | 0.3 (0.3) | 0.3 (0.3) | 0 | 0.5 (0.6) | 0.3 (0.3) | 0 | 0.8351 | 0.41 |
| ***Cephalobus* spp.** | 1.5 (3) | 1.4 (3) | 1.1 (2) | 1.5 (3) | 2.1 (7) | 2.2 (8) | 0.8737 | 0.35 |
| ***Chiloplacus* spp.** | 0 | 0 | 0.7 (1) | 0.3 (0.3) | 1.3 (2) | 0.5 (0.6) | 0.2964 | 1.32 |
| ***Cruznema* spp.** | 0. 5 (0.6) | 0 | 0 | 0 | 0.7 (1) | 0 | 0.2933 | 1.33 |
| ***Diploscapter* spp.** | 0 | 0.3 (0.3) | 0 | 0 | 0 | 0 | 0.4430 | 1 |
| **Doryliamidae** | 1.3 (2) | 2.3 (9) | 0.3 (0.3) | 1.2 (2) | 1.6 (4) | 1.4 (3) | 0.2012 | 1.62 |
| ***Eucephalobes* spp.** | 1 (2) | 0 | 0.3 (0.3) | 0.8 (1) | 0.3 (0.3) | 0.8 (1) | 0.5482 | 0.82 |
| ***Mesorhabditis* spp.** | 0.3 (0.3) | 0 | 0.5 (0.6) | 0 | 0.3 (0.3) | 0.8 (1) | 0.5680 | 0.79 |
| ***Panagrolaimus* spp.** | 0.3 (0.3) | 0.7 (1) | 0.8 (1) | 0.8 (1) | 0.5 (0.6) | 0.8 (1) | 0.9599 | 0.2 |
| ***Rhabditis* spp.** | 2.1 (7) | 1.4 (3) | 1.3 (2) | 2.1 (7) | 1.5 (3) | 0.5 (0.6) | 0.2321 | 1.51 |
| ***Tylenchus* spp.** | 0 | 0.3 (0.3) | 0 | 0 | 0 | 0.3 (0.3) | 0.5878 | 0.76 |
| **Total NPN** | 3.8 (43) | 3.8 (43) | 3.6 (35) | 3.1 (21) | 4 (53) | 3.9 (48) | 0.7502 | 0.53 |

| Sampling interval 2 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nematode genera/family** | **Untreated control (T 1)** | **Terranova (T 2)** | **Doublet (T 3)** | **Nemat (T 4)** | **Calienté (T 5)** | **EDB® (T 6)** | ***P* value** | **F ratio** |
| ***Acrobeles* spp.** | 0.4 (0.5) | 0.1 (0.1) | 0 | 0.3 (0.3) | 0.3 (0.3) | 0.4 (0.5) | 0.5919 | 0.76 |
| ***Acrobeloides* spp.** | 0 | 0.3 (0.6) | 0 | 0.1 (0.2) | 0.1 (0.2) | 0.2 (0.2) | 0.4430 | 1 |
| ***Cephalobus* spp.** | 1 (2) | 1 (2) | 0.8 (1) | 0.6 (1) | 0.8 (1) | 0.6 (1) | 0.8566 | 0.38 |
| **Dorylaimidae spp.** | 0.4 (0.5) | 0.4 (0.5) | 0.6 (1) | 0.2 (0.2) | 0.5 (1.6) | 0.2 (0.2) | 0.8648 | 0.37 |
| ***Eucephalobus* spp.** | 0.4 (0.5) | 0.4 (0.5) | 0.5 (0.6) | 0.1 (0.1) | 0.2 (0.4) | 0.3 (0.3) | 0.8652 | 0.37 |
| ***Mesorhabditis* spp.** | 0.3 (0.3) | 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | 0.8990 | 0.31 |
| ***Rhabditis* spp.** | 0.4 (0.5) | 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | 0.5 (1) | 0.4 (0.6) | 0.5225 | 0.86 |
| **Total NPN** | 1.8 (5) | 1.6 (4) | 1.4 (3) | 1.2 (2) | 1.6 (4) | 1.4 (3) | 0.6724 | 0.64 |
| **Sampling interval 3** | | | | | | | | |
| ***Acrobeles* spp.** | 1.1 (2) | 1 (2) | 1.4 (3) | 1.1 (2) | 1 (2) | 1.2 (2) | 0.5246 | 0.86 |
| ***Cephalobus* spp.** | 1.1 (2) | 1 (2) | 1 (1.4) | 0.3 (0.6) | 1 (2) | 0.5 (2) | 0.5906 | 0.76 |
| **Dorylaimidae** | 0.1 (0.1) | 0.5 (0.6) | 1 (2) | 1 (2) | 0.5 (0.6) | 1 (2) | 0.6199 | 0.71 |
| ***Eucephalobus* spp.** | 0.2 (0.2) | 0.4 (0.5) | 0.1 (0.1) | 0 | 1 (2) | 0.3 (0.3) | 0.1219 | 2 |
| ***Mesorhabditis* spp.** | 0.1 (0.1) | 0.2 (0.2) | 0 | 0 | 0 | 0.2 (0.2) | 0.6278 | 0.7 |
| ***Rhabditis* spp.** | 0.8 (1) | 0.7 (1) | 0.3 (0.3) | 0.7 (1) | 0.8 (1) | 1 (2) | 0.7238 | 0.57 |
| **Total NPN** | 2.2 (8) | 2 (6) | 2 (6) | 2 (6) | 2 (6) | 2.2 (8) | 0.8624 | 0.37 |

| Sampling interval 4 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nematode genera/family** | **Untreated control (T 1)** | **Terranova (T 2)** | **Doublet (T 3)** | **Nemat (T 4)** | **Calienté (T 5)** | **EDB®[a] (T 6)** | ***P* value** | **F ratio** |
| ***Acrobeles* spp.** | 1.7 (4) | 0 | 1.2 (2) | 0 | 0.5 (0.6) | 0.8 (1) | 0.1393 | 1.9 |
| ***Aphelenchoides* spp.** | 1.8 (5) | 1.5 (3) | 0 | 0 | 0 | 0.4 (0.5) | 0.2784 | 1.37 |
| ***Cephalobus* spp.** | 2.4 (10) | 3.7 (39) | 3 (19) | 2.5 (11) | 3 (19) | 3.7 (39) | 0.5381 | 0.84 |
| ***Diploscapter* spp.** | 0 | 1.2 (2) | 0 | 0 | 0 | 0 | 0.4430 | 1 |
| **Dorylaimidae** | 3.4 (29) | 2.6 (12) | 3.4 (29) | 1.5 (3) | 3.4 (29) | 1.4 (3) | 0.1085 | 2.09 |
| ***Rhabditis* spp.** | 7.3 (1479) | 5 (147) | 6.8 (897) | 5.3 (199) | 7 (1095) | 7.5 (1807) | 0.2998 | 1.31 |

[a]EDB®= ethylenedibromide