INTEGRATED MANAGEMENT STRATEGIES FOR *MELOIDOGYNE* SPECIES IN *SOLANUM LYCOPERSICUM* PRODUCTION SYSTEMS

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DISSERTATION SUBMITTED IN FULLFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF AGRICULTURAL MANAGEMENT (PLANT PROTECTION), DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agricultural Management (Plant Protection) has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

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DEDICATION

To my beloved kids, Thutoentle Princess and Mooki Thlalefo Mabuka.

I say may The Mighty God bless you.

ACKNOWLEDGEMENTS

I fervently dispatch my gratitudes and heartfelt praises to the Mighty Almighty God of Mount Zion Who knew me before I was formed. Who spiritually helped, guided and enlightened my ways, thereby enabling me to work consistently and very hard, without which I could not have completed this project. My sincere and pensive thanks to my beloved mother, Mrs Malekgo Julia Mabuka, my uncle Mr Joseph Mabatha, my sisters, Kgomotso, Rakgadi and Tumelo Mabuka and my brothers, Lethlokwa, Lebokgang and Monntle Mabuka, for their individual and collective unwavering support and guidance throughout my studying life, may the Great God duly bless you and make your future a sounding success. Very special thanks to my late and beloved brother Mr Takalani Steven Mabuka, who seamlessly sacrificed in life for my future. Brother, you will eternally be in my thoughts. My gratitude and endless thanks to Professor P.W. Mashela ─ my supervisor for his diligent training and assistance in various areas of this project. I heartily appreciate the work-discipline instilled in me without favour. There was so much to learn in research and scientific writing. I also send my cordial gratitudes to Dr M.O. Pelinganga, Mr M.P. Maloka, Mr P.E. Tseke and Mr Z.P. Dube for their much appreciated assistance throughout the study. My sincere gratitudes are directed to the National Research Foundation (NRF) of South Africa and the Land Bank Chair of Agriculture ─ University of Limpopo, for financial support which sustained and materialised this project.

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sorghum var. ndendane-X1 at six levels of nemafric-BG phytonematicide under *Meloidogyne* infested field (n =78).

ABSTRACT

Tomato (*Solanum lycopersicum* L.) production had been ranked as the most important commodity in terms of job and wealth creation within the auspices of the National Development Plan (NDP) framework in Limpopo Province. However, soil-borne diseases including plant-parasitic nematodes preclude the successful monoculturing of this commodity and therefore inducing instability in job creation. Generally, after growing a tomato crop for one season in commercial tomato-production systems, the land is being fallowed for 3-5 years under natural grasses. Attempts are being initiated to ensure that during the 3-5 years the land be occupied by an economic alternative crop in order to level off job instability as broadly articulated in the NDP framework. The production of sweet stem sorghum (*Sorghum bicolor* L.) for ethanol production during the 3-5 years fallowing period could potentially be attractive to commercial tomato-producing famers. Preliminary agronomic evaluations demonstrated that sweet stem sorghum var. ndendane-X1 had attributes to fulfil the identified need. However, the degree of nematode resistance of the variety to *Meloidogyne incognita* race 2 and *M. javanica*, which are dominant in Limpopo Province, along with the compatibility of var. ndendane-X1 to phytonematicides used in tomato production had not been documented. The objectives of the study were, therefore, to determine whether sweet stem sorghum var. ndendane-X1: (1) had any degree of nematode resistance to *M. incognita* race 2 under both greenhouse and microplot conditions, (2) had any degree of nematode resistance to *M. javanica* under greenhouse conditions, and (3) would be compatible with phytonematicides used in suppression of population densities of

Meloidogyne species in tomato production under field conditions. In the greenhouse trials, seeds were sown in 20-cm-diameter plastic pots and each seedling inoculated with 0, 600, 1 000, 1 400, 1 800 and 2 200 eggs and second-stage juveniles (J2s) of *M. incognita* race 2 or *M. javanica*. Treatments were arranged in a randomised complete block design (RCBD), with 10 replicates (n = 60). In the microplot trial, seeds were sown in 30-cm-diameter plastic pots and buried 75% deep in a 0.30-m intra-row and 0.25-m inter-row spacing. Treatments, namely, 0, 200, 600, 1 000, 1 400, 1 800 and 2 200 J2s of *M. incognita* race 2 were arranged in RCBD, with 14 replications (n = 98). In a *Meloidogyne*-infested field trial, seeds were sown at 0.2-m inter-row and 0.3-m intra-row spacing, with treatments 0, 2, 4, 6, 8 and 10 g nemafric-BG phytonematicide/plant, arranged in RCBD, with 13 replications (n = 78). The degree of nematode resistance was measured using host-status and host-sensitivity, which provide information on reproduction of the target nematode and plant damage due to nematode infection, respectively. Nematode reproduction was measured through the reproductive factor (RF), which is a proportion of final nematode population density (Pf) to initial nematode population density (Pi), summarised as RF = Pf/Pi. In all nematode resistance trials, RF was equivalent to zero, which implied that var. ndendane-X1 was a non-host to both *M. incognita* race 2 and *M. javanica*. Additionally, in both greenhouse and microplot trials, sweet stem sorghum var. ndendane-X1 did not suffer any significant damage due to infection by *Meloidogyne* species. Using nematode-plant relation concepts, sweet stem sorghum var. ndendane-X1 was resistant to *M. incognita* race 2 and *M. javanica* under greenhouse and microplot conditions. Under field conditions, nemafric-BG phytonematicide reduced eggs and J2s of *Meloidogyne* species in root and soil samples

by 76-85% and 24-65%, respectively, without nematode effect on plant growth, suggesting that nemafric-BG could be integrated with nematode resistance in var. ndendane-X1 to manage nematode population densities. In conclusion, pilot projects where sweet stem sorghum var. ndendane-X1 could be used during the 3-5 years fallowing period in a tomato-sweet stem sorghum crop rotation system should be established to assess: (i) the economics of the proposed cropping system, (ii) the effect of the cropping system on soil-borne diseases, including plant-parasitic nematodes, and (iii) the effect of the cropping system on soil health.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Options to manage population densities of plant-parasitic nematodes in tomato (*Solanum lycopersicum* L.) production using nematode-resistant genotypes in crop rotation systems are limited by factors which include: (i) lack of empirically-based information on the degree of nematode resistance in alternative crops, (ii) existence of biological races in *Meloidogyne* species, (iii) wide host ranges for *Meloidogyne* species, (iv) limited information on economic potential of highly nematode-resistant indigenous plants, and (v) limited information on the compatibilities of the available nematode-management options (Nzanza *et al*., 2013). Generally, after tomato production in commercial systems, the land cannot be replanted to tomato crop for several years, but to alternative crops without direct economic value. Such alternative crops are intended to prevent soil erosion and therefore improve soil fertility for the next round of a tomato crop. In commercial farming systems where tomato is the sole primary crop like at ZZ2 Boerdery (Pty) Ltd, large tracts of land are necessary to allow for the use of one piece of land every three to five years for tomato production (Nzanza *et al*., 2013).

1.1.1 Description of the research problem

Build-up of soil-borne diseases, including plant-parasitic nematodes, prevents monoculture in tomato production (Nzanza *et al*., 2013). Due to the scarcity of land, using a piece of land for commercial production once every 3-5 years in tomato

production, while in-between non-economic crops occupy the land, could be uneconomical. At ZZ2, for instance, the field is left fallowed to allow for natural grasses to grow before the next cropping cycle. During the non-tomato period, grasses are constantly baled to allow for regeneration, with hay being used as basal ingredient for composting. In that context, the aim of fallowing is to mitigate the negative effect of monoculture, while improving soil health. Crop rotations with economically suitable crops would ameliorate the challenge of occupying the land with uneconomic crops. However, crops intended for inclusion in crop rotation systems are limited by their host-status and host-sensitivity to *Meloidogyne* species.

1.1.2 Impact of the research problem

The exact degree of damage caused by plant-parasitic nematodes is frequently underestimated due to the presence of multiple pathogens, which makes the diagnosis rather confusing (Spaul and Cadet, 1990). However, numerous estimates on damage caused by nematodes in crops on a worldwide basis exist. According to the Society of Nematologists, which undertook a major crop loss estimation project in 1971, losses due to nematodes were approximately 10%, with extension to 50% and total crop failure in certain crops. The total annual loss due to nematodes in 16 field crops, 23 fruit and nut crops and 24 vegetable crops was up to US $1.6 billion, while in vegetables alone it was estimated to be about US $267 million per annum (Feldmesser *et al*., 1971). Also, Sasser and Freckman (1987) reviewed crop losses on the basis of worldwide surveys and suggested average yield losses of major crops due to plant-parasitic nematodes at 12.3%. In monetary terms, worldwide annual crop losses due to plant-parasitic

nematodes had been estimated at US $100 billion (Oka and Yermirahu, 2002). Prior to withdrawal of methyl bromide in 2005, Chitwood (2003) estimated that worldwide annual crop losses due to nematode damage was at US $125 billion.

1.1.3 Possible causes of the research problem

Tomato is a major vegetable crop in Limpopo Province, which is home to *M. javanica* and *M. incognita* race 2. After having been relied upon for many years in management of population densities of plant-parasitic nematodes, the withdrawal of fumigant nematicides from agro-chemical markets left a serious void in tomato production systems (Mashela, 2007). *Meloidogyne* species have a wide host-range, wide distribution and interaction with other soil-borne pests, which limit the use of nematode-resistance in crop rotation systems. Alternative crops available for use in crop rotation systems are limited by their host-status to *Meloidogyne* species.

1.1.4 Proposed solutions

The inclusion of economically important alternative crops in crop rotation with tomato as primary crop could be more appealing to most tomato-producing farmers. For instance, ethanol-producing crops, provided the farmers are willing to invest in ethanol-producing equipment, could be ideal in tomato production systems as a measure to ameliorate lengthy fallowing periods prior to the next cropping cycle of tomato.

1.2 Problem statement

Long fallowing periods with uneconomic natural grasses prior to the next cropping cycle in tomato production could be ameliorated through integrated management strategies that are intended to manage soil-borne pests. The approach should also attempts to assess the substitution of uneconomic natural grasses with nematode-resistant ethanol-producing alternative crops. The researcher proposed to determine the degree of nematode resistance in a local sweet stem sorghum (*Sorghum bicolor* L.) to local *Meloidogyne* species with the eventual aim of having such a variety to be included in tomato-crop rotation systems which will include the use of plant crude extracts for the management of nematodes.

1.3 Motivation

Sucrose-producing crops like sweet stem sorghum are the most preferred in ethanol production systems. Trials are underway to evaluate the agronomic of local sweet stem sorghum varieties for ethanol production. However, the degree of nematode-resistance in the varieties is not documented. Thus, information on the degree of nematode-resistance of high sucrose-producing sweet stem sorghum varieties to *M. incognita* race 2 and *M. javanica* would help farmers in decision-making with regard to viewing the inclusion of sweet stem sorghum as an alternative crop in tomato-sweet stem sorghum crop rotation systems.

1.4 Aim

The aim of this study was to investigate the degree of nematode-resistance in a selected sweet stem sorghum variety to *Meloidogyne* species which are predominant in Limpopo Province for possible use as an integrated nematode management strategy in tomato production.

1.5 Objectives

1. To determine whether sweet stem sorghum var. ndendane-X1 was a host to *M. incognita* race 2 and the host-sensitivity of the variety to *M. incognita* race 2 under greenhouse and microplot conditions.

2. To investigate whether sweet stem sorghum var. ndendane-X1 was a host to *M. javanica,* along with the host-sensitivity of the variety to *M. javanica* under greenhouse conditions.

3. To determine whether sweet stem sorghum var. ndendane-X1 would be compatible with nemafric-BG phytonematicide in suppression of population densities of *Meloidogyne* species under field conditions.

1.6 Format of dissertation

The dissertation was designed using the Senate-approved technical format of the University of Limpopo. Following the description of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the three subsequent chapters addressed each of the objectives in

sequence (Chapter 3-5). Finally, findings in all chapters would be summarised and integrated to provide the significance of the findings, recommendations with respect to future research and culminated in conclusions.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Worldwide, economic benefits of indigenous plants are being evaluated as alternative crops after centuries of neglect in favour of exotic crops (Mashela and Mollel, 2001). Due to centuries of adaptation in specific localities, indigenous plants have over the years, developed attributes which had since been envious to sustainable food security proponents (Mashela and Mollel, 2001). Sweet stem sorghum (*Sorghum bicolor* L.) is indigenous to Africa (Martin *et al*., 1975), with agronomic attributes which include: (1) high photosynthetic efficiency as a C4 crop that does not have photorespiration, (2) high water use efficiency with capabilities to grow in high temperatures, (3) drought-resistance which is attributed to various adaptations, (4) high tolerance to stressful environments and (5) exceling in poor and marginal soils (Sasaki and Antonio, 2009).

At ZZ2 Boerdery (Pty) Ltd in Mooketsi, South Africa, cover cropping in tomato (*Solanum lycopersicum* L.) is recognised as one of the best practice for improving soil health (Nzanza *et al*., 2013). Under nature farming systems, because of the quick regeneration of natural grasses, cover cropping with exotic crops is only resorted to in special circumstances. At ZZ2 on-farm trials were widely conducted to determine the best cover crops for the region (Nzanza *et al*., 2013). Much attention had been given to evaluating the performance of vetch (*Vicia unguiculata* L.) and oat (*Avena sativa* L.) cropping mixtures for N-fixation and organic matter addition or cowpea (*Vigna unguiculata* L.) and

oat mixtures for N-fixation and disease suppression. Sunn hemp (*Crotolaria juncea* L.) and pearl millet (*Pennisetum glaucum* L.) were separately tested for their individual deep root systems that bring Ca and K up when the plants are eventually mulched.

Sweet stem sorghum had been identified as one of the most suitable alternative crops for ethanol production since it requires fewer chemical reaction steps and less energy from primary inputs to the end-product of ethanol, when compared to using grain and forage plants as primary inputs (Reddy *et al*., 2005). Ethanol produced from sweet stem sorghum is carbon neutral which implies that the amount of carbon dioxide that sweet stem sorghum fixes during its growing cycle offsets the carbon dioxide produced during crop production, processing and ethanol utilisation (Reddy *et al*., 2005). Generally, it costs only US $0.46 to produce 1 L of ethanol from sweet stem sorghum, while for sugarcane and maize the coasts are US $0.58 and US $0.56, respectively (Doggett, 1988). The successful production of sweet stem sorghum, however, could be hampered by the root-knot (*Meloidogyne* species) nematodes, which are notorious for reducing crop yields by as high as 50% to complete crop failure in crops such as watermelon (*Citrullus lanatus* Thumb.) (Lamberti, 1979). In South Africa, the widely distributed root-knot nematodes include *M. javanica* and *M. incognita* races 2 and 4 (Kleynhans *et al*., 1996). In plant nematology, races are nematodes which are morphologically similar, but could be separated using differential hosts and molecular markers (Pofu, 2012). In Limpopo Province, sweet stem sorghum var. ndendane-X1 was selected as a potential candidate for use in biofuel production due to its high brix content, which averages over 20% (Mashela, P.W.: Personal communication).

2.2 Concepts related to the research problem

2.2.1 Nematode-plant interactions

Seinhorst (1967) introduced the concepts of host-status and host-sensitivity to describe nematode-plant relations, which had since been widely used in plant-parasitic nematology. Host-status was described using the proportion of the final nematode population density (Pf) and the initial nematode population density (Pi), referred to as the reproductive factor (RF = Pf/Pi). Using the RF concept, when Pf = Pi, the population is at equilibrium (E) point, beyond which nematodes have intensive competition for resources, while RF is invariably less than unity (Seinhorst, 1967). Generally, before E point, nematodes are at the lowest competition for resources and if the plant is a host, RF is invariably greater than unity. Ferris (1981) and later Duncan and McSorley (1987), expounded the host-status concepts using mathematical models, which although theoretical in nature, assist nematode practitioners in better understanding of nematode reproduction and density-dependent growth patterns (Salisbury and Ross, 1992) and thereby improving nematode management tactics.

Host-sensitivity was described in relation to damage inflicted by nematodes to plants, with Seinhorst (1965) using a model to coin three helpful concepts: (i) tolerance, (ii) damage threshold and (iii) minimum yield, which had since been widely used in nematode-plant relations (Duncan and McSorley, 1987; Ferris, 1981). Tolerance in susceptible hosts occurs at the Pi where nematode infections have not yet started to inflict yield reduction, damage threshold is the Pi level where yield reduction emerges, while minimum yield occurs at the Pi where there is maximum competition due to

nematode infection (Duncan and McSorley, 1987; Ferris, 1981). The concept of minimum yield suggests that nematodes do not kill their hosts, which agrees with cyclic nature in population growth of plant-parasitic nematodes (Mashela, 1992) and density-dependent growth patterns (Salisbury and Ross, 1992).

Host-sensitivity measures the responses of a plant to nematode infection and is a function of (i) nematode type, (ii) inoculum level, (iii) plant type, (iv) age of plant and (v) biotic and abiotic factors (Seinhorst, 1965). Generally, certain nematode species, for example, the root-knot nematodes, the burrowing nematodes (*Radopholus similis* Cobb), the sting nematode (*Belonolaimus longicaudatus* Rau) and the root-lesion nematode (*Pratylenchus penetrans* Cobb) are more aggressive than others, for example, the citrus nematode (*Tylenchulus semipenetrans* Cobb), and result in greater yield losses (Duncan, 2009; Mashela, 1992). When interacting with abiotic factors like salinity, plant-parasitic nematodes like *T. semipenetrans*, the sting nematode and *M. incognita,* induce maximum damage in crops (Duncan, 2009; Duncan *et al*., 1995; Mashela and Nthangeni, 2002). Review of host-status and host-sensitivity of plants to nematodes would in this study be limited to the description of three concepts: (1) susceptible plants, (2) tolerant plants and (3) resistant plants (Seinhorst, 1967; Trudgill, 1992).

Susceptible hosts: These are plants that have the ability to build up nematode populations and suffer subsequent damage in terms of growth reduction (Trudgill,

1992). Generally, host types respond to attack by root-knot nematodes by forming galls on roots, referred to as root galls (Agrios, 2005). Feeding cells induced by root-knot nematodes, termed giant cells, are formed from host root cells during parasitism to sustain the growth, development and reproduction of the nematode (Hussey and Grundler, 1998).

Tolerant hosts: Seinhorst (1967) defined tolerance to nematodes as the capacity of the plant to withstand nematode damage. Most nematodes can reproduce in tolerant hosts without causing any significant reduction in growth and yield (Seinhorst, 1967; Trudgill, 1985). However, tolerant hosts are not suitable for use in crop rotation systems since they invariably increase nematode population densities, which may eventually produce virulent biological races.

Resistant hosts: Resistant plants neither allow nematode reproduction nor suffer nematode damage (Seinhorst, 1967; Taylor and Sasser, 1978). Resistance to nematodes is usually associated with the inability of the nematode to induce a normal feeding site or reproduce inside the host (Miller and Guyla, 1987). Introduction of well-adapted and high-yielding nematode-resistant cultivars is currently the focus in nematode management strategies. Genotypes with superior levels of resistance to a particular plant-parasitic nematode are continuously being selected for planting and/or breeding efforts to minimise nematode population increases and crop damage (Miller

and Guyla, 1987). Traditionally, nematode resistance genes were introduced into susceptible hosts through a process called introgression (Kaplan and Davis, 1987).

Plant breeders introgress natural nematode resistance genes from resistant landraces into nematode-susceptible crops to improve their resistance to nematodes (Lambrides and Miller, 1998). For instance, successful introgression of Mi resistance genes in tomato cultivars resulted in intensive use of the Mi genes in agriculture (Thurau *et al*., 2010). However, due to the pathogenic variability of the root-knot nematodes with multiple biological races, introgression of resistance genes raised concerns with respect to the durability of the engineered resistant genes (Faghihi *et al*., 1995). Although the Mi genes blocked nematode development at an early stage, due to the occurrence of biological races, successful development of infective stages and their subsequent reproduction on Mi-resistant tomato genotypes among *Meloidogyne* species occurred (Roberts and Thomason, 1989). Also, virulent nematode biotypes against the Mi gene were observed and described in various tomato-producing regions (Roberts and Thomason, 1989). Generally, introgressed nematode resistance lost its practical usefulness when, under certain conditions, resistant cultivars were easily challenged by new virulent nematode races, which could also be exacerbated by environmental factors such as high temperature and high salinity (Duncan, 2009; Mashela, 1992).

2.2.2 Mechanisms of nematode resistance

Bird (1974) identified the infection cycle in the root-knot nematodes, which comprises: (i) probing of host for suitability, (ii) cell wall perforation by thrusting the stylet, and (iii) ingestion of cell contents. Adjacent undifferentiated cells, around the one in which the stylet is inserted, are coalesced through the dissolution of cell walls, with mitosis occurring without cytokinesis, followed by the breakdown of cell walls, with the consequent multinucleate condition and increase in size of giant cells, which are externally visible as root galls. In *Meloidogyne* species, giant cell formation occurs through either hyperplasia or hypertrophy (Bird, 1974). In hyperplasia a cell increases in size due to division of organelles during mitosis, without cytokinesis taking place, with the result that the enlarged cell contains multi-organelles (Bird, 1974). In contrast, hypertrophy is an increase in size due to the enlargement of organelles. In *Hederodera* species, giant cell formation occurs through a process syncytium, where cell walls of adjacent cells coalesce, resulting in an enlarged cell with multi-organelles. Resistance may occur during initial stages of infection cycle or long thereafter, as explained from the ensuing mechanisms of resistance to plant-parasitic nematodes.

Pre-infectional resistance: Pre-infectional resistance is mainly due to pre-formed chemicals, which are fully expressed in root tissues before infection and do not rise to higher levels in response to attacks by invading nematodes (Ferraz and Brown, 2002). Marigold species (*Tagetes* species) suppress populations of the lesion and the root-knot nematodes through pre-formed chemical compounds, which were identified as alpha-terthienyl and bi-thienyl (Veech, 1981). Among 175 plant species from different families

surveyed for resistance to *P. penetrans*, resistance in 70 species was closely correlated with pre-infectional resistance (Gommers and Voor In't Holt, 1976). In the same study, populations of *P. penetrans* were reduced by 99%, 55% and 63% in *Tagetes patula* L., *T. erecta* L. and *T. minuta* L., respectively. Asparagus (*Asparagus officinalis* L.) contains glycosides, which have nematicidal properties responsible for pre-infectional resistance (Rohde, 1972). Similarly, Griffin and Waite (1971) noted that certain varieties of alfalfa (*Medicago sativa* L.) released substances that were repellent to the tulip root nematode (*Ditylenchus dipsaci* Kuhn).

Post-infectional resistance: Post-infectional resistance is the ability of a plant to defend itself against nematode parasitism by releasing chemicals present in low levels to higher levels in the host tissues after penetration of nematodes (Kaplan and Davis, 1987). Induced chemicals are triggered to higher levels by the invading nematodes, where the antimicrobial chemicals either inhibit feeding, development or kill the invading nematode. Induced chemicals, called phytoalexins, are believed to confer resistance to most plant-parasitic nematodes (Harborne, 1999). Generally, post-infectional nematode resistance is introgressible (Kaplan and Davis, 1987), with three distinct variations, *viz.* (a) phytoalexins, (b) time-unlinked genetic resistance (instant hypersensitivity) and (c) time-linked genetic resistance (gradual hypersensitivity).

(a) Phytoalexins

Phytoalexins are defensive phytochemicals present in cells in inactive forms and are instantly activated by the penetration of pathogens (Veech, 1981). Generally,

phytoalexins represent effective plant resistance mechanisms to nematodes, particularly the sedentary nematode types like the root-knot nematodes (Huang, 1985; Veech, 1981). Giebel (1974) and Roy (1981) each suggested that enzymes might influence changes in plant growth regulators, free bound phenols and composition of amino acids in plants, with changes inducing lignifications in order to limit nematode development.

(b) Time-unlinked genetic resistance

Time-unlinked genetic resistance is usually referred to as hypersensitivity since there is, upon penetration of roots by a pathogen, a rapid dying of host cells that produce localised necrosis around the pathogen, thus, limiting its spread and growth (Wallace, 1971). Hypersensitivity can be in response to facultative fungi, bacteria, viruses and nematodes (Klement *et al*., 1964).

(c) Time-linked genetic resistance

In some resistance genes, the host response appears to occur at different timing than it does in post-infectional resistance genes of Mi in *Meloidogyne* species. For instance, an *H7*-mediated resistance of potato to (*Globodera rostochiensis* Behrens) was characterised by gradual necrosis of tissues around the invading nematode (Rice *et al*., 1987). Despite the initial necrosis, feeding sites began to develop and the nematode developed and became sedentary (Rice *et al*., 1987). Later on, the feeding site was surrounded by necrotic tissues and eventually collapsed. Few nematodes that

continued to develop on *H7* potato plants were mostly males (Rice *et al*., 1987), which was an indication of poor nutrition for the nematode.

2.2.3 Nematode-resistance in sorghum species

Multi-cultivars of sorghum for use as rotational crops and cover crops include sorghum-sudangrass hybrid (*Sorghum bicolor* x *Sorghum bicolor var. sudanense*) (Clark, 2007; McSorley *et al*., 1994a; McSorley and Gallaher, 1991). This hybrid has the potential to smother weeds, suppress nematode species and penetrate compacted sub-soils (Clark, 2007). Also, *S*. *bicolor* hybrids had been reported to inhibit some species of nematodes in subsequent crops. Suppressive activities of the hybrids were primarily due to the production of natural nematicidal compounds (Clark, 2007), their poor host-status, general stimulation of microbial antagonists and the release of toxic products during decomposition (Magdoff and Van Es, 2009). For maximum suppression of soil-borne pathogens, cut or chopped sorghum stover should immediately be incorporated into the soil (Clark, 2007). Grain sorghum and sweet stem sorghum when grown as cover crops or green manure reduced population densities of most *Meloidogyne* species (McGuidwin, and Layne, 1995). Presumably, *Meloidogyne* species are inhibited because these crops are poor hosts for nematode reproduction, while in the case of green manures, leaves contain chemicals that hydrolyze to form nematicidal compounds (Magdoff and Van Es, 2009).

Summer and winter plant species within *Sorghum* genus were evaluated in greenhouse experiments, with differences being observed among summer and winter plants to *M. ethiopica* (Roberts, 1992). Among the 32 summer evaluated plants, *S*. *bicolor* cv. 'SARA' was non-host to *M. ethiopica* (RF = 0). Similar results were observed by McSorley *et al*. (1994a) for *M. javanica* and *M. incognita* in cv. 'SARA'. Under field conditions, Rodriguez-Kabana *et al*. (1991) observed that crop rotation of soybean with sorghum increased soybean productivity and was effective in controlling various nematodes, among them *M. arenaria*, *javanica* and *M. incognita.* Rotations with castor (*Ricinus communis* L.), velvet bean (*Mucuna pruriens* L.), American joint vetch (*Aeschynomene americana* L.) and sorghum *bicolor* were most effective in maintaining the lowest population densities of *Meloidogyne* species (Rodriguez-Kabana *et al*., 1991).

2.3 Alternatives to nematode resistance

Conventional organic amendments are bulky and have relatively low concentrations of nutrients, while they can contain high nitrates, soluble salts, heavy metals, and plant pathogens (Usman, 2013). Often, they have odours and other nuisances associated with health hazards. Use of conventional organic amendments requires a waiting period and thus, time investment from application to effective decomposition. Also, they are required in large quantities to give effective control and therefore, transport costs could be high. Some organic amendments take up to 10 years to completely mineralise for efficient management of nematodes (Usman, 2013). Storage of organic amendments could result in loss of as much as 90% of N content and much of the K within three

weeks, due to leaching, freezing, volatilisation at high temperatures and ammonia formation (Usman, 2013). Approximately 50% of N is available during the first season, 10-25% the next year, 10% the year after, *etc*. Organic amendments breakdown and N transformations require the same conditions as N fertilizers for best assimilation - good aeration, optimum moisture and mineral balance (Plaster, 1992). The Indigenous Cucurbitaceae Technology (ICT) Research programme was initiated, researched and developed by the Land Bank Chair of Agriculture – University of Limpopo under five themes: (1) ground leaching technology, (2) nematode resistance technology, (3) inter-generic grafting technology (4) agronomic technology and (5) botinemagation technology to mitigate the drawbacks of organic amendments.

2.3.1 Ground leaching technology

The ground leaching technology (GLT) was initially developed for use in ameliorating the drawbacks of using conventional organic amendments in managing plant-parasitic nematodes include: (1) inconsistent results in nematode suppression, (2) large quantities (10-500 mt/ha) are required to effect nematode suppression, (3) unavailability of organic materials in sufficient quantities, (4) when high quantities were available far from the site of use, this translated to high transport costs, (5) waiting period to enhance decomposition and, therefore, to avoid negative period, and (6) reduction of soil pH, which invariably increases the (un)availability of certain nutrient elements from the soil (Mankau, 1968; Mankau and Minteer, 1962; McSorley and Gallaher, 1995). Consequently, originally the GLT system was developed and researched for use in

post-emergent applications, but later on the system was adapted for pre-emergent applications.

In post-emergent application systems, mature fruits of wild watermelon (*Cucumis African*us L.) and wild cucumber (*Cucumis myriocarpus* Naude.) were cut into pieces, dried at 52°C (Makkar, 1999) for 72 h, ground in a Wiley mill and passed through a 1-mm-pore sieve (Mashela, 2002). The materials were separately applied at transplanting without first undergoing any microbial degradation activity (Mashela, 2002; Mashela and Mphosi, 2002). Crude extracts were spread in a shallow hole around the base of the stem of the transplant at 2-5 g/plant and then covered with soil. The amount translates to 20-71 kg ground material/ha (0.20-0.71 mt/ha) for 4 000 tomato plants/ha, which is much less when compared with quantities (10-500 mt/ha) required in conventional organic amendment systems (Stirling, 1991). Incidentally, the small quantities precluded high transport costs to haul the materials to the fields. Also, when used at transplanting, the waiting period for microbial decomposition was not necessary and the material did not reduce soil pH (Mashela and Nthangeni, 2002). Most importantly, suppression of nematode numbers was consistently achieved, regardless of the environment where the study was conducted.

In GLT systems microbial decomposition had negligible role in the efficacy of crude extracts from *C. myriocarpus* fruit, as shown by lack of interaction between this product and *Bacillus* species (Mphosi *et al*., 2004). Mashela and Pofu (2012) also demonstrated

that the material promoted nodulation of *Bradyrhizobium japonicum* (Kirchner) in cowpea (*Vigna unguiculata* L.). Also, independence of GLT system from microbial activity was demonstrated through elimination of *Bacillus* species in predictive stepwise regression models when using crude extracts of castor bean (*Ricinus communis* L.) fruit (Mashela and Nthangeni, 2002; Mofokeng *et al*., 2005) and fever tea (*Lippia javanica* L.) leaves. The concept, GLT emanated from the fact that the plant organ was ground (present tense: grind), with potent chemicals being leached out of crude extracts through irrigation or rain water.

Active gredients in *C. myriocarpus* fruit were cucurbitacin A, which comprises cucumin and leptidermin (Chen *et al*., 2005). Cucumin also suppresses the division of cancer cells in animals (Van Wyk *et al*., 1997). However, the suppression occurred at concentrations which were toxic to healthy cells, while at reduced concentrations, the material stimulated division of cancer cells. Quadratic relationships between cell divisions and concentrations ascribed to cucumin characterised responses of biological systems to extrinsic factors, which are referred to as density-dependent growth patterns (Salisbury and Ross, 1992). Using the observation of stimulation effect on cells, concentrations were reduced *in vitro* from 0 to 2.25 g/plant, with germination of tomato, watermelon and butternut squash having quadratic relationships (Mafeo and Mashela, 2009). In the trials, concentrations of crude extracts of *C. myriocarpus* fruit explained 91%, 97% and 91% to total treatment variation in inhibition of seed germination in tomato, watermelon and butternut squash, respectively. Results suggested that crude extracts of *C. myriocarpus* fruit had allelopathic effect on seed germination of test plants

and therefore, the material was not suitable for use as a pre-emergent bio-nematicide. Various studies were initiated using the Curve-Fitting Allelochemical Response Dosage (CARD) computer model (Liu *et al*., 2003) to determine concentrations where crude extracts of *C. myriocarpus* fruit stimulated germination of various crops in order to establish the pre-emergent quantities (Mafeo and Mashela, 2009).

Not all plant materials are suitable for use in GLT system. For instance, crude extracts of oleander (*Nerium indicum* L.) leaves, chilli pepper (*Capsicum annuum* L.) fruit and tamboti (*Spirostachys africana* L.) bark did not suppress nematode population, while those of fever tea (*Lipia javanica* L.) suppressed nematode population, but also reduced soil pH (Mashela *et al*., 2008). Apparently, there is a link between the suitability of a plant material in GLT and its being suitable for use in aqueous form. Crude extracts of fruits from the two *Cucumis* species, *L. javanica* leaves and *R. communis* fruit were highly successful in GLT systems (Mashela *et al*., 2011). Crude extracts of *C. myriocarpus* fruit and *R. communis* fruit had been successful when used in aqueous form as bionematicide in tomato and carrot (*Daucus carota* L.) production, respectively (Mashela, 2007). The stimulation of tomato plant growth due to the application of crude extracts from fruit of *C. myriocarpus* in the GLT system was referred to as a fertiliser effect (Mashela, 2002). However, due to the small quantity used in GLT systems, the treatment had no significant effect on nutrient elements in leaf tissues (Mashela, 2002).

In GLT system, the material is applied for 56 days, thereafter, there is no guarantee that the material could still be effective in suppressing nematodes, since results beyond this period were inconsistent (Mashela, 2007). When harvested at 120 days after initiating treatment with *Cucumis* species in GLT system, *T*. *semipenetrans* numbers were significantly higher in *Cucumis*-treated soil than in untreated controls (Maile, 2013). This observation suggested that reapplication after 56 days was necessary, which might add to operational costs.

2.3.2 Nematode resistance technology

The family Cucurbitaceae contains four genera of economic importance in agriculture, namely, *Citrullus*, *Cucumis*, *Cucurbita* and *Lagenaria* (Pitrat *et al.*, 1999). Limpopo Province, South Africa, is considered as the centre of diversity for wild *Cucumis* species (Kristkova *et al*., 2003), mainly *C. africanus* and *C. myriocarpus*. Host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *Meloidogyne* species were investigated in greenhouse, microplot and field trials (Pofu, 2012). Both *C. africanus* and *C. myriocarpus* were shown to be highly resistant to *M. incognita* races 2 and 4 and *M. javanica,* which are dominant in South Africa (Kleynhans *et al*., 1996).

2.3.3 Inter-generic grafting technology

Inter-generic grafting technology had incompatibility challenges due to different stem diameter sizes of scions and rootstocks in various crops (Tiederman, 1989). Grafts of watermelon with relatively thick stem diameters and *C. africanus* and *C. myriocarpus*

with relatively thin stem diameters had mortality rates of 64% (Pofu and Mashela, 2011). Procedures were developed to optimise the stem diameters of *Citrullus* and *Cucumis* genera, resulting into 100% survival of the intergrafts (Pofu and Mashela, 2011). In a subsequent greenhouse study (Pofu, 2012), all intergrafts survived and *C. africanus* and *C. myriocarpus* seedling rootstocks retained their capabilities to reduce population densities of *M. incognita*. Under field conditions the procedure was also successful, with intergrafts flowering earlier and producing higher fruit yield than intact plants (Pofu, 2012).

2.3.4 *Cucumis* agronomic technology

The technology involves all aspects of the agronomy of the two *Cucumis* species. Originally, fruits used in GLT systems were collected from the wild, since plants were difficult to propagate due to auto-allelopathy. Mafeo (2005) developed sexual propagation procedures and determined fertilisation requirements of *C. myriocarpus*. The procedures included the leaching of allelochemicals in running tapwater to improve germination (Mafeo, 2005). Nkgapele *et al*. (2011) also investigated irrigation and fertilisation requirements of *C. africanus* and *C. myriocarpus* in pot trials, while Mafeo and Mashela (2009) tested these requirements for *C. myriocarpus* under field conditions. In both trials, results suggested that moderate irrigation and fertilisation were required for achieving optimum fruit yield. Attempts are being made to use *in vitro* propagation in order to eliminate auto-allelopathic effects and the resultant poor emergence and therefore, non-uniformity in plant population stands (Maile, 2013).

2.3.5 Botinemagation technology

This technology involves the application of botanicals in the management of nematode population densities through irrigation water (Mashela *et al*., 2011). Incidentally, active ingredients from fruits of *Cucumis* species are extracted through a fermentation process which involves the use of pieces of dried fruits and EMROSA effective micro-organisms (EM) over a period of 14 days (Pelinganga and Mashela, 2012). Fermented crude extracts of 500 g fresh fruits from *Cucumis* species per 16 L of water were tested and reduced *M. incognita* race 2 population levels in roots and soil by 89% (range 80 – 100%) and 69% (range 52 – 79%) (Pelinganga *et al*., 2012). At low concentrations, both products had stimulatory effects on tomato plant growth, while at high concentrations they inhibited plant growth. Pelinganga and Mashela (2012) developed the mean concentration stimulation range (MCSR) using a series of dilutions from 40 g and 80 g dried fruits of *C. africanus* and *C. myriocarpus,* respectively. From both *Cucumis* species, the MCSR was approximately 3%, while nematode numbers were suppressed from 78-97% from *C. africanus* fruit and from 87-97% in *C. myriocarpus* fruit.

2.4 Crop rotation

Seasonal rotations of susceptible crops with non-host or poor-host crops in the same area of land remain one of the most important techniques used for nematode management (Usman, 2013). The occurrence of polyphagous nematode species with wide host ranges, such as *Meloidogyne* species, limits the potential inclusion of non-hosts in crop rotation systems (Viaene and Abawi, 1998). Hence, it is necessary to determine the host-status of individual crop cultivars for local nematode populations

before a rotation scheme is recommended for a particular field. Rotations using poor hosts or tolerant crops together with highly susceptible vegetable crops had been successfully used for management of nematodes (Wallace, 1971). However, crop rotations have economic costs for the grower. Use of witch-grass (*Panicum capillare* L.) in a peanut (*Arachis hypogaea* L.) rotation has beneficial effects on soil, reducing parasitic nematode populations and also causing shifts in rhizosphere microbial soil. Some fungi that affect the development of nematodes are dependent on specific plants to support their endophytic development or growth in the rhizosphere and so can only be used in certain crop rotations. Similarly, rotation crops, such as beans (*Phaseolus vulgaris* L.), maize (*Zea mays* L.) and cabbage (*Brassica oleracea* L.) that support extensive growth of the nematophagous fungus (*Pochonia chlamydosporia*) in their rhizospheres but supported limited reproduction of root-knot nematodes (Viaene and Abawi, 1998).

Antagonistic crops to nematodes are those that are considered to produce toxic substances, usually, while the crops are growing or after incorporation into the soil. In practical nematode management strategies, the use of this approach relies on pre-plant cover crops, intercropping or green manures. Marigold, neem, sunn hemp, castor bean, partridge pea, asparagus, rape seed and sesame have been extensively studied and used as antagonistic crops for nematode control. Sunn hemp (*Crotalaria juncea* L.) is often cultivated as a cover crop for direct seeding, intercrops or soil amendment and is considered an antagonistic crop for most plant-parasitic nematodes, especially root-knot nematodes (Wang *et al*., 2002). Population densities of *M. incognita* were affected by

previous cover crops of *C. juncea* in North Florida (Wang *et al*., 2002). Viaene and Abawi (1998) recommended the use of some *Crotalaria* species from Senegal as pre-crops for providing green manure while at the same time decreasing the level of root-knot nematode and increasing the level of beneficial mycorrhizal fungi. Marigolds (*Tagetes* species), has been shown to suppress plant parasitic nematodes, such as root-lesion and root-knot nematodes. Most antagonistic plants cultivated as pre-plant cover crops may be followed by soil incorporation of the biomass with a subsequent reduction of plant parasitic nematode numbers and the enhancement of nematode antagonists. However, it should be noted that grower acceptance of new strategies using antagonistic plants is based on economic and logistical considerations, as well as efficacy. Too often the large amounts of biomass required restricted the use of the approach to cheap sources of local species/waste products. Although some empirical tests have been made, the combined use of antagonistic plants and biological control agents has been little studied.

2.5 Gaps on the research problem

Preliminary studies demonstrated that sweet stem sorghum var. ndendane-X1 was suitable for use in ethanol production. However, host-status and host-sensitivity of sweet stem sorghum var. ndendane-X1 to *M. incognita* race 2 and *M. javanica* under diverse conditions are not documented. Also, the role of ICT in tomato-sweet stem sorghum cropping systems is also not documented.

CHAPTER 3
INFLUENCE OF SWEET STEM SORGHUM ON TWO *MELOIDOGYNE* SPECIES UNDER GREENHOUSE CONDITIONS

3.1 Introduction

The southern root-knot nematode (*Meloidogyne incognita* Kafoid and white) is widely distributed in tomato (*Solanum lycopersicum* L.) systems and causes enormous yield losses (Nzanza *et al*, 2013). The management of this nematode in tomato production using nematode resistance within a crop rotation system is complicated by the intra- and inter- continental existence of biological races (Sasser, 1977; Taylor and Sasser, 1978), which are morphologically similar with different host preferences. Worldwide, *M. incognita* race 1 constitutes 44% of the total *M. incognita* populations (Sasser, 1979). In contrast, *M. incognita* race 2, 3 and 4 constitutes 13%, 4% and 2% of the total *M. incognita* populations, respectively (Sasser, 1979). Generally, *M. incognita* race 1 is predominant in Europe (Robertson *et al*., 2006), while *M. incognita* races 1, 2 and 3 are widely distributed in the USA (Sasser, 1982). *Meloidogyne incognita* race 4 is widely distributed in West Africa (Olowe, 2010), while the predominant South African *M. incognita* races are 2 and 4 (Kleynhans *et al.*, 1996). The intra- and inter-continental existence of biological races in *M. incognita* implies that indigenous alternative crops should be adequately nematode-tested for inclusion in crop rotation systems for managing nematodes, which could be achieved through differential host tests or molecular markers (Robertson *et al*., 2006).

Globally, population densities of *M. javanica* contribute 73% of all *Meloidogyne* species, while *M. incognita*, *M. arenaria* and *M. hapla* contribute 17.2%, 7.1% and 2.7%, respectively (Taylor *et al*., 1982). However, in South Africa the distribution *M. incognita* and *M. javanica* is equivalent, while *M. javanica* is more aggressive than *M. incognita* (Kleynhans *et al*., 1996). Both *Meloidogyne* species should be managed in order to improve crop yields (Pofu, 2012).

Sweet stem sorghum (*Sorghum bicolor* L.) is indigenous to Africa (Bryan, 1990; Saballos, 2008), with the potential of serving as an ethanol-producing alternative crop. Sweet stem sorghum var. ndendane-X1 produces over 20% brix and 60 tons/ha of striped cane (Mashela, unpublished data) and therefore, qualifies to serve as an alternative crop for ethanol production as proposed for sweet stem sorghum varieties (Modiba and Mokoena, 2013). However, the major limiting factor to the successful inclusion of sweet stem sorghum var. ndendane-X1 in crop rotation systems would be yield losses by *Meloidogyne* species. Thus, a study was conducted to determine the host-status and host-sensitivity of sweet stem sorghum var. ndendane-X1 to *M. incognita* race 2 and *M. javanica* under greenhouse conditions.

3.2 Materials and methods

3.2.1 Study location

The experiment was initiated in the greenhouse at the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Ambient temperatures

averaged 28/21ºC, with maximum temperatures reduced using thermostatically-activated fans, while the minima were controlled by the greenhouse effect. The trial for *M. incognita* race 2 was conducted during summer (October-December) 2012, while that of *M. javanica* during autumn (January-March) 2013 (Figure 3.1).

Figure 3.1 Various views of *Meloidogyne*-sweet stem sorghum trials under greenhouse conditions.

3.2.2 Experimental design, treatments and procedures

Twenty-cm-diameter plastic pots were filled with 2 700 ml steam-pasteurised sand (300ºC for 1 h) and Hygromix (Hygrotech, Pretoria West) in a 3:1 (v/v) ratio. Pots were placed inside the greenhouse at 45-cm inter-row and 30-cm intra-row spacing. Sweet stem sorghum var. ndendane-X1 seeds were sown at two seeds/pot and thinned to one a week after emergence. When required, *M. incognita* race 2 or *M. javanica* inocula were prepared by extracting eggs and second-stage juveniles (J2s) from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.).

Three days after thinning, plants were each fertilised with 5 g of 2:3:2 (22) fertilizer mixture per plant to provide a total of 310 mg N, 210 mg P and 260 mg K, along with half-strength Hoagland solution (Murashige and Skoog, 1962). Plants were irrigated with 250 ml tapwater/plant every other day and each inoculated by dispensing approximate numbers of *M. incognita* or *M. javanica* eggs and second-stage juveniles J2s using a 20-ml-plastic syringe. Inoculums were placed into 5-cm-deep holes around the cardinal points of plant stems per replication. Treatments, namely, 0, 600, 1 000, 1 400, 1 800 and 2 200 eggs and J2s, were arranged in a randomised complete block design, with 10 replicates (n = 60).

3.2.3 Data collection

At 56 days after inoculation, chlorophyll content on flag leaves was measured using a digital chlorophyll meter (Minolta chlorophyll meter SPAD-502). Plant height was measured from the soil surface to the tip of the flag leaf, shoots were cut at the soil surface and stem diameters measured 5 cm above the severed ends using a digital vernier caliper. Shoots were oven-dried at 70°C for 72 h and weighed. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density per total roots per plant. Nematodes were extracted from 5 g roots per plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey and Barker, 1973) and passed through top-down nested 75- and 25-µm-pore-sieves. Contents of the 25-µm-pore-sieve were poured into 100-ml-plastic containers for counting under a stereomicroscope (Leica zoom 2000). Soil per pot was thoroughly mixed and a 250-ml soil sample was collected. Nematodes were

extracted from soil samples using the modified sugar-floatation and centrifugation method (Coolen and D'Herde, 1972). The soil sample was washed through top-down nested 150-, 75- and 25-µm-pore-sieves. Contents of the 25-µm-pore-sieve were poured into 50-ml centrifuge tubes.

Approximately 5 g kaolin powder was added in each tube and contents centrifuged at approximately 1 800 RPM for 5 minutes, with eggs and nematodes having settled at the bottom of the tubes with soil particles. The solution containing kaolin and debris was then decanted. A 469 g sugar/L tapwater was poured into centrifuge tubes and stirred once prior to centrifuging at 1 800 RPM for 60 seconds. The aliquot was then decanted onto 25-µm-pore-sieve, with sugar solution rinsed off eggs and nematodes, followed by washing into 100-ml-plastic containers for counting under a stereomicroscope. During counting, which was completed in 2 days, samples were temporarily stored at 5°C. Nematode numbers from roots were converted to nematode per total root system per plant, while soil nematode numbers were converted to 2 700-ml soil per pot. Reproductive factor (RF) values, described as a proportion of final nematode population density (Pf) and initial nematode population density (Pi), were computed. Relative penetration index (RPI) was also computed (Pofu and Mashela, 2012).

3.2.4 Data analysis

Prior to analysis of variance (ANOVA), nematode data were transformed through $\log_{10}(x + 1)$ to normalise the variances (Gomez and Gomez, 1984), but untransformed data

were reported. Data were subjected to ANOVA through the SAS software (SAS Institute, 2008) to determine the effects of Pi on RF values and plant growth components (Appendices 3.1-3.2). Mean separation for significant ($P \leq 0.05$) treatment means was achieved through the Waller-Duncan multiple-range test. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

3.3 Results

The RF and RPI values of *M. incognita* race 2 or *M. javanica* were zero and infinite, respectively, at all levels of inoculation (Table 3.1). Nematode inocula levels had no significant ($P \geq 0.05$) effect on overall plant growth, which was evaluated through chlorophyll content, dry root mass, dry shoot mass, plant height and stem diameter (Table 3.2).

Table 3.1 Responses of final nematode population densities (Pf), the reproductive factor (RF) and relative penetration index (RPI) of *Meloidogyne incognita* race 2 and *Meloidogyne javanica* to five levels of initial nematode population densities (Pi) on sweet stem sorghum var. ndendane-X1 (*Sorghum bicolor*) under greenhouse conditions (n = 60).

Meloidogyne incognita race 2					
Nematode (Pi)	Pf_{roots}	Pf_{soil}	Pf_{total}	RF	RPI
600	0	0	0	0	∞
1000	9	0	9	0,009	∞
1400	4	0	4	0,003	∞
1800	12	0	12	0,007	∞
2200	7	0	7	0,003	∞
$LSD_{0.05}$	21.32	-	21.32	0,139	∞

Meloidogyne javanica					
600	11	0	11	0,018	∞
1000	12	0	12	0,012	∞
1400	28	0	28	0,020	∞
1800	19	0	19	0,011	∞
2200	26	0	26	0,012	∞
$LSD_{0.05}$	21.32	-	21.32	0,670	∞

$RF = Pf_{total}/Pi$, $RPI = Pf_{roots}/Pf_{soil} - 1$.

Table 3.2 Responses of dry shoot mass (DSM), dry root mass (DRM), plant height (PHT), stem diameter (SDR) and chlorophyll content (CHLC) to six levels of initial population densities (Pi) of *Meloidogyne incognita* race 2 and *Meloidogyne javanica* on sweet stem sorghum var. ndendane-X1 under greenhouse conditions (n = 60).

Meloidogyne incognita race 2					
Nematode (Pi)	DSM (g)	DRM (g)	PHT (cm)	SDR (mm)	CHLC
0	0.82	0.58	68.52	3.76	36.42
600	1.30	0.55	63.53	3.63	36.10
1000	0.97	0.53	61.84	3.46	33.10
1400	0.73	0.57	66.34	3.77	37.73
1800	0.79	0.57	67.84	3.73	35.26
2200	0.95	0.61	66.89	3.86	38.09
$LSD_{0.05}$	0.58	0.19	9.23	0.67	6.37
Meloidogyne javanica					
0	1.27	0.46	61.35	4.37	33.81
600	1.22	0.67	67.41	4.38	30.57
1000	1.21	0.58	55.46	4.12	34.06
1400	1.35	0.63	66.28	4.42	33.77
1800	1.30	0.59	59.79	3.95	32.27
2200	1.16	0.57	59.00	4.07	33.36
$LSD_{0.05}$	0.50	0.14	10.08	0.71	7.90

3.4 Discussion

The RF and RPI values on sweet stem sorghum var. ndendane-X1 were primarily zero or indefinite. In plant-parasitic nematodes, there are some plants where nematode penetration into roots is allowed (Acedo, 1984; Huang, 1986; Ibrahim *et al*., 1980). In plants like sunn hemp (*Crotolaria junceae* L.), rye (*Secale cereal* L.), marigolds (*Tagetes species*), velvet bean (*Mucuna deeringiana* L.) and some cowpea (*Vigna unguiculata* L.) cultivars, penetration into roots is prevented (Caswell *et al*., 1991; McSorley and Gallaher, 1991; Ploeg, 1999; Roberts, 1993). Nematode resistance can occur after penetration or before penetration of nematodes into the root system. When nematode resistance occurs after penetration of J2s into roots it is post-infectional resistance, while that before penetration is pre-infectional resistance (Kaplan and Davis, 1987). In this study, none of the two forms of resistance were apparent as shown by indefinite RPI values.

Host-status in plant-parasitic nematodes is described in four forms: immune, resistant, tolerant or susceptible plants (Kaplan and Davis, 1987). Generally, resistant plants restrict nematode feeding and reproduction (Barker, 1993; Cook, 1991; Trudgill, 1991), while immune plants prevent nematode feeding and reproduction (Taylor and Sasser, 1978). Tolerant plants allow nematodes to feed and reproduce, but there is no reduction in plant growth and yield (Seinhorst, 1967). In contrast, susceptible plants allow nematodes to reproduce in roots, but also express plant growth and yield losses due to nematode infection (Seinhorst, 1967). Host-sensitivity measures the responses of a

plant to nematodes infection and is shown through reduction in plant growth and/or yield loss (Seinhorst, 1967).

3.4.1 *Meloidogyne incognita* race 2

Observations in this study suggested that sweet stem sorghum var. ndendane-X1 was immune to *M. incognita* race 2 under greenhouse conditions. *In vivo* studies for host-status of sweet stem sorghum on *M. incognita* under greenhouse conditions are scant. However, Mojtahedi *et al.* (1993) in *in vitro* trials observed that leaves of grain sorghum and sweet stem sorghum when chopped and incorporated as green manure suppressed population of densities of *M. incognita* when compared with untreated controls. Buried sorghum leaves inhibited migration of J2s (Mojtahedi *et al*., 1993), which suggested that the decomposing leaves had nematicidal properties. The chemical responsible for inhibition of mobility was presumed to be hydrogen cyanide (H−C≡N), which is generally produced when a chemical constituent called dhurrin is hydrolyzed by glucosidase (McGuidwin and Layne, 1995). Once leaf tissues are disrupted, the potent hydrogen cyanide produced by decomposing sorghum organs is toxic to a wide range of organisms and therefore, no nematode species should be immune to nematicidal effects of sorghum green manures.

In vivo studies population densities of *M. incognita* were inhibited due to the crop being non-hosts, while in cases of green manures microbial degradation of leaves might have increased allelochemicals in the soil. In greenhouse trial of host-status in selected

tropical rotation crops with *Meloidogyne* species and races, sorghum cultivar 'SX-17' did not support the reproduction of *M. incognita* races 1 and 3, *M. arenaria* race 1 or *M. javanica* (McSorley and Gallaher, 1991; McSorley *et al*., 1994a). In both studies, egg masses were not observed on roots, which supported the view that certain sorghum species might be immune to infection by *M. incognita*. McSorley *et al*. (1994b) recommended sorghum cv. 'SX-17' for use in crop rotation systems for the management of population densities of *Meloidogyne* species. In the USA, sorghum cv. 'Trudan' was also shown to suppress population densities of *M. hapla,* with subsequent increases in yield of vegetable produced as successor crops (Widmer and Abawi, 2002).

3.4.2 *Meloidogyne javanica*

Sweet stem sorghum var. ndendane-X1 was immune to *M. javanica* in greenhouse conditions, which confirmed findings of McSorley *et al*. (1994b). In another study, Rodriguez-Kabana *et al*. (1992) observed that sorghum cv. 'ST6E' reduced population densities of *M. javanica* to allow for the successful production of okra and sesame crops. Cultivar 'ST6E' was also effective as cover crop for controlling *M. javanica* and *M. arenaria* in Alabama (McSorley *et al*., 1994a; Rodriguez-Kabana *et al*., 1992). *Meloidogyne javanica* could potentially be managed with *S*. *bicolor* hybrids and crotalaria (*Crotolaria pumila* L.) in Florida (McSorley *et al*., 1994a). Similar results were obtained when sweet and grain sorghums were used as cover crops to suppress and manage *M. javanica* in Hawaii (McSorley *et al*., 1994a). Marigold, sesame, sweet stem

sorghum and sunn hemp were beneficial in controlling *M. javanica* under dryland conditons (Mojtahedi *et al*., 1993).

In vitro, studies at Oregon potato trials, sweet stem sorghum cv. 'Trudan 8', grain sorghum hybrids Sordan 79, SS-222 and Bravo II, when incorporated into the soil as green manure reduced population densities of *M. javanica* and *M. chitwoodi* (Mojtahedi *et al*., 1993). In Florida, grain sorghum cv. 'FS25E' and sweet stem sorghum cv. 'SX-17' did not reduce population densities of plant-parasitic nematodes such as lesion nematode (*Pratylenchus scribneri* Steiner) and ring nematodes (*Mesocriconema* species) (McSorley and Gallaher, 1991). However, Thies *et al*. (1995) suggested that although forage sorghum and sweet stem sorghum were hosts to *P. penetrans*, the crops were less suitable hosts than white clover (*Trifolium repens* L.), oat (*Avena sativa* L.) and rye forage crops. McSorley *et al*. (1994b) demonstrated that stubby-root nematode (*Trichodorus obtusus* Cobb) populations decreased when sorghum species were used in crop rotation systems.

3.5 Conclusions

Sweet stem sorghum var. ndendane-X1 was apparently immune to *M. incognita* race 2 and *M. javanica*. Thus, the variety has the potential to serve in crop rotation systems which are intended to suppress plant-parasitic nematodes as an economically important alternative crop that can be used in ethanol production.

CHAPTER 4
EFFECTS OF SWEET STEM SORGHUM ON *MELOIDOGYNE INCOGNITA* RACE 2 UNDER MICROPLOT CONDITIONS

4.1 Introduction

Sweet stem sorghum (*Sorghum bicolor* L.) var. ndendane-X1 appeared to be an appropriate industrial crop for biofuel production due to its immunity to *Meloidogyne incognita* race 2 and *M. javanica* as previously shown under greenhouse conditions (Chapter 3). Elsewhere, most nematode-studies in sorghum were conducted under field conditions (Mojtahedi *et al*., 1993; McSorley, 1994b). Generally, results from greenhouse and field studies may differ due to the existence of distinctly different conditions (Perry *et al*., 2009). In most cases microplot studies integrates field and greenhouse conditions at different degrees – except for soil variation where in both greenhouse and microplot studies it can remain constant. The host-status and host-sensitivity of sweet stem sorghum var. indendane-X1 to *M. incognita* race 2 under microplot conditions is not documented. Therefore, the objective of this study was to determine the host-status and host-sensitivity of sweet stem sorghum var. ndendane-X1 to *M. incognita* race 2 under microplot conditions.

4.2 Materials and methods

The experiment was initiated as a microplot trial at the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). The trial was conducted during spring (August-October) in 2012 (Figure 4.1). Thirty-cm-diameter plastic pots

were filled with 10 000 ml steam-pasteurised sand (300ºC for 1 h) and Hygromix in a 3:1 (v/v) ratio.

Figure 4.1 Various views of the microplot experiment on *Meloidogyne*-sweet stem sorghum trial.

4.2.1 Experimental design, treatments and procedures

Pots were 75% buried and arranged in 0.30 m intra-row and 0.25 m inter-row spacing. The seven treatments, namely 0, 200, 600, 1 000, 1 400, 1 800 and 2 200 J2s of *M. incognita* were arranged in a randomised complete block design, with 14 replications (n = 98). One week after sowing sweet stem sorghum var. ndendane-X1, seedlings were thinned and fertilised with 5 g of 2:3:2 (22) per plant to provide the previously described nutrient elements (Chapter 3). When necessary, plants were irrigated with 500-ml tapwater/plant every other day. *Meloidogyne incognita* race 2 was prepared and appropriate plants inoculated as explained previously (Chapter 3).

4.2.2 Data collection

At 56 days after inoculation, plant and nematode variables were collected, prepared and recorded as previously described (Chapter 3). In addition to reproductive factor (RF) and relative penetration index (RPI), effect of sweet stem sorghum on nematodes was also measured using the reproductive potential (RP), which is total eggs and J2s/g roots (Matabane, 2013).

4.2.3 Data analysis

Data were subjected to analysis of variance (Appendices 4.1-4.3) as previously described (Chapter 3).

4.3 Results

Treatments had no effect on Pf (Appendix 4.1), RF, RPI and RP were each affected by the treatments.

4.3.1 Effect on nematode numbers

4.3.1.1 Reproductive factor

In sweet stem sorghum var. ndendane-X1, RF values were less than one at all levels of inoculation (Table 4.1). The RF values decreased quadratically with increasing $\log_{10}$ (Pi + 1) values, with the variation being explained by 98% of the model (Figure 4.2).

4.3.1.2 Reproductive potential

The RP values of *M. incognita* race 2 on var. ndendane-X1 were different ($P \leq 0.05$) at different levels of inoculation and were slightly higher than one but none was greater than 6 units (Table 4.1).

4.3.1.3 Relative penetration index

The RPI values were also affected by different levels of inoculation although there was no clear direction of the effects (Table 4.1).

4.3.2 Effect on plant growth

Treatments had no significant effects on chlorophyll content, dry root mass, dry shoot mass and sucker number of sweet stem sorghum var. ndendane-X1 (Table 4.2).

Table 4.1 Responses of final population densities (Pf) of *Meloidogyne incognita* race 2 as expressed by the reproductive factors (RF), reproductive potential (RP) and relative penetration index on sweet stem sorghum var. ndendane-X1 under microplot conditions (n = 98).

Nematode level	Fresh root (g)	Final nematode population density			Proportions		
		Total Pf/ Root	Total Pf/10 000 ml soil	Total nematode	RF	RP	RPI
200	56.57	72	71	143	0.71	2.44	1.01
600	54.21	95	142	237	0.40	4.55	0.66
1000	53.20	111	168	279	0.32	5.94	0.66
1400	54.57	104	100	204	0.14	3.62	1.04
1800	50.54	107	100	207	0.11	4.02	1.07
2200	49.95	96	128	224	0.10	4.71	0.75
LSD $_{0.05}$	8.11	106.23	253.12	261.24	0.06	0.22	0.24

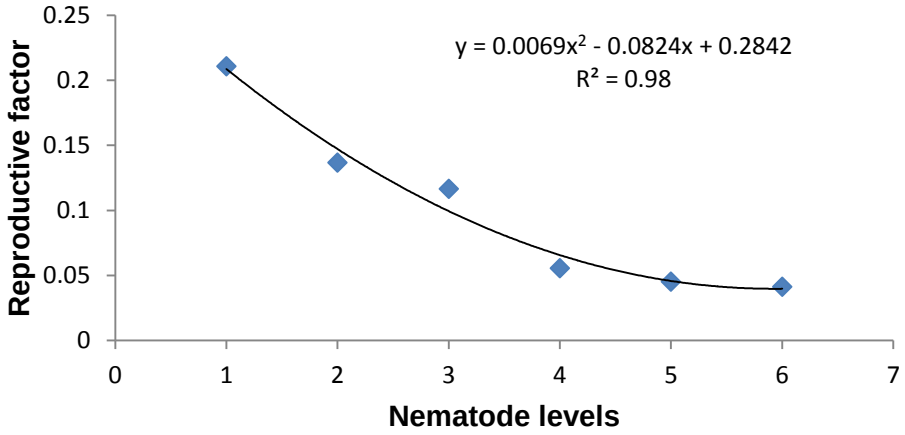

Figure 4.2 Responses of reproductive factors *of Meloidogyne incognita* to increasing initial nematode numbers in sweet stem sorghum var. ndendane-X1.

Table 4.2 Responses of chlorophyll content (CHLC), dry root mass (DRM), dry shoot mass (DSM) and number of suckers to seven levels of initial population densities (Pi) of *Meloidogyne incognita* race 2 on sweet stem sorghum (*Sorghum bicolor*) var. ndendane-X1 under microplot conditions (n = 98).

Treatment (Pi)	CHLC	DRM (g)	DSM (g)	Suckers
0	14.68	10.25	29.78	0.81
00	16.68	10.01	27.29	0.83
600	15.15	9.59	26.43	0.78
1000	14.38	9.41	26.66	0.82
1400	15.00	9.66	27.02	0.80
1800	15.17	8.94	26.91	0.79
2200	14.48	8.84	25.63	0.78
$LSD_{0.05}$	2.75	1.43	3.59	0.05

4.4 Discussion

The RF values of less than one suggested that sweet stem sorghum var. ndendane-X1 was a non-host to *M. incognita* race 2, which agreed with previous observations under greenhouse conditions (Chapter 3). McSorley (1994a) observed that sweet stem sorghum cv. 'SARA' was a non-host to *M. javanica* and *M. incognita* under both greenhouse and field conditions. The concave quadratic relationship between RF values and the transformed Pi values suggested that var. indendane-X1 had high degree of nematode resistance to *M. incognita* race 2. Previously, Pofu (2012) demonstrated that RF values and the transformed Pi values on certain indigenous *Cucumis* species had convex quadratic relationships where RF values started by increasing, reaching a peak and then decreasing. Results of the current study

suggested that the optimum RF values of *M. incognita* race 2 on sweet stem sorghum var. ndendane-X1 was at the lower-end of the used inoculum levels. In initial studies, Pofu (2012) also had similar observations, with appropriate adjustments of inoculation levels providing convex quadratic relationships, which characterise density-dependent growth patterns.

Generally, the quadratic relationships are the main feature of density-dependent growth patterns, which have three growth responses: stimulation, no response and inhibition responses to increasing levels of chemical concentrations (Salisbury and Ross, 1992). Plants use various secondary metabolites, referred to as allelochemicals, in defense (Mojtahedi *et al*., 1993; McSorley, 1994b). Various studies (Pelinganga, 2013; Tseke, 2013) demonstrated that nematode populations respond to increasing concentrations of allelochemicals in density-dependent growth patterns.

The RP values in this study, which were not equivalent to zero, do not agree with the previous greenhouse observations which suggested that sweet stem sorghum var. ndendane-X1 was immune to *M. incognita* race 2 (Chapter 3). Generally, immunity to nematodes in plants is rare, while it is common in animals (Menezes and Jared, 2002). The positive RPI values observed on var. ndendane-X1 for *M. incognita* race 2 suggested that more nematode juveniles were inside roots than in soil (Pofu and Mashela, 2012). Apparently, nematode resistance in this variety to *M. incognita* race 2 was of the post-penetration form, which is common in various plant species (Kaplan and

Davis, 1987). The post-penetration nematode resistance is referred to as active resistance (Kaplan and Davis, 1987) and it can be introgressed into highly nematode-susceptible plants (Kaplan and Davis, 1987). However, this type of nematode resistance is also highly susceptible to environmental conditions.

The failure of various inoculum levels of *M. incognita* race 2 to reduce growth of sweet stem sorghum var. ndendane-X1 is another feature which suggests that the variety was resistant to *M. incognita*. Generally, for nematodes to reproduce and therefore increase population nematode densities, feeding is a pre-requisite (Ferraz and Brown, 2002). Generally, when population nematode densities increase beyond the damage threshold level, plant growth is drastically reduced. In this study, failure of nematode infection to reduce growth of var. ndendane-X1 is another evidence that this variety is highly resistant to *M. incognita* race 2. According to the nematode resistance classification (Seinhorst, 1967), the target variety is resistant to *M. incognita* race 2. Resistance in sweet stem sorghum var. ndendane-X1 confirm those in other sorghum varieties (Chapter 3; Rodriquez-Kabana, where the inclusion in crop rotation systems intended to reduce population densities invariably improved yield of successor crops. Thus, var. ndendane-X1 is highly suitable for use in crop rotation systems.

4.5 Conclusions

Sweet stem sorghum var. ndendane-X1 is resistant to *M. incognita* race 2 and could therefore, be used in crop rotation systems intended to suppress population densities of *M. incognita* race 2.

CHAPTER 5
RESPONSES OF *MELOIDOGYNE* SPECIES AND GROWTH OF SWEET STEM SORGHUM TO NEMAFRIC-BG PHYTONEMATICIDE UNDER FIELD CONDITIONS

5.1 Introduction

Sweet stem sorghum (*Sorghum bicolor* L.) was shown to be highly resistant to the southern root-knot (*Meloidogyne incognita* Kafoid and White) under greenhouse and microplot conditions (Chapter 3; Chapter 4), while similar results were observed for *M. javanica* under greenhouse conditions (Chapter 3). The unusual high level of nematode resistance in sweet stem sorghum var. ndendane-X1 suggested that this variety could serve as an alternative crop for the production of ethanol, in attempts to eliminate the 3-5 years of fallowing in tomato (*Solanum lycopersicum*) production (Nzanza *et al*., 2013). In the three separate trials (Chapter 3; Chapter 4), plants were exposed to pure cultures of *Meloidogyne* species, while under field conditions the nematode occurs as a mixture of species which in South Africa comprise *M. incognita* races 2 and 4 and *M. javanica* (Kleynhans *et al*., 1996). In addition to the use of nematode resistance in plants to manage plant-parasitic nematodes, other products are being tested as alternatives to methyl bromide at the University of Limpopo. Methyl bromide had since been withdrawn from the agro-chemical markets in 2005 due to its environment-unfriendliness (Mashela *et al*., 2011).

In Limpopo Province, South Africa, alternatives to methyl bromide include the use of nemarioc-AG and nemafric-BG, which are phytonematicides derived from fruits of wild

cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.), respectively (Mashela *et al*., 2011). The two plants, from within the Cucurbitaceae Family (Pitrat *et al*., 1999), have a center of diversity in Limpopo Province, South Africa (Kristkova *et al*., 2003). Mafeo (2012) demonstrated that nemarioc-BG phytonematicide was highly phytotoxic to various economic crops, particularly when applied prior to seed germination. However, when applied after transplanting, the product was less phytotoxic, with cases of stimulated growth reported in some cases (Mashela, 2002; Mashela *et al*., 2011). Nemafric-BG phytonematicide had been hardly tested against population densities of *Meloidogyne* species, but had been tested against those of the citrus nematode (*Tylenchulus semipenetrans* Cobb) (Maile, 2013). Also, responses of population densities of *Meloidogynes* species and growth of sweet stem sorghum var. ndendane-X1 to nemafric-BG phytonematicide are not documented. The objective of this study, therefore, was to determine the responses of *Meloidogyne* species and growth of sweet stem sorghum to nemafric-BG phytonematicide under field conditions.

5.2 Materials and methods

5.2.1 Study location and conditions

The experiment was conducted under field conditions at the Plant Protection Skills Centre, University of Limpopo, South Africa (23˚ 53'10"S, 29˚ 44'15"E). The site is characterised by summer (Oct-Dec) rainfall, with mean annual rainfall of less than 500 mm. Maximum/minimum temperatures average 28/19^{o}C. The trial was conducted in summer 2012 (Figure 5.1).

Figure 5.1 Sweet stem sorghum var. ndendane-X1 under field conditions.

The site contained Hutton soil (65% sand, 30% clay, 5% silt), which had 1.6% organic C, EC_e 0.148 ds/m and pH (H_2O) of 6.5. The site has high population densities of *Meloidogyne* species.

5.2.2 Experimental design, treatments and cultural practices

The 2-week-old sweet stem sorghum seedlings var. ndendane-X1, planted at 0.2 m inter-row and 0.3 m intra-row spacings, were thinned to one. The site was sampled for initial population density (Pi) after tomato production, with 3 700 second-stage juveniles/250 ml sample soil. Treatments, namely 0, 2, 4, 6, 8 and 10 g nemafric-BG phytonematicide/plant were arranged in a randomised complete block design, with 13 replicates (n = 78). After thinning, plants were each fertilised with 5 g of 2:3:2 (22) per plant, to provide a total of 310 mg N, 210 mg P and 260 mg K and half-strength Hoagland solution. When necessary, plants were irrigated with 1 L/plant of tapwater

every other day. Plants were treated for the seedling cutworm, termites and locust with cutworm-bait.

5.2.3 Data collection

At 56 days after thinning, plant height, chlorophyll content and sucker numbers were recorded as previously described (Chapter 3). Shoots were cut at the soil surface and oven-dried at 70°C for 72 h for dry matter determination. Root systems were removed using a garden fork and immersed in water to remove soil particles, blotted dry and 5 g roots sampled for nematode extraction as described previously (Chapter 3). A 250-ml soil sample/plant was collected for nematode extraction and assessment (Chapter 3).

5.2.4 Data analysis

Prior to analysis of variance (ANOVA), nematode and number of suckers were separately transformed through $\log_{10}(x + 1)$ to normalise the variances (Gomez and Gomez, 1984), but untransformed data were reported. Data were subjected to ANOVA through the SAS software (SAS Institute, 2008) to determine the effects of nemafric-BG phytonematicide on juveniles in soil and eggs and juveniles on root, chlorophyll content, dry shoot mass, plant height and sucker number were recorded (Appendices 5.1-5.2). Mean separation for significant ($P \leq 0.05$) treatments was achieved through the Fisher's least significant different test. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

5.3 Results

Phytonematicide effects were highly significant ($P \leq 0.01$) on population densities of eggs and juveniles in root and juveniles in soil, contributing 40% and 40% in total treatment variation of nematodes in root and soil, respectively (Appendix 5.1). Relative to untreated control, nemafric-BG phytonematicide reduced eggs and juveniles in root and juveniles in soil by 76-85% and 24-65%, respectively (Table 5.1). Relative to Pi (≈ x juveniles) in sweet stem sorghum without nemafric-BG phytonematicide, Pf was reduced in roots and soil by 77-85% and 24-60% respectively (Table 5.1). Nematode effects on chlorophyll content, dry shoot mass, plant height and sucker number were not significant at 5% level of probability (Appendix 5.2; Table 5.2).

Table 5.1 Responses of final nematode population densities (Pf) in 250-ml soil and roots to six levels of nemafric-BG phytonematicide on growth of sweet stem sorghum var. ndendane-X1 in the field conditions (n = 78).

Nemafric-BG	Pf 5 g roots		Pf 250 ml soil	
(g)	Variable	Impact (%)	Variable	Impact
0	141[a]	-	123[a]	-
2	32[b]	-77	93[b]	-24
4	29[b]	-79	50[c]	-59
6	29[b]	-79	56[c]	-55
8	33[b]	-76	43[c]	-65
10	21[c]	-85	49[c]	-60

Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's least significant difference test.

Table 5.2 Responses of chlorophyll content (CHLC), dry shoot mass (DSM), plant height (PHT) and sucker number (SU) on growth of sweet stem sorghum var. ndendane-X1 with nemafric-BG phytonematicide to *Meloidogyne* species under field conditions (n = 78).

Nemafric-BG (g)	CHLC	DSM (g)	PHT (cm)	SU. #
0	8.71	33.75	100.68	0.57
2	8.45	33.78	104.77	0.67
4	8.87	35.75	103.73	0.61
6	9.90	36.02	99.42	0.62
8	9.58	39.98	108.58	0.50
10	10.69	35.83	100.77	0.63
$LSD_{0.05}$	1.96	7.87	11.71	0.24

5.4 Discussion

The sweet stem sorghum var. ndendane-X1 kept population densities of *Meloidogyne* species relatively low as shown by the phytonematicide-untreated control. Nemafric-BG phytonematicide and the variety kept population densities of *Meloidogyne* at even lower levels than when the variety was used alone. The lower population densities of *Meloidogyne* species in soil and in root of var. ndendane-X1 alone agreed with findings under greenhouse (Chapter 3) and microplot (Chapter 4) conditions. Also, the current observation confirmed those of other studies where sorghum varieties under diverse conditions were highly resistant to plant-parasitic nematodes (McGuidwin and Layne, 1995; McSorley *et al*., 1994a).

The suppression of population densities of *Meloidogyne* species by nemafric-BG phytonematicide confirmed other observations under greenhouse conditions where the

product reduced population densities of *T. semipenetrans* (Maile, 2013). Generally, the product had been tested widely in liquid formulation as nemafric-BL, where it reduced population densities of *Meloidogyne* species in tomato roots under greenhouse conditions by 87-96%, microplot conditions by 64-99% and field conditions by 79-85% (Pelinganga, 2013). Similarly, in the soil the material reduced juveniles by 93-97%, 41-91% and 79-85% under greenhouse, microplot and field conditions, respectively (Pelinganga, 2013). The lower nematode magnitudes in the current study when compared to those of others (Pelinganga, 2013) could be attributed to the high degree of nematode resistance in the variety which was being protected from nematode damage by nemafric-BG phytonematicide.

In other studies (Pelinganga, 2013), a nematode-susceptible tomato cultivar was used, resulting in high population densities in untreated controls, while in the current study the opposite was true. Due to the high degree of nematode resistance in sweet stem sorghum var. ndendane-X1, the untreated control had low nematode numbers, which were reduced by the protected plants. Although the design of the study did not allow for the determination of interaction between var. ndendane-X1 and nemafric-BG phytonematicide, it was clear in the current study that the two complemented each other in the suppression of population densities of *Meloidogyne* species. Generally, nemafric-BG phytonematicide at the concentrations used had no effect on growth of sweet stem sorghum var. ndendane-X1, which suggested that the two were compatible and therefore, suitable for integration in the purpose of suppressing population densities of *Meloidogyne* species.

Generally, phytonematicides either stimulate plant growth, have no effect on plant growth or inhibit plant growth (Mafeo, 2012; Maile, 2013; Pelinganga, 2013). The three different responses characterise density-dependent growth patterns (Liu *et al*., 2003; Salisbury and Ross, 1992), which is an important concept in the determination of mean stimulation concentration range (MSCR) ─ a non-phytotoxic concentration of phytonematicides (Pelinganga *et al*., 2012).

5.5 Conclusions

Both sweet stem sorghum var. ndendane-X1 and nemafric-BG phytonematicide reduced population densities of *Meloidogyne* species under field conditions. Additionally, nemafric-BG phytonematicide at the used concentrations did not have inhibition effects on growth of ndendane-X1. In conclusion, var. ndendane-X1 and nemafric-BG phytonematicide could be used to complement each other in integrated management of population densities of *Meloidogyne* species.

CHAPTER 6
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

6.1 Summary

Sweet stem sorghum (*Sorghum bicolor* L.) var. ndendane-X1 has the potential of serving as an ethanol-producing plant. The variety was shown to be highly resistant to *Meloidogyne incognita* race 2 and *M. javanica* under diverse conditions. Also, the variety is compatible with nemafric-BG phytonematicide, which is being developed for management of population densities of *Meloidogyne* species in tomato production.

6.2 Significance of findings

The build-up of soil-borne diseases, including plant-parasitic nematodes, precludes monoculture in tomato-production. The tomato field has a 3 to 5-year cycle without the primary crop, which are currently being used for fallowing with natural grasses or uneconomic alternative exotic crops. Sweet stem sorghum var. ndendane-X1 was shown to be highly resistant to *M. incognita* race 2 and *M. javanica*, which are widely distributed in Limpopo Province. Also, the nematode resistance in the variety was compatible with the ground leaching technology, which is currently used for managing population densities of nematodes in tomato production. Thus, the production of sweet stem sorghum during the 3-5 years for the production of ethanol would improve soil health, while also improving the economic suitability of commercial tomato farming systems as commercial tomato-sweet stem sorghum farming systems.

6.2 Recommendations

A pilot project of a commercial tomato-sweet stem sorghum farming systems is necessary to establish the economic potential of the proposed system. The pilot project would also be used to provide empirical information on soil health of the proposed system in order to assess the long-term sustainability of the system.

6.3 Conclusions

Sweet stem sorghum var. ndendane-X1 is resistant to *M. incognita* race 2 and *M. javanica*. The variety has the attributes to serve in biofuel industries for ethanol production. The proposed tomato-sweet stem sorghum cropping systems might result in spin offs which could benefit the country in terms of sustainable environment impact, job creation and wealth creation.

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APPENDICES

Appendix 3.1 Sum of squares for roots, soil and total nematode on sweet stem sorghum var. ndendane-X1 under greenhouse conditions (n = 60).

Meloidogyne incognita race 2

Source of variation	DF	Total roots		Total soil		Total	
		SS	**%**	SS	**%**	SS	**%**
REP	9	1.632	13^{ns}	0	0	1.632	13^{ns}
TRT	5	0.578	3^{ns}	0	0	0.578	3^{ns}
ERROR	45	12.378	84	0	0	12.378	84
TOTAL	59	14.589	100	-	-	14.589	100

Meloidogyne javanica

REP	9	9.428	31^{ns}	0	0	9.428	31^{ns}
TRT	5	1.042	4^{ns}	0	0	1.042	4^{ns}
ERROR	45	19.681	65	0	0	19.681	65
TOTAL	59	30.152	100	-	-	30.152	100

Values with ^{ns} were not significant at $P \leq 0.05$.

Appendix 3.2 Sum of squares for dry shoot mass (DSM), dry root mass (DRM), plant height (PHT), stem diameter (SDR) and chlorophyll content (CHLC) of sweet stem sorghum var. ndendane-X1 to *Meloidogyne incognita* race 2 and *Meloidogyne javanica* under greenhouse conditions (n = 60).

Meloidogyne incognita race 2

Source of variation	DF	DSM (g)		DRM (g)		PHT (cm)		SDR (mm)		CHLC	
		SS	%	SS	%	SS	%	SS	%	SS	%
REP	9	0.16	16^{ns}	0.06	31^{ns}	0.13	27^{ns}	0.23	47^{ns}	0.29	25^{ns}
TRT	5	0.7	7^{ns}	0.00	1^{ns}	0.06	14^{ns}	0.00	1^{ns}	0.09	8^{ns}
ERROR	45	0.78	77	0.14	68	0.29	59	0.26	52	0.78	67
TOTAL	59	1.01	100	0.21	100	0.50	100	0.50	100	1.17	100

Meloidogyne javanica

REP	9	0.33	37^{ns}	0.10	49^{ns}	0.09	19^{ns}	0.16	41^{ns}	0.71	33^{ns}
TRT	5	0.01	1^{ns}	0.01	9^{ns}	0.05	13^{ns}	0.01	4^{ns}	0.04	2^{ns}
ERROR	45	0.56	62	0.08	42	0.32	68	0.21	55	1.37	65
TOTAL	59	0.91	100	0.20	100	0.47	100	0.39	100	2.13	100

Values with ^{ns} were not significant at $P \leq 0.05$.

Appendix 4.1 Sum of squares for roots, soil and total nematode of sweet stem sorghum var. ndendane-X1 to *Meloidogyne incognita* race 2 under microplot conditions (n = 98).

Source of variation	DF	Nematode root		Nematode in soil		Total nematode	
		SS	%	SS	%	SS	%
REP	13	30.009	20^{ns}	12.236	16^{ns}	7.362	15^{ns}
TRT	5	7.300	5^{ns}	4.315	5^{ns}	3.541	7^{ns}
ERROR	65	115.586	75	60.815	79	39.532	78
TOTAL	83	152.894	100	77.367	100	50.437	100

Values with ^{ns} were not significant at $P \leq 0.05$.

Appendix 4.2 Sum of squares for reproductive factor, reproductive potential and relative penetration index of *Meloidogyne incognita* race 2 on sweet stem sorghum var. ndendane-X1 under microplot conditions (n = 98).

Source of variation	DF	Reproductive factor		Reproductive potential		Relative penetration index	
		SS	%	SS	%	SS	%
REP	13	0.099	12	1.534	20	0.876	15
TRT	5	0.311	38	0.718	10	0.931	17
ERROR	65	0.416	50	5.350	70	3.911	68
TOTAL	83	0.827	100	7.603	100	5.720	100

Appendix 4.3 Sum of squares for chlorophyll content (CHLC), dry root mass (DRM), dry shoot mass (DSM) and number of suckers to *M. incognita* race 2 on sweet stem sorghum var. ndendane-X1 under microplot conditions (n = 98).

Source of variation	DF	CHLC		DRM (g)		DSM (g)		SUCKERS	
		SS	%	SS	%	SS	%	SS	%
REP	13	0.164	14^{ns}	0.181	25^{ns}	0.046	10^{ns}	0.082	17^{ns}
TRT	6	0.039	3^{ns}	0.037	5^{ns}	0.025	5^{ns}	0.026	5^{ns}
ERROR	78	1.004	83	0.501	70	0.387	85	0.391	78
TOTAL	97	1.207	100	0.720	100	0.458	100	0.500	100

Values with ^{ns} were not significant at P ≤ 0.05.

Appendix 5.1 Sum of squares for population densities of *Meloidogyne* species under six levels of nemafric-BG phytonematicide on sweet stem sorghum var. ndendane-X1 under field conditions at 56 days (n = 78).

Source of variation	DF	Roots		Soil	
		SS	%	SS	%
Replication	12	1.4759	8^{ns}	1.1286	14^{ns}
Treatment	5	7.1911	40^{**}	3.1195	40^{**}
Error	60	9.4785	52	3.6374	46
Total	77	18.1555	100	7.8856	100

Values with ** were significant at P ≤ 0.01, while, ^{ns} values were not significant at P ≤ 0.05.

Appendix 5.2 Sum of squares for chlorophyll content (CHLC), dry shoot mass (DSM), plant height (PHT) and sucker number of sweet stem sorghum var. ndendane-X1 at six levels of nemafric-BG phytonematicide under *Meloidogyne* infested field (n =78).

Source of variation	Chlorophyll content			DSM		PHT		Sucker No	
	DF	SS	%	SS	%	SS	%	SS	%
Replication	12	0.260	29^{ns}	0.333	23^{ns}	0.120	26^{ns}	0.483	8^{ns}
Treatment	5	0.073	8^{ns}	0.056	4^{ns}	0.019	4^{ns}	0.226	3^{ns}
Error	60	0.554	63	1.061	73	0.326	70	5.632	89
Total	77	0.888	100	1.451	100	0.465	100	6.342	100

Sources of variation followed by ^{ns} were not significant at $P \leq 0.05$.