NEMATODE RESISTANCE AND RESISTANCE MECHANISM IN SWEET POTATO CULTIVARS 'BOPHELO', 'BOSBOK' AND 'MVUVHELO' TO *MELOIDOGYNE INCOGNITA*

MMBONISENI MESHACK MAKHWEDZHANA

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SUPERVISOR   :   DR. K.M. POFU (ARC)

CO-SUPERVISOR :   PROF. P.W. MASHELA (UL)

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DECLARATION

I, Mmboniseni Meshack Makhwedzhana declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agricultural Management 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.

Candidate: M.M. Makhwedzhana   Signature   Date

DEDICATION

To my brothers and sisters with Love

ACKNOWLEDGEMENTS

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ABSTRACT

Meloidogyne incognita race 2 is internationally recognised as one of the most aggressive *Meloidogyne* species and it is also widely distributed in Limpopo Province, where it occurs alone or as mixed populations with other *Meloidogyne* species. Traditionally, *Meloidogyne* species had been managed using synthetic chemical nematicides, most of these products had been withdrawn from agro-chemical markets due to their environment-unfriendliness. Following the withdrawal of synthetic chemical nematicides, nematode resistance had been the most preferred strategy for managing high nematode population densities. The availability of nematode resistant genotypes in sweet potato (*Ipomoea batatas*) would enhance the use of resistance in managing *Meloidogyne* species and races in Limpopo Province. Generally, should post-infectional nematode resistance be available in the test sweet potato cultivars, the information would be relayed to plant breeders for use as source of introgression in various commercial cultivars where nematode-resistant genotypes do not exist. The objectives of the study, were to determine: (1) Host-status and host-sensitivity in sweet potato cv. 'Bophelo', 'Bosbok' and 'Mvuvhelo' to *M. incognita* race 2. (2) the existing nematode resistance mechanism in any of the test cultivars that had resistance to *M. incognita* race 2. For achieving Objective 1, eight treatments namely, 0, 25, 50, 125, 250, 625, 1250 and 3125 eggs and second stage-juveniles (J2) *M. incognita* race 2 were used under greenhouse trials for each cultivar. To achieve Objective 2, sweet potato plants were inoculated with 100 J2 with four plants harvested every other day for 30 days counting to 15 harvesting times. At 56 days after inoculation, cv. 'Bophelo' had

reproductive factor (RF) values above unity for *M. incognita* race 2 and plant growth variables were reduced. Therefore, the cultivar was a susceptible host to *M. incognita* race 2 and mechanism trial was not conducted for this cultivar. *Meloidogyne incognita* race 2 failed to reproduce on cultivars 'Bosbok' and 'Mvuvhelo' whereas nematode infection did not affect plant growth and therefore, the two cultivars were resistant to *M. incognita* race 2. Mechanisms of resistance to *M. incognita* race 2 on cultivars 'Bosbok' and 'Mvuvhelo' demonstrated significance existence of (1) necrotic spots, (2) poorly developed giant cells, (3) formation of rootlet interferences (4) absence of root galls and (5) non-detectable J2 in roots. All these features suggested the existence of post-infectional nematode resistance in the two cultivars to *M. incognita* race 2. In conclusion, cultivar 'Bophelo' was susceptible to *M. incognita* race 2, whereas cultivars 'Bosbok' and 'Mvuvhelo' were resistant to *M. incognita* race 2, with the evidence of post-infectional nematode resistance to the nematode species.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Sweet potato (*Ipomoea batatas*) is a major staple food in Africa, Asia, the Caribbean, and South America, where it is declared highly nutrient-rich in calories and biological active phytochemicals such as dietary fibre, pectin, beta-carotene, carbohydrates, vitamin A and C, iron, potassium and protein (Ana *et al.*, 2010; Omotobora *et al.*, 2014; Sun *et al.*, 2012). However, it has been found to suffer severe damage caused by root-knot (*Meloidogyne* species.) nematodes, with significant reduction on quality of storage root and yield (Cervantes-Flores, 2000). *Meloidogyne* species are the most widely spread and damaging nematode species to sweet potato, and occur in most areas where sweet potato is cultivated (Cervantes-Flores and Yencho, 2002). *Meloidogyne incognita* races 2 and 4 and *M. javanica* are widely distributed in South Africa and could result in crop yield losses up to 14% (Kleynhans *et al.*, 1996). The international withdrawal of the highly effective fumigant nematicides from the agro chemical markets in 2005 resulted in a serious void in crop production systems (Mashela *et al.,* 2015). Incidentally, there is a need to find alternative strategies to manage high population densities of *Meloidogyne* species in sweet potato production. One of the most preferred nematode management strategy is the use of nematode resistance, which, like all other strategies, has its advantages and disadvantages.

1.1.1 Description of research problem

Nematode-plant resistance has received increasing attention since the suspension of the ozone-depleting fumigant nematicides in 2005 (Mashela *et al.,* 2015). Generally, nematode resistance offers environment-friendly solutions and is compatible with other interventions such as biological agents, phytonematicides and crop rotations (Starr *et al.*, 2002). However, the challenge of using nematode-resistant genotypes as an option to manage population densities of plant-parasitic nematodes in sweet potato is that *Meloidogyne* species have a wide host-range, global distribution, multiple races and lack of empirically-based information on the degree of nematode resistance in alternative crops. Also, there is limited information on economic potential of highly nematode-resistant indigenous plants, except that Pofu (2012) demonstrated that there are certain wild *Cucumis* species indigenous to Limpopo Province which were highly resistant to *Meloidogyne* species.

In other crops, nematode resistance under high soil temperatures above 28°C (Dropkin, 1969), under salinity (Mashela *et al.,* 1992) and under attack by honeydew-producing insects (Pofu *et al.,* 2013), was shown to be lost. Other limitations of resistant varieties are that when they are repeatedly planted, virulent races of *Meloidogyne* species that are capable of overcoming the resistance would develop (Mashela *et al.,* 2016). Nonetheless, with the withdrawal of soil fumigants and synthetic nematicides, nematode resistance is still playing an important role in nematode management. The host-status and host-sensitivity of locally-available biofortificated and consumer-preferred sweet

potato cultivars, and their mechanisms of resistance to *M. incognita* race 2 in South Africa had not been documented.

1.1.2 Impact of research problem

Meloidogyne species have a wide host range (Dawar *et al.*, 2007; Goodey *et al.*, 1965) and could reduce yield of 40 major world cash crops by an average of 12.3 % (Sasser, 1987). On average, crop losses due to root-knot nematodes in sweet potato were estimated at 6, 15, 24 and 6% for South Africa, South America, West Africa, and Southeast Asia, respectively (Kleynhans, 1991). The total annual loss due to nematodes in 16 field crops, 23 fruits, nuts 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%. Estimated annual crop yield losses due to nematodes prior to the global cut-off date for withdrawal of methyl bromide (MB) technology in 2005 stood at US$125 billion (Chitwood, 2003). Three and eight years after the withdrawal, the estimated yield losses stood at US$157 and US$173 billion, respectively (Abad *et al.*, 2008; Elling, 2013).

1.1.3 Possible causes of the research problem

The control of root-knot nematodes is rather difficult, with the widely used synthetic chemical nematicides being environment-unfriendly. Synthetic nematicides were also shown to be detrimental to human health and non-target organisms (Hay, 2015). The

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withdrawal of methyl bromide from the agrochemical markets after it had been relied on for over 50 years for suppressing nematode population densities left a serious void (Mashela *et al.*, 2015). After the withdrawal of fumigant nematicides, much effort was redirected to the use of nematode resistance in suppression of nematodes, particularly because this alternative is compatible with most management options and cultural practices.

1.1.4 Proposed solutions of research problem

The use of resistant varieties is the most promising method of managing plant-parasitic nematodes (Khanzada *et al.*, 2012). Resistant cultivars do not only reduce the cost of production, but also safeguard the environment against pollution from chemical residues associated with nematicides (Onkendi *et al.*, 2013). The combination of most management strategies of controlling nematodes with cultural and mechanical methods could be a solution to most challenges ascribed to nematode management. Thus, if crops with resistance to root-knot nematode eventually become available, they would be rotated with susceptible varieties and with other crops. Such rotations would reduce the likelihood that resistance-breaking races of the nematode would occur (Mashela *et al.,* 2015).

1.1.5 General focus of the study

The study focused on the host-status and host-sensitivity of the three sweet potato cultivars 'Bophelo', 'Bosbok' and 'Mvuvhelo'. Mechanism of nematode resistance to *M.*

incognita race 2 was also investigated in those cultivars which were resistant to this nematode species.

1.2 Problem statement

Information on nematode resistance in sweet potato cultivars in South Africa had been scanty due to the heavy reliance on synthetic nematicides. Recent screening of 12 sweet potato cultivars demonstrated that most cultivars were hosts to *M. incognita* races 2 and 4, whereas cultivars 'Bophelo', 'Bosbok' and 'Mvuvhelo' were non-hosts (Pofu e*t al*., 2016). Generally, non-host status does not provide information on the degree of nematode resistance, which should be established using the Seinhorst (1965) model. In nematode resistance, a series of nematode levels are used which include measurements of nematode reproduction factor (RF) and plant damage due to nematode infection, whereas in screening only one level of nematode infection is used and assessment could generally be done through reproductive potential, which is the number of eggs and juveniles/fresh root system (Pofu *et al.,* 2016). The advantage of using nematode resistance is that plant-nematode relations can be described either as susceptible, tolerant or resistant (Seinhorst, 1965). Lack of information on the mode of nematode resistance in sweet potato cultivars to *M. incognita* race 2 reduces the potential uses of these cultivars in plant breeding against nematodes. Generally, only post-infectional mechanism of nematode resistance can be used in plant breeding (Kaplan and Davis, 1987; Thurau *et al.*, 2010).

1.3   Hypotheses

1. Cultivars 'Bophelo', 'Bosbok' and 'Mvuvhelo' would be resistant to *M. incognita* race 2.

2. Nematode resistance in cv. 'Bosbok' and 'Mvuvhelo' to *M. incognita* race 2 would be post-infectional mechanism of resistance.

1.4 Rationale

Meloidogyne incognita race 2 is internationally recognised as one of the most aggressive *Meloidogyne* species (Hussey and Janssen, 2002), whereas in South Africa it is second to *M. javanica*. However, *M. incognita* is widely distributed in Limpopo Province, where it occurs alone or as mixed populations with *M. javanica* (Kleynhans *et al.*, 1996). In crops without nematode resistance like watermelon (*Citrullus lanatus*), *M. incognita* reduces yield by as high as 50% to complete crop failure (Thies and Levi, 2007). The availability of nematode resistant genotypes in sweet potato would enhance the use of resistance in managing *Meloidogyne* species and races in Limpopo Province. The nematode resistance technology is cost effective and sustainable, and therefore, could be the most preferred among other alternative nematode management strategies (Mashela *et al.,* 2015). Generally, should post-infectional nematode resistance be available in any of the tested sweet potato cultivars, the information would be relayed to plant breeders for use as source of introgression in various commercial cultivars where nematode resistant genotypes do not exist.

1.5 Purpose of the study

1.5.1 Aim

The aim of the study was development of nematode resistance and mechanism protocols in sweet potato cultivars on *M. incognita* race 2.

1.5.2 Objectives

1. To determine host-status and host-sensitivity in sweet potato cv. 'Bophelo', 'Bosbok' and 'Mvuvhelo' to *M. incognita* race 2.

2. To determine the existing nematode resistance mechanism in any of the test cultivars that had resistance to *M. incognita* race 2.

1.6 Reliability, validity and objectivity

Reliability was ensured by using appropriate statistical levels of significance ($P \leq 0.05$). Validity was ensured by conducting the same experiment at the same location during different times. Objectivity was achieved by discussing the findings on the basis of empirical evidence as shown by statistical analyses, with findings compared and contrasted with findings in other studies (Little and Hills, 1981).

1.6 Bias

Bias is described as any influence, conditions or set of conditions that singly or altogether distort the data (Leedy and Ormrod, 2005). In the current study, bias was minimised by ensuring that experimental error in each experiment being reduced through increased replications and randomization.

1.7 Scientific significance of the study

The findings of the current study would enhance decision of whether to advocate for or against the use of this sweet potato cultivars as an alternative crop in areas with high population densities of the root-knot nematodes, particularly *Meloidogyne* species.

1.8 Structure of dissertation

Following the General Introduction (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the two subsequent chapters addressed each of the two objectives in sequence (Chapter 3-4). Finally in (Chapter 5), findings in all chapters were summarised and integrated to provide the significance of the findings, recommendations with respect to future research and culminated in conclusions which tied the entire study together. Literature citation and referencing followed the Harvard style as prescribed by Senate-approved policy framework of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Worldwide, economic benefits of nematode resistance in plants are being evaluated as alternative to synthetic chemical nematicides (Mashela *et al.,* 2016). Three years prior to withdrawal of methyl bromide in 2005, yield loses due to nematodes were estimated at US$125 billion (Chitwood, 2003). Three and eight years after the withdrawal, several studies are underway to develop crops with resistance genes against various *Meloidogyne* species (Norshie *et al.*, 2011).

Recent screening of 12 sweet potato cultivars (Pofu e*t al.*, 2016) demonstrated that most cultivars were hosts to *M. incognita* races 2 and 4, whereas 'Bophelo', 'Bosbok' and 'Mvuvhelo', which are South African orange and cream-fleshed cultivars respectively, were non-hosts to all *Meloidogyne* species and races. Generally, host-status in plant-parasitic nematodes is assessed using reproductive factor (RF), which is quotient of final nematode population densities (Pf) and initial nematode population densities (Pi): RF = Pf/Pi (Seinhorst, 1965). Host-status and host-sensitivity concepts are both used as indicators of whether a host is resistant, tolerant or susceptible to plant-parasitic nematodes (Seinhorst, 1967). Based on nematode-chemical interaction, nematode resistance mechanisms had been concentrated to two, concepts, pre-infectional and post-infectional nematode resistance.

The nematode bodies are covered with sensory organs, which are used to detect chemicals in small quantities, therefore influencing the direction to which nematode should move. Chemo-attractants and chemo-repellents attract and repel nematodes, respectively (Wuyts *et al*., 2006; Zhao *et al*., 2000). However, a recent literature review on mechanisms of nematode resistance in nematode-resistant transgenic plants suggested that at a molecular level, plants used three different strategies to prevent nematode infection (Mashela *et al.,* 2016).

2.2 Work done on problem statement

2.2.1 Nematodes-resistance in sweet potato

Assessments of sweet potato cultivars for resistance to *M. incognita* populations showed that galls occurred on roots as an indication of host-status, suggesting that the nematode was able to reproduce within the tested cultivars (Olabiyi, 2007). Osunlola and Fawole (2015) investigated the pathogenicity of *M. incognita* on sweet potato and showed that the nematode caused reduction in growth, yield and quality reduction in sweet potato. In other studies, yield losses due to *Meloidogyne* species in sweet potato production had been estimated at (6%) for South Africa (Kleynhans *et al*., 1996).

Pofu e*t al*. (2016) screened 12 sweet potato cultivars against *Meloidogyne* species and races and demonstrated that most cultivars were host to *M. javanica* and *M. incognita* races 2 and 4, whereas 'Bophelo', 'Bosbok' and 'Mvuvhelo' were non-hosts to all *Meloidogyne* species and races. Karuri *et al.* (2017) conducted a survey of root-knot nematodes resistance in sweet potato varieties and observed that 68.0% tested sweet

potato varieties were highly resistant to *M. incognita*, whereas 11.1% were classified as susceptible. Similar results were observed on the resistance of sweet potato clones to *M. incognita* races 1 and 2, were among 63 analysed clones, 78% were resistant to *M. incognita* race 1, 79% to *M. incognita* race 3 and 67% showed multiple resistance to all *M. incognita* races (Gomes *et al.,* 2015).

The Japanese Sweet Potato Breeding Programme, conducted work with a putative hexaploid *I. trifida* (K123) wild relative, where it was crossed with various genotypes to establish nematode-resistant cultivars. After several generations of backcrossing, the *M. incognita* resistant and high-yielding cultivar called 'Minamiyutaka' was developed (Iwanaga, 1988). That was the first successful use of wild sweet potato germplasm to transfer desirable traits like root-knot nematode resistance into sweet potato cultivars, with the success having a major impact on sweet potato breeding programme in Japan (Iwanaga, 1988). Dean and Struble (1953) observed that root-knot nematode resistance was of high frequency in seedling populations from selected sweet potato resistant parents. Similar observations were made by Giamalva *et al.* (1961) when they crossed sweet potato lines with different degrees of resistance to *M. incognita*, where different ratios in resistance responses among the progeny were obtained.

Jones and Dukes (1980) studied the inheritance of resistance in sweet potato to *M. incognita* and *M. javanica* and found that resistance to the two species was not correlated, suggesting the independent inheritance theory. Jones and Dukes (1980) used three measures of resistance, namely, number of egg masses, gall index and

necrosis index they reported heritability estimates of 0.69, 0.78 and 0.72, respectively. Gasapin (1984) suggested that the different degrees of resistance showed by different sweet potato cultivars to *M. incognita* and *M. javanica* could be attributed to the differences in genes for resistance possessed by the different cultivars. Although nematode resistant genotypes hardly existed in the tested orange-fleshed sweet potato cultivars for South Africa nematode populations, the findings suggested the existence of resistant genotypes in local cream-fleshed cultivars (Pofu *et al*., 2016). In the trials, Pofu *et al*. (2016) used the reproductive potential, which does not provide an indication of whether the tested plants were resistant or tolerant to the tested nematode.

2.2.2 Nematode resistance in other crops

In sweet stem sorghum (*Sorghum bicolor*) J2 failed to penetrate roots in *S. bicolor* cv. 'Ndendane-X1' which led to the conclusion that the cultivar had pre-infectional mechanism of nematode resistance to both *M. incognita* race 2 and *M. javanica* (Mashela and Pofu, 2016). Host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *Meloidogyne* species were investigated and both *C. africanus* and *C. myriocarpus* were shown to be resistant to *M. incognita* races 2 and 4 and *M. javanica,* which are dominant in South Africa (Pofu *et al.,* 2010). Nematode races are morphologically identical within the same species, but can be separated using differential hosts and/or molecular approaches (Mashela *et al.,* 2015).

According to Dayan *et al*. (2010), *S. bicolor* produces chemical compound, sorgoleone ($C_{22}H_{29}O_4$), which has nematicidal properties that result in inhibition of nematode

mobility. Out of 30 tested cabbage (*Brassica oleracea*) cultivars for nematode resistant, seven white head cabbage cultivars were reported to be highly resistant to *Heterodera cruciferae* (Aydinli and Mennan, 2012). Cruciferous plants from Brassicaceae family contain glucosinolate ($C_{17}H_{32}O_{11}NS_3$) chemical compounds which release nematode toxic products such as thiocyanate (SCN) and isothiocyanate (C_4H_5NS) when they decompose (Brown *et al.*, 1991; Matthiesen and Kirkegaard, 2006; Ntalli and Caboni, 2012), which are toxic to nematodes (Larkin, 2013; Petersen *et al.*, 2001). Dry bean (*Phaseolus vulgaris*) cultivars 'Apore' and 'Talisma' were highly resistant to *M. javanica*, whereas snap bean cultivars 'Macarrao atibaia' and 'Macarrao preferido' were moderately resistant to *M. javanica* (Ferreira *et al.*, 2010). Common bean cv. 'Polder' was shown to be resistant to *M. chitwoodi* and *M. fallax* (Wesemael and Moens, 2012). Resistant Lima bean (*P. lunatus*) inoculated with *P. penetrans* produced the phytoalexin, coumestrol ($C_{15}H_8O_5$) (Veech, 1982).

Resistance to *M. arenaria* was expressed in soybean (*Glycine max*) as small, poorly formed giant cells, with reduced cell number and cell size of cells surrounding selected feeding cell (Pedrosa *et al.*, 1996). *Meloidogyne* species produced glyceollin ($C_{20}H_{18}O_5$), a nematode-toxic chemical compound (Veech, 1982). Carrot (*Daucus carota*) cv. 'Brasilia' was shown to be resistant to *M. javanica* (Huang, 1986; Huang *et al.,* 1986). Generally, when *M. chitwoodi* J2 penetrated roots of carrot cv. 'Parmex' and cv. 'Berlanda', fewer egg masses were observed on each cultivar (Sone, 2010), with high male to female ratio and numerous rootlets. Wesemael and Moens (2008) also reported egg masses of less than 20% in cv. 'Parmex' and cv. 'Berlanda' infected with *M.*

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chitwoodi. According to Osei *et al.* (2010), leguminous plants contain numerous chemicals, some of which were nematostatic or influence nematode behaviour. The absence of galls on the roots of cowpea (*Vigna unguiculata*) varieties led to the conclusion that *V. unguiculata* varieties had the ability to inhibit the formation of feeding sites that are required to support the reproduction of females after penetration. The gene responsible for resistance to *M. incognita* in *V. unguiculata* appeared to confer resistance to other *Meloidogyne* species (Fery, 1980).

Mashela and Pofu (2012) conducted a trial on host response of *Capsicum frutens* cultivar 'Capistrano' to *M. incognita* race 2, the reproductive factor was less than unity, while nematode infection had no effect on plant growth and concluded that the cultivar was resistant to *M. incognita* race 2. The reproductive factor of *M. javanica* on hemp cultivars were greater than one, without the cultivars suffering damage from the nematode infection. Results suggested that the four cultivars were tolerant to *M. javanica* (Pofu *et al.,* 2010). Growth of beetroot cultivar 'Detroit Dark Red' was significantly stimulated and inhibited at low and high nematode infection levels, respectively. In contrast, RF values for *M. javanica* on cultivar 'Crimson Globe' were below unity, without any significant effects on plant growth, it was concluded that, 'Detroit Dark Red' was tolerant to *M. incognita*, whereas 'Crimson Globe' was resistant to *M. javanica* (Mashela, 2017). Reproductive factors suggested that three open-pollinated varieties, namely: OBATAMPA, QPM-SR and QS-OBA were non-host to both *M. incognita* race 2 and *M. javanica*. Penetration indices suggested that OBATAMPA

had post-infectional non-host status, whereas QPM-SR and QS-OBA had pre-infectional non-host status (Ngobeni *et al.,* 2012).

2.2.3 Assessment concepts in nematode-plant resistance

The concepts of host-status and host-sensitivity was introduced to describe nematode-plant relations (Seinhorst, 1967), 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), explained the host-status concepts using various mathematical models, which, assist nematode specialists in better understanding of nematode reproduction and density-dependent growth patterns (Salisbury and Ross, 1992) and thereby improving nematode management tactics. Also, E point, assist in selecting appropriate inoculation levels, since excessively high levels could result in RF being lower than zero due to competition as opposed to nematode resistance.

Host-sensitivity was described in relation to damage inflicted by nematodes to plants, with Seinhorst (1965) using a model to formulate three concepts: (i) susceptible, (ii) tolerance and (iii) resistance, which had since been widely used in nematode-plant

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relations (Mashela, 2017). Susceptible hosts are plants that have the ability to build up nematode populations and suffer subsequent damage in terms of growth reduction (Trudgill, 1992). 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 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).

2.2.4 Mechanisms of nematode resistance

Active nematode resistance responses occur post-infection, following penetration into the root or other host tissues by the nematodes (Mashela *et al.,* 2016). Post-infectional resistance is expressed by delayed or retarded development of the nematodes after penetration into the plant, or by non-development of the nematode to maturity in the plants (Gasapin, 1986). In post-infectional resistance, plant have the ability 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). Naturally, resistant plants carrying major R genes for resistance to nematodes are invaded like susceptible plants. Pre-infectional resistance may be manifested as physical or chemical barriers, or as nutritional inadequacies. 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). Failure of J2 to penetrate roots in *S. bicolor* cv. 'Ndendane-X1' led to the conclusion that the cultivar had pre-infectional mechanism of nematode resistance to both *M. incognita* race 2 and *M. javanica* (Mashela and Pofu, 2016). Asparagus (*Asparagus officinalis*) also possesses pre-infectional nematode resistance (Gommers, 1981). Sunn hemp (*Crotalaria juncea*) released chemicals into the rhizosphere that prevented infection by *M. incognita* J2 prior to penetration (McSorley and Gallaher, 1991; Roberts, 1992). Tomato cv. 'Nemared' was reported to be pre-infectional resistant to *M. incognita* and *Pratylenchus. penetrans* (Hung and Rohde, 1973). The J2 of *M. incognita* and *P. penetrans* could not penetrate roots of cv. 'Nemared' (Ohri and Pannu, 2010).

In other plants, resistance is post-infectional. In this mode of resistance, a hypersensitive response (HR) and accumulation of toxic metabolites, and some resistance results in the degeneration of nematode feeding sites (Veech, 1981). After invasion into the plant, the activation of incompatible interactions can result in nematodes emigrating from the roots or nematode death within resistant roots (Roberts *et al.*, 1998). This results in a lower number of egg masses and reduced size of egg laying females in resistant cultivars as compared to susceptible cultivars (Gasapin, 1986). Dean and Struble (1953) reported that on resistant and susceptible sweet potato varieties, juveniles (J2) enter the roots in equal number, but fewer nematodes develop to egg-laying maturity on resistant varieties. The two workers suggested that resistance

to nematode in sweet potato was related to an extensive necrosis of root tissues. Histological studies of 'Porto Rico' sweet potato showed that the primary root penetration by J2 occurred at the tips of young roots in the region of tissue differentiation. Another major nematode penetration site in sweet potato occurred through the loose ruptured cells of enlarging roots where lateral roots emerged (Krusberg and Nielsen, 1958). Several types of host-parasite reactions are related with root-knot nematode resistance in sweet potato. These are: none to trace amounts of galling on the host, moderate to severe root tip necrosis, general inability of nematode larvae to reach mature stages, little or no reproduction by the nematode, and reduced number of eggs where reproduction does occur (Davide and Struble, 1966). Recent mechanism of resistance study suggested that post-infectional nematode resistance was in place in the two wild indigenous *Cucumis* species. The results confirmed that the identified nematode resistance in *C. africanus* and *C. myriocarpus* to *Meloidogyne* species was post-infectional (Ramatsitsi, 2017).

2.2.5 Molecular approaches in nematode resistance

Molecular approaches suggested that there were three strategies for nematode resistance namely (i) RNA-resistance strategy, (ii) anti-gene products strategy and (iii) anti-plant gene strategy which were recently reviewed by Mashela *et al.* (2016) in nematode-resistant transgenic plants.

RNA-resistance strategy: Hewezi and Baum (2015) reported that the RNA interference (RNAi) disrupts the nematode gene products through host-induced gene silencing

approach. The RNAi genes had revealed accurate selectivity for the target organisms with slight side effects (McDowell and Woffenden, 2003). Cathepsin L-like cysteine proteinases, produced by R genes in nematode resistant transgenic plants, were shown to be an attractive group of candidate genes for RNAi-induced downregulation due to their high level of specificity to the target nematode gene products (McDowell and Woffenden, 2003), resulting in silencing effects on host-induced gene products. Also, the host-produced RNAi of Mi-cpl-1 gene confers resistance to *M. incognita* by inducing negative effects on nematode infection, development and the subsequent reproduction (McDowell and Woffenden, 2003).

Anti-gene products strategy: Plant-parasitic nematodes secret chemical compound called gene products through the sub-ventral and dorsal gland cells during migration and sedentary phases, respectively (Gheysen and Fenoll, 2002; Tripathi *et al.*, 2015). The secretion of gene products is important, especially during the formation of nematode feeding sites, which allow for nematode development to subsequent stages (Curtis, 2008; Siddique *et al.*, 2014). During migratory phases, roots are wounded upon which, chemical compounds referred to as defence plant genes, comprising peroxidase, chitinase, lipoxygenase, extension and proteinase inhibitors are activated (Gheysen and Fenoll, 2002; Hewezi and Baum, 2015). The anti-gene products strategy in nematode resistant plants, ranged from those during both migratory and sedentary phases, in respect to those that silence the expression of the gene products (Mashela *et al.*, 2016).

Anti-plant gene strategy: The host plant genes that respond to nematode feeding and secretions to allow for successful partnerships between gene products and gene plants are silenced in anti-plant gene strategy (Mashela *et al*., 2016). Thus, the phytotoxic chemical compounds that destroy the feeding structures, syncytium and giant cells, are upregulated (Mashela *et al*., 2016). Mostly the plant releases certain plant genes in order to protect the nematode and such chemicals could be suppressed, thereby leaving the bodies of nematodes exposed (Hewezi and Baum, 2015). The anti-plant gene strategy had been successfully used in certain transgenic plants (Mashela *et al*., 2016).

2.3 Work not yet done on problem statement

The distinction between screening for host-status and nematode had been re-emphasised recently (Mashela *et al.,* 2016). In screening, one inoculum level of nematode is used, with results expressed using the concept of reproductive potential (RP) (Mashela *et al.,* 2016), whereas in nematode resistance, a series of nematode levels are used, with findings being expressed using reproductive factors (RF) (Seinhorst, 1965). Although screening results suggest that sweet potato cultivars 'Bophelo', 'Bosbok' and 'Mvuvhelo', were non-host to all tropical *Meloidogyne* species in South Africa, it would not be wise to conclude that the three sweet potato cultivars were resistant to *Meloidogyne* species. Hence, empirical-based information on the degree of nematodes resistance in the three sweet potato cultivars would be necessary.

CHAPTER 3
NEMATODE RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN THREE SWEET POTATO CULTIVARS

3.1 Introduction

The use of nematode resistant genotypes in sweet potato may offer a powerful and reliable strategy for sustainable management of nematode population densities (Mashela *et al.*, 2016). Prior to introducing nematode resistant traits, screening for non-hosts is done among a large number of cultivars, followed by establishing the degree of nematode resistance. A previous screening study suggested that sweet potato cv. 'Bophelo', 'Bosbok' and 'Mvuvhelo' were non-host to *Meloidogyne incognita* race 2 (Pofu *et al.*, 2016). Among the major sweet potato pests, root-knot nematodes, *Meloidogyne* species, are one of the most destructive for the crop. *Meloidogyne incognita* races 2 widely distributed in tropical areas of South Africa and the yield losses due to *Meloidogyne* species in sweet potato production had been estimated at (6%) for South Africa (Kleynhans, 1991). Worldwide, withdrawal of highly effective synthetic fumigants used in the management of plant parasitic nematode population densities has had negative impact in many crop production systems. Three and eight years after the withdrawal several studies are underway to develop crops with resistance genes against various *Meloidogyne* species with intentions of using them as alternatives for nematode management (Norshie *et al.*, 2011). The objective of this study, therefore, was to determine host-status and host-sensitivity in sweet potato cv. 'Bophelo', 'Bosbok' and 'Mvuvhelo' to *M. incognita* race 2.

3.2 Materials and methods

3.2.1 Description of the study site

The study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, Limpopo Province of South Africa (23°53'10"S, 29°44'15"E) under greenhouse conditions. Ambient day/night temperatures averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans. The trial for *Meloidogyne incognita* race 2 was conducted during autumn (January-March) 2016.

3.2.2 Treatments and research design

Treatments, namely, 0; 25; 50; 125; 250; 625; 1250; 3125 and 3125 eggs and J2 *M. incognita* race 2, were arranged in a randomised complete block design, with 5 replications. Cultivar 'Beauregard' served as nematode susceptible standard (Legend 3.1).

3.2.3 Procedures

Thirty-cm-diameter pots were filled with steam-pasteurised loam soil and Hygromix-T at 4:1 (v/v) ratio. Thirty centimeter diameter pots were placed inside the greenhouse benches, with inter row and intra row spacing of 0.30 m. Cuttings of cultivar 'Bophelo', 'Bosbok' and 'Mvuvhelo', were transplanted in plastic pots containing the growing mixture described above. *Meloidogyne incognita* race 2 inoculum was prepared from roots of nematode-susceptible tomato cv. 'Floradade' (Hussey and Barker, 1973). Each plant was inoculated by dispensing approximate numbers of *M. incognita* eggs and

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second-stage juveniles J2 using a 20-ml-plastic syringe. Inoculums were placed into 5-cm-deep holes around the cardinal points of plant stems per replication.

Legend 3.1 Establishment of cv. 'Bosbok' inoculated with *Meloidogyne incognita* race 2.

3.2.4 Cultural practices

Cuttings were irrigated with 250 ml tapwater every other day. Approximately 5 g Multifeed (Nulandies, Johannesburg) was applied at transplanting to provide 1.21 Mg, 0.43 K, 0.47 N, 0.43 P;1 Fe, 4.02 Mg per ml; 0.47 Zn, 0.10 Cu, 1.34 B and 0.09 Mo mg per ml. The cuttings were also fertilised with 2.5 g N: P: K 2:3:2 (22) to provide a total of 155 mg N, 105 mg P and 130 mg K. Addition to that, 5 g of 2:3:2 (26) + 0.5% ZN + 5% S + 5% Ca fertiliser mixture per plant was applied which provided 155 mg of N, 105 mg P, and 130 mg K per ml of water. Pest management comprised regular monitoring and application of control measures when necessary.

3.2.5 Data collection

At harvest, 56 days after inoculation (Mashela *et al*., 2015), the variables measured included: vine length; stem diameter; fresh root mass; fresh root tuber mass; fresh shoots mass and gall nematodes rating. Stem diameter was measured with digital vernier caliper, plant height was measured from the crowns to the tips, and shoots then cut at the soil level. The root galls were assessed using the North Carolina Differential Scale of 0 to 4 scale, 1 = no galls; 2 = 1 – 10 galls; 3 = 11 - 100 galls and 4 = being greater than 100 galls per root system (Taylor and Sasser, 1978). Nematodes were extracted from roots per plant by maceration and blending for 30 seconds in 1% $NaOCl_2$ (Hussey and Barker, 1973). The material was passed through top-down nested 63 µm and 25 µm mesh sieves. Contents of the 25 µm was poured into 10 ml plastic containers for nematode counting under a stereomicroscope. Soil in each pot was mixed and a 250 ml soil sample was collected for nematode extraction using the sugar-

floatation and centrifugation method (Jenkins, 1964). Nematodes were counted out of 10 ml aliquot with the use of stereomicroscope. Reproductive factors (RF) were computed using proportion of final nematode numbers (Pf) to initial nematodes numbers (Pi).

3.2.6 Data analysis

Prior to analysis of variance (ANOVA), nematode data were transformed through $\log_{10}$ (x + 1) to homogenise the variances (Gomez and Gomez, 1984). Data were subjected to ANOVA through the SAS software to determine the effects of initial nematode population densities (Pi) on RF values and yield components. Sum of squares was partitioned to determine the contribution of sources of variation to the total treatment variation (TTV) in plant and nematode variables (Gomez and Gomez, 1984). Mean separation for significant (P ≤ 0.05) treatments was achieved through the Waller-Duncan Multiple Range test.

3.3 Results

3.3.1 *Meloidogyne incognita* race 2 on cv. 'Bophelo'

In cv. 'Bophelo' inoculation levels had no significant effect in all nematode variables except for reproductive factor (RF = Pf/Pi) where treatment had highly significant effect and contributed 60 % of the total treatment variation (Table 3.1). The inoculation levels 25 and 50 had RF above one, whereas inoculation levels above 125, 250, 625, 1250 and 3125 Pi had RF less than one (Table 3.2). Further, as inoculum levels increased, mean RF values gradually declined with the potential increase on the Pi above 3125

(Table 3.2). Mean RF value on cv. 'Beauregard' was above unity (Table 3.2). Treatments had a significant effect on stem diameter, dry root mass and dry tuber mass contributing 42, 40 and 54% of the total treatment variation. The treatments had no significant effect on vine length and dry shoot mass (Table 3.3). Generally, there was a decrease in stem diameter and dry root mass with increase in inoculum levels while dry tuber mass was increased with increase in inoculum levels (Table 3.4).

3.3.2 *Meloidogyne incognita* race 2 on cv. 'Bosbok'

In cv. 'Bosbok', inoculation levels had significant effect on J2 in soil, final nematode population density (Pf) and reproductive factor (RF) while J2 in roots had no significant effect (Table 3.5). The reproductive factor (RF = Pf/Pi) was less than one at all levels of inoculation (Table 3.6). Mean RF value on cv. 'Beauregard' was greater than one (Table 3.6). All plant variables were not affected by inoculation level of *M. incognita* race 2 (Table 3.7). Responses of plant variables to *M. incognita* was not significantly different in all levels of inoculation (Table 3.8).

3.3.3 *Meloidogyne incognita* race 2 on cv. 'Mvuvhelo'

In cv. 'Mvuvhelo', all nematode variables were not affected by inoculation levels of *M. incognita* race 2 (Table 3.9). The RF values were below unity at all levels of inoculation (Table 3.10). On cv. 'Beauregard' mean RF value was above unity (Table 3.10) Inoculation levels of *M. incognita* race 2 had no effects in all plant variables (Table 3.11). Responses of plant variables to *M. incognita* race 2 on cv. 'Mvuvhelo' was not significant different in all levels of inoculation (Table 3.12).

Table 3.1 Analysis of variance for second-stage juveniles (J2) in roots and soil, final nematode population density (Pf) and the reproduction factor (RF) of *Meloidogyne incognita* race 2 on 'Bophelo' cultivar at 56 days after inoculation.

Source	DF	J2 in roots		J2 in soil		Pf		RF	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.04995	33	3.71423	58	3.11556	55	11.6279	27
Treatment	6	0.04995	34[ns]	1.59808	25[ns]	1.43581	25[ns]	25.2000	60***
Error	24	0.04995	33	1.08554	17	1.11375	20	5.6036	13
Total	34	0.14985	100	6.39785	100	5.66512	100	42.4315	100

*** Denotes highly significance differences at P ≤ 0.01 and [ns], denotes not significant at P > 0.05; Pf = Nematodes per root system + nematodes per 5 L soil.

RF = (Pf) ÷ (Pi).

Table 3.2 Responses of final eggs, final population second-stage juvenile (J2) in roots and soil, final nematode population density (Pf) and the reproductive factor (RF) of *Meloidogyne incognita* race 2 on sweet potato cultivar 'Bophelo' at 56 days after inoculation.

Treatment	Eggs	J2 in roots	J2 in soil	Pf	RF[y]
25	4	0	144	148	5.92a
50	0	4	144	148	2.96ab
125	4	0	0	4	0.03b
250	0	0	240	240	0.96b
625	8	0	48	56	0.09b
1250	0	0	48	48	0.04b
3125	0	0	240	240	0.08b
Cultivar 'Beauregard'					
[z]B3125	4100	3168	3212	10480	3.35

[y]RF=Pf/Pi and [z]Beauregard as a susceptible standard.

Table 3.3 Analysis of variance for vine length, stem diameter, dry shoot mass, dry root mass and dry tuber mass of 'Bophelo' infested with *Meloidogyne incognita* race 2 after 56 days of inoculation.

Source	DF	Vine length		Stem diameter		Dry shoot mass		Dry root mass		Dry tuber mass	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	134.607	58	1.475	41	135.708	67	70.827	45	2.448	29
Treatment	7	41.500	18^{ns}	1.525	42^{**}	33.762	16^{ns}	62.937	40^{**}	4.516	54^{**}
Error	28	54.463	24	0.636	17	34.404	17	22.441	15	1.406	17
Total	39	230.57	100	3.64	100	203.87	100	156.20	100	8.37	100

** Denotes significance differences at $P \leq 0.05$, while, ^{ns} values not significant at $P \leq 0.05$.

Table 3.4 Responses of vine length (VL), stem diameter (SD), dry shoot mass (DSM), dry root mass (DRM) and dry tuber mass (DTM) of cv. 'Bophelo' to initial population of *Meloidogyne incognita* race 2.

Pi	VL Variable	SD Variable	SD R.I. (%)	DSM Variable	DRM Variable	DRM R.I. (%)	DTM Variable	DTM R.I. (%)
0	48.54	8.60[abcd]	–	33.44	27.34[a]	–	1.22[b]	–
25	39.76	9.56[a]	11.16	39.88	21.41[abc]	−21.69	0.13[b]	−89.34
50	48.90	9.38[ab]	9.07	34.09	18.67[bc]	−31.71	0.16[b]	−86.89
125	44.18	9.22[abc]	7.21	31.61	17.30[c]	−36.72	0.61[b]	−50
250	46.12	8.2[cd]	−4	32.64	21.71[abc]	−20.59	0.39[b]	−68.03
625	44.40	9.06[abcd]	5.35	35.89	16.32[c]	−40.31	0.26[b]	−78.69
1250	46.00	8.36[bcd]	−2.79	36.01	23.50[ab]	−14.05	0.00[b]	−100
3125	46.38	8.16[d]	−5.12	33.45	20.99[bc]	−23.23	2.86[a]	134.45
LSD$_{0.05}$	9.56	–		7.59	–		–	

Table 3.5 Analysis of variance for second-stage juveniles (J2) in roots and soil, final nematode population density (Pf) and the reproduction factor (RF) of *Meloidogyne incognita* race 2 on 'Bosbok' cultivar at 56 days after inoculation.

Source	DF	J2 in roots		J2 in soil		Pf		RF	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.09107	33	0.30831	22	0.31425	22	0.001148	21
Treatment	6	0.09107	34^{ns}	0.82216	57**	0.83788	57**	0.003044	57**
Error	24	0.09107	33	0.30831	21	0.31425	21	0.001148	22
Total	34	0.27321	100	1.43878	100	1.46638	100	0.00534	100

** Denotes significance differences at $P \leq 0.05$ and ^{ns} denotes not significant at $P > 0.05$; Pf = Nematodes per root system + nematodes per 5 L soil.

RF = (Pf) ÷ (Pi).

Table 3.6 Responses of final eggs, final population second-stage juvenile (J2) in roots and soil, final nematode population density (Pf) and the reproductive factor (RF) of *Meloidogyne incognita* race 2 on sweet potato cultivar 'Bosbok' at 56 days after inoculation.

Treatment	Eggs	J2 in roots	J2 in soil	Pf	RF[y]
25	0	0	0	0	0.00
50	0	0	0	0	0.00
125	0	0	0	0	0.00
250	0	0	0	0	0.00
625	0	0	0	0	0.00
1250	0	0	0	0	0.00
3125	0	12	192	204	0.07
Cultivar 'Beauregard'					
[z]B3125	7864	7048	2592	17504	5.60

[y]RF=Pf/Pi and [z]Beauregard as a susceptible standard.

Table 3.7 Analysis of variance for vine length, stem diameter, dry shoot mass, dry root mass and dry tuber mass of 'Bosbok' infested with *Meloidogyne incognita* race 2 after 56 days of inoculation.

Source	DF	Vine length		Stem diameter		Dry shoot mass		Dry root mass		Dry tuber mass	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	373.969	37	1.352	11	28.764	14	10.110	12	50.59	38
Treatment	7	264.825	27^{ns}	5.169	44^{ns}	91.765	45^{ns}	39.549	46^{ns}	22.83	17^{ns}
Error	28	355.871	36	5.376	45	82.221	41	36.748	42	59.50	45
Total	39	994.67	100	11.89	100	202.75	100	86.40	100	132.93	100

^{ns} Not significant at $P \leq 0.05$.

Table 3.8 Responses of vine length (VL), stem diameter (SD), dry shoot mass (DSM), dry root mass (DRM) and dry tuber mass (DTM) of cv. 'Bosbok' to initial population of *Meloidogyne incognita* race 2.

Pi	VL (cm)	SD (mm)	DSM (g)	DRM (g)	DTM (g)
0	42.88	6.48	18.89	10.61	5.92
25	44.20	4.88	20.62	7.93	6.07
50	52.04	5.68	26.13	13.75	4.99
125	27.08	3.64	13.58	7.97	6.24
250	37.08	5.26	12.82	11.56	6.11
625	43.18	6.70	21.25	13.55	7.48
1250	45.04	6.12	17.17	15.93	11.85
3125	38.66	4.76	18.59	10.87	5.98
LSD $_{0.05}$	24.44	3.00	11.75	7.85	99.99

Table 3.9 Analysis of variance for second-stage juveniles (J2) in roots and soil, final nematode population density (Pf) and the reproduction factor (RF) of *Meloidogyne incognita* race 2 on 'Mvuvhelo' cultivar at 56 days after inoculation.

Source	DF	J2 in roots		J2 in soil		Pf		RF	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.56060	38	0.16211	33	0.48653	30	0.02560	21
Treatment	6	0.45159	31^{ns}	0.16212	34^{ns}	0.53765	34^{ns}	0.06616	53^{ns}
Error	24	0.45346	31	0.16211	33	0.57808	36	0.03251	26
Total	34	1.46565	100	0.48633	100	1.60226	100	0.12427	100

^{ns} Denotes not significant at P ≤ 0.05; Pf = Nematodes per root system + nematodes per 5 L soil.

RF = (Pf) ÷ (Pi)

Table 3.10 Responses of final eggs, final population second-stage juvenile (J2) in roots and soil, final nematode population density (Pf) and the reproductive factor (RF) of *Meloidogyne incognita* race 2 on sweet potato cultivar 'Mvuvhelo' at 56 days after inoculation.

Pi	Eggs	J2 in roots	J2 in soil	Pf	RF[y]
25	0	8	0	8	0.32
50	0	0	0	0	0.00
125	0	4	0	4	0.03
250	0	24	0	24	0.10
625	0	0	0	0	0.00
1250	0	28	0	28	0.02
3125	0	8	48	56	0.01
Cultivar 'Beauregard'					
[z]B3125	6968	9108	864	13864	5.42

[y]RF = Pf/Pi and [z]Beauregard as a susceptible standard.

Table 3.11 Analysis of variance for vine length, stem diameter, dry shoot mass, dry root mass and dry tuber mass of 'Mvuvhelo' infested with *Meloidogyne incognita* race 2 after 56 days of inoculation.

Source	DF	Vine length		Stem diameter		Dry shoot mass		Dry root mass		Dry tuber mass	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	87.545	65	0.494	22	8.063	15	2.921	22	59.22	43
Treatment	7	30.857	23^{ns}	0.714	32^{ns}	27.183	51^{ns}	3.384	26^{ns}	32.40	24^{ns}
Error	28	16.393	12	1.009	46	18.039	34	6.932	52	46.21	33
Total	39	134.79	100	2.22	100	53.28	100	13.24	100	137.82	100

^{ns} Not significant at $P \leq 0.05$.

Table 3.12 Responses of vine length (VL), stem diameter (SD), dry shoot mass (DSM), dry root mass (DRM) and dry tuber mass (DTM) of cv. 'Mvuvhelo' to initial population of *Meloidogyne incognita* race 2.

Pi	VL (cm)	SD (mm)	DSM (g)	DRM (g)	DTM (g)
0	35.24	7.82	20.44	5.14	22.86
25	37.10	8.10	20.76	6.71	22.11
50	34.82	8.64	17.49	6.28	17.87
125	31.16	8.16	17.67	6.15	24.34
250	32.62	7.70	13.84	4.88	21.14
625	29.20	7.88	15.67	4.60	24.92
1250	32.76	8.76	16.58	5.43	24.45
3125	32.48	8.12	18.63	6.66	19.37
$LSD_{0.05}$	5.25	1.30	5.50	3.41	8.81

3.4 Discussion

3.4.1 *Meloidogyne* species on cv. 'Bophelo'

In plant-parasitic nematodes, nematode resistance is described using two concepts: (a) host-status and (b) host-sensitivity (Seinhorst, 1965). Host-status is described using the reproductive factor (RF), which is a measure of the reproductive potential of a nematode on a given host (Windham and Williams, 1988). The RF values below one suggest that the nematodes failed to feed and reproduce on a given plant host, whereas values above one suggest that the nematode established a feeding site and reproduced (Windham and Williams, 1988).

In cv. 'Bophelo' RF values decreased with increase in initial population densities and furthermore, the RF values of *M. incognita* race 2 were above one at Pi levels lower than 125 and less than one at Pi levels above 125. The high mean nematode numbers and RF above unity on cv. 'Beauregard' indicates that the nematodes were viable and able to establish the feeding site and reproduce on cv. which served as a susceptible standard. The observed RF did not agree with those of Sano and Iwahori (2001) who observed that J2 in *M. incognita*, *M. arenaria* and *M. javanica* trials penetrated roots of all test sweet potato cultivars well, with more than 50% penetrated J2 reproducing as early as five days after inoculation on cultivars with egg masses. In the current study, the equilibrium point (E) of *M. incognita* race 2 on cv. 'Bophelo' might be between 50 and 125 Pi, above which RF started to decline. The observed response on RF suggested that density-dependent growth (DDG) principles were in place (Pofu and

Mashela, 2013). The DDG principles are depicted through three responses to abiotic and biotic factors, namely, stimulation, neutral and inhibition (Pofu and Mashela, 2013). In other studies, it was shown that E point for *M. incognita race 2* on *Cucumis* species was less than Pi = 500 and E point for the citrus nematodes (*Tylenchulus semipenetrans*) was at Pi = 10 000 nematodes (Kwaye *et al*., 2008). A test plant that is inoculated with levels below the E point, nematode population will increase if the test plant is a host but if not a host the nematode population will decline (Pofu *et al.,* 2010). If a test plant is inoculated with nematode level above the E point regardless of whether the test plant is a host or non-host the population of the nematode will decrease due to high competition in the feeder roots.

Host-sensitivity refers to host responses to nematode infection (Seinhorst, 1967). Generally, when the RF value is greater than one and the plant suffers yield loss the plant is described as a susceptible host, when the RF value is less than one and there is no yield loss, the test plant is said to be resistant, whereas, a host that allows nematodes to reproduce but does not incur yield loss is referred to as a tolerant host (Seinhorst, 1967). The two concepts, host-status and host-sensitivity, are essential in describing the degree of nematode resistance in plants. In cv. 'Bophelo' stem diameter and dry root mass were reduced with the increase in Pi, suggesting that *M. incognita* race 2 caused damage on cv. 'Bophelo'. However, dry tuber mass was increased with the increasing Pi numbers, which could be described as nematode infection having stimulation effects on this variable. Generally, within the context of DDG patterns, different variables can respond to the same factor in any of the three phases, namely,

stimulation, neutral or inhibition, as observed in various trials (Mashela *et al.*, 2015). Growth of beetroot cultivar 'Detroit Dark Red' was significantly stimulated and inhibited at low and high nematode infection levels, respectively (Mashela, 2017).

The reduction in stem diameter in 'Bophelo' confirmed observations where infection by *Meloidogyne* species reduced stem diameter in *Cleome gynandra* (Rabothata, 2017). Generally, reduction in stem diameter due to infection by *Meloidogyne* species is common in plants (Mashela *et al.*, 2011). Various abiotic and biotic factors that increase root/shoot ratio, with increased accumulation of non-structural carbohydrates in roots, result in reduced stem diameter as a physical mechanism to restrict the flow of carbohydrates to roots, thereby regulating osmotic potential in root cells (Mashela and Nthangeni, 2002). This physical regulatory mechanism for reducing the concentration of non-structural carbohydrates was further demonstrated under infection by high population densities of the citrus nematode (*Tylenchulus semipenetrans*) and in a split-root pruning trial (Mashela and Nthangeni, 2002). Literature is replete with reports on nematode inhibition of plant growth, which is characterised by reduced biomass and yield, depending on the aggressiveness of the nematode species (Mashela and Nthangeni, 2002; Osunlola and Fawole, 2015). Generally, *Meloidogyne* species are among the most aggressive nematodes, with high infections characterised by stunted plant growth (Windham and Williams, 1988).

Using the Seinhorst (1967) model for the description of host-status and host-sensitivity, cv. 'Bophelo' was susceptible to *M. incognita* race 2 since RF was above unity and plant

variables were affected by nematode infection. Results on the current study agreed with Olabiyi (2007) who demonstrated the existence of both nematode resistance and susceptibility sweet potato cultivars for resistance to *M. incognita* populations. Susceptibility of sweet potato varieties to *M. incognita* is probably due to the presence of previously reported unfavorable alleles that reduce the level of resistance (Cervantes-Flores *et al.*, 2008).

3.4.2 Nematode resistance in cv. 'Bosbok'

Mean RF values provide an indication of whether a plant is a host or a non-host to the test nematode, with less than one values suggesting non-host status, while greater than one values indicate host status (Seinhorst, 1967). Generally, mean RF values of less than one suggested that *M. incognita* race 2 failed to feed on cv. 'Bosbok', therefore, to reproduce on the tested sweet potato cultivar (Windham and Williams, 1988). Cultivar 'Beauregard' which served as a susceptible standard had high mean nematode numbers and RF above unity indicates that the nematodes were viable and able to feed and reproduced. In plant-parasitic nematodes, feeding is a prerequisite to development and reproduction, without which juveniles are converted to non-feeding males (Windham and Williams, 1988). Host sensitivity describes the responses of plant growth to nematode infection, which could be either inhibition or neutral or stimulation (Seinhorst, 1967). Infection of *M. incognita* at all levels had no effect on growth of cv. 'Bosbok'. At certain appreciable levels, nematode infections have no effect on plant growth (Sikora and Fernàndez, 2005). Because *M. incognita* race 2 juveniles failed to develop and reproduce on cv. 'Bosbok', and plant variables were not affected by

nematode infection cv. 'Bosbok' is therefore a non-host to *M. incognita* race 2. Similar results were observed, notably on wild cucumber (*Cucumis myriocarpus*), wild watermelon (*Cucumis africanus*) and bitter gourd (*Momordica balsamina*) as being non-hosts to *M. incognita* race 2 (Pofu *et al.,* 2015; Pofu *et al.*, 2010).

3.4.3 Nematode resistance in cv. 'Mvuvhelo'

The lower number of nematode juveniles in the roots of sweet potato cv. 'Mvuvhelo' disagrees with the view that nematodes locate and penetrate root of nematode-susceptible and nematode-resistant plants equally (Pofu *et al.,* 2010). However, there are some cases where penetration was prevented. In plant-parasitic nematodes, there are some plants where nematode penetration into roots is allowed (Acedo *et.al.,* 1984). In plants like sunn hemp (*Crotolaria junceae*), rye (*Secale cereal*), marigolds (*Tagetes* species), velvet bean (*Mucuna deeringiana*) and some cowpea (*Vigna unguiculata*) cultivars, penetration into roots is prevented (Caswell *et al.,* 1991).

The RF values on cultivar 'Mvuvhelo' were less than one suggesting that the nematode failed to reproduce on the tested plant. Susceptible standard cv. 'Beauregard' had high mean nematode numbers and RF above unity indicates that the nematodes were viable and managed to feed and reproduced on the given host. The less than unity in RF values of *M. incognita* race 2 on the sweet potato cultivar 'Mvuvhelo' also suggested that this nematode did not have the potential to feed on the plant species, since feeding is a pre-requisite for nematode development and reproduction (Ferraz and Brown, 2002). Also, *M. incognita* race 2 failed to damage or reduce yield components of sweet

potato cv. 'Mvuvhelo' at all levels of inoculation. Using the Seinhorst (1967) model, cv. 'Mvuvhelo' was, therefore, resistant to *M. incognita* race 2. The findings of this current study confirmed the potential existence of resistance to *Meloidogyne* species and races in local cream-fleshed sweet potato cultivars, 'Bosbok' and 'Mvuvhelo' (Pofu *et al*., 2016). Also, the findings of the current study agreed with Mashela and Pofu (2012) on *Capsicum frutens* cultivar 'Capistrano' it was found being resistant to *M. incognita* race 2, also beetroot cultivar 'Crimson Globe' was resistant to *M. javanica* (Mashela, 2017).

3.5 Conclusion

Sweet potato cv. 'Bophelo' was susceptible to *M. incognita* race 2. Consequently, this cultivar is not suitable to be used as alternate crops for managing population densities of *M. incognita* race 2, since the nematode build-up would cause damage in subsequent crops. However, cultivars 'Bosbok' and 'Mvuvhelo' were resistant to *M. incognita* race 2. Consequently, these cultivars are suitable for use in crop rotations intended to manage population densities of *M. incognita* race 2. However, further studies are necessary to establish the form of nematode resistance in order to determine whether the two cultivars could be used in introgression.

CHAPTER 4
MECHANISM OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN TWO SWEET
POTATO CULTIVARS

4.1 Introduction

Plants which are resistant to root-knot (*Meloidogyne* species) nematodes may exhibit pre- or post-infectional mechanisms of nematode resistance. The use of genetic materials from one plant to another is referred to as introgression and may become important in climate-smart agriculture for the introduction of pathogen resistance in resilient cultivars (Haussmann *et al.*, 2004). However, pre- infectional nematode resistance does not allow for gene introgression, only post-infectional nematode resistance can be introgressed (Kaplan and Davis, 1987; Thurau *et al.*, 2010), dictating the need to establish the mechanism of nematode resistance in any nematode resistant plant species in order for it to serve as a candidate of introgression.

Failure of J2 to penetrate roots in *Sorghum bicolor* cv. 'Ndendane-X1' led to the conclusion that the cultivar had pre-infectional mechanism of nematode resistance to both *M. incognita* race 2 and *M. javanica* (Mashela and Pofu, 2016). Pre-infectional nematode resistance was also suggested on other crops including, *Tagetes erecta* (Huang, 1985), *Allium sativum* (Fadzirayi *et al.*, 2010), *Phaseolus lunatus* (Veech, 1982), *Fragaria ananassa* (Oslo, 2010), *Rudbeckia hirta* (Freeman *et al.*, 1993) and *Brassica napus* (Matthiesen and Kirkegaard, 2006; Ntalli and Caboni, 2012).

On the basis of presence of necrotic spot, failure of giant cell development, proliferation of rootlet interference and proliferation of small undeveloped root galls, Ramatsitsi (2017) suggested that *Cucumis africanus* and *Cucumis myriocarpus* had post-infectional nematode resistance to *Meloidogyne* species, respectively. Also, *Glycine max* (Pedrosa *et al*., 1996), *Poncirus trifoliata* (Kaplan, 1981), *Vitis vinifera* (Anwar and McKenry, 2000), *Vigna unguiculata* (Williamson and Kumar, 2006), *Solanum lycorpersicum* (Kwara *et al*., 2014) and coffee (Silva *et al*., 2010) have had post-inflectional nematode resistance.

The host-status and host-sensitivity results suggested that cultivars 'Bosbok' and 'Mvuvhelo' were resistance to *M. incognita* race 2 (Chapter 3). However, information on mechanism of nematode resistance in two sweet potato cv. 'Bosbok' and 'Mvuvhelo' against *M. incognita* race 2 remains scanty. Generally, only post-infectional mechanism of nematode resistance can be used in plant breeding (Kaplan and Davis, 1987; Thurau *et al*., 2010). Information on mechanism of nematode resistance in the two sweet potato cultivars 'Bosbok' and 'Mvuvhelo' would improve their utility in plant breeding programmes. The objective of this study was to determine the existing nematode resistance mechanism in any of the test cultivars that had resistance to *M. incognita* race 2.

4.2 Materials and methods

4.2.1 Description of the study site

The study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E) under greenhouse conditions. The trial for mechanism of resistance was conducted during summer (October-December) 2016. Ambient day/night temperatures during each season averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans.

4.2.2 Treatments and research design

Treatments comprising of 15 harvesting times, was laid out in randomized complete block design (RCBD), with four replications (Legend 4.1). Harvesting was done every other day, for a period of 30 days.

4.2.3 Procedures

The sterilised cuttings of sweet potato cultivars namely, 'Bosbok' and 'Mvuvhelo' were transplanted into 250 ml polystyrene cups containing 200 ml steam-pasteurised fine sand. Pots were placed on greenhouse benches at 10 cm inter and intra row spacing. Sweet potato cuttings and nematodes were obtained from Green Biotechnologies Research Centre. Isolates of *M. incognita* race 2 were raised on nematode-susceptible tomato cv. 'Floradade' seedlings and roots collected for egg masses when needed. Uniform egg masses were hand-picked using a tooth pick and put in tapwater for over

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72 hours to allow for the development of all eggs to J2 (Powers *et al.*, 1992). A day after transplanting, each seedling was infested with *M. incognita* J2 by dispensing 100 *M. incognita* race 2 each using a 20 ml plastic syringe by placing into 5 cm deep holes on the cardinal points of the stem per treatment. Seedlings were irrigated with 40 mL tapwater every second day.

Legend 4.1 Establishment of cv. 'Mvuvhelo' inoculated with *Meloidogyne incognita*.

4.2.4 Data collection

Vines were harvested every other day for thirty days. At each harvest, shoots of the harvested cuttings were separated from roots, and the roots were rinsed in water to remove the soil particles. Total roots was stained in 3.5% acid fuchsin (Byrd *et al.*, 1983). Roots were immersed in 1.5% NaOCl solution for 5 minutes followed by rinsing in tapwater to remove excess NaOCl solution. The roots sample were stained in 30 ml tapwater mixed with 1 ml acid fuchsin, which was heated to boiling in an autoclave for 10 minutes, and then cooled to room temperature. Excess stain was removed by decanting, with total root system remaining in the base of the flask. Roots were distained in acidified glycerine mixed with few drops of hydrochloric acid and boiled for 1 minute. Total distained root system was placed in a 9 cm petri dish, with the top lid on, assessed under a microscope at 45 × magnification for (a) necrotic spot, (b) giant cell number, (c) rootlet interference at feeding site and (d) root gall number (Legend 4.2b).

Legend 4.2 (a) Stained and (b) distained root sample of cv. 'Mvuvhelo' infected with *Meloidogyne incognita* race 2

4.2.5 Data analysis

Data were subjected to analysis of variance using SAS software as previously described (Chapter 3)

4.3 Results

4.3.1 Nematode resistant and quadratic relations on cv. 'Bosbok'

In cv. 'Bosbok' harvest time (days) had highly significant (P ≤ 0.01) effects on necrotic spots, giant cell number and rootlet interference. The treatments contributed 86, 51 and 90% in TTV for necrotic spots, giant cell number and rootlet interference, respectively (Table 4.1). Starting from Day 2 after inoculation, only necrotic spots were noticeable on the first harvest, whereas giant cell number and rootlet interference were noticeable from Days 4 to 28 (Table 4.2). Necrotic spot, giant cell and rootlet interference over sampling period each exhibited positive quadratic relations, with the model having 73, 65 and 86% associations, respectively (Figure 4.1). Using the relation $x = -b_1/2b_2$, the optimum necrotic spots, giant cells and rootlet interference were attained at Days 14, 7 and 17 after inoculation (Table 4.4).

4.3.2 Nematode resistant and quadratic relations on cv. 'Mvuvhelo'

In cv. 'Mvuvhelo' periodic harvest time (days) had highly significant (P ≤ 0.01) effects on necrotic spot, giant cell number and rootlet interference. The treatments contributed 95, 72 and 74% in TTV for necrotic spot, giant cell number and rootlet interference, respectively (Table 4.1). Also, starting from Day 2 after inoculation, only necrotic spots were noticeable on the first harvest, whereas, giant cell number and rootlet interference

were noticeable from Days 4 to 28 (Table 4.3). Also, necrotic spots, giant cells and rootlet interference over sampling period each exhibited positive quadratic relations, with the models having 94, 72 and 76% associations, respectively (Figure 4.2). Using the relation $x = -b_1/2b_2$, the optimum necrotic spots, giant cells and rootlet interference were attained at Days 13, 8 and 40 after inoculation (Table 4.4).

Table 4.1 Total treatment variation (TTV) on necrotic spot, giant cell number and rootlet interference in sweet potato cultivars 'Bosbok' and 'Mvuvhelo' infected by *Meloidogyne incognita* race 2 under greenhouse conditions at 30 days after inoculation (n = 60).

		Necrotic spot		Giant cell number		Rootlet interference	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Cultivar 'Bosbok'							
Replication	3	0.02423	7	0.14836	26	0.00276	1
Treatment	14	0.30390	86[***]	0.29141	51[***]	0.33076	90[***]
Error	42	0.02358	7	0.12942	23	0.03517	9
Total	59	0.35171	100	0.56919	100	0.36869	100
Cultivar 'Mvuvhelo'							
Replication	3	0.01364	2	0.06567	13	0.03970	13
Treatment	14	0.54667	95[***]	0.35793	72[***]	0.22229	74[***]
Error	42	0.01768	3	0.07720	15	0.04021	13
Total	59	0.57799	100	0.5008	100	0.3022	100

[ns]Not significant at $P \leq 0.05$, [**]Significant at $P \leq 0.05$, [***]Highly significant at $P \leq 0.01$.

Table 4.2 Mean separation for necrotic spot, giant cell number, rootlet interference and root mass in sweet potato cultivar 'Bosbok' infected by *Meloidogyne incognita* under greenhouse conditions at 30 days after inoculation (n = 60).

Days	Necrotic spot[z]	Giant cell number[z]	Rootlet interference[z]
2	0.325[d]	0.000[e]	0.000[d]
4	1.029[c]	0.517[abcd]	0.226[cd]
6	1.152[bc]	0.406[bcde]	0.150[d]
8	1.042[bc]	0.850[ab]	0.445[bc]
10	1.069[bc]	0.780[abc]	0.075[d]
12	1.078[bc]	0.870[ab]	0.464[bc]
14	1.160[bc]	0.949[a]	0.639[ab]
16	1.138[bc]	0.778[abc]	0.584[ab]
18	1.245[abc]	0.612[abcd]	0.656[ab]
20	1.256[ab]	0.376[bcde]	0.612[ab]
22	1.425[a]	0.489[abcde]	0.826[a]
24	1.391[a]	0.445[abcde]	0.802[a]
26	1.389[a]	0.420[bcde]	0.806[a]
28	1.401[a]	0.325[cde]	0.795[a]
30	1.427[a]	0.195[de]	0.806[a]
P ≤	0.01	0.05	0.01

[z]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

Table 4.3 Mean separation for necrotic spot, giant cell number, rootlet interference and root mass in sweet potato cultivar 'Mvuvhelo' infected by *Meloidogyne incognita* under greenhouse conditions at 30 days after inoculation (n = 60).

Days	Necrotic spot[z]	Giant cell number[z]	Rootlet interference[z]
2	0.294[g]	0.000[f]	0.000[f]
4	0.639[f]	0.359[ef]	0.301[e]
6	0.894[e]	0.519[bcde]	0.389[de]
8	1.065[e]	0.758[abcd]	0.461[cde]
10	0.931[e]	0.926[a]	0.588[cd]
12	1.265[d]	0.967[a]	0.539[cde]
14	1.319[cd]	0.775[abc]	0.595[cd]
16	1.325[cd]	0.938[a]	0.464[cde]
18	1.363[bcd]	0.834[ab]	0.656[bcd]
20	1.358[bcd]	0.775[abc]	0.508[cde]
22	1.420[abcd]	0.369[def]	0.639[bcd]
24	1.487[abc]	0.325[ef]	0.595[cd]
26	1.521[ab]	0.413[cde]	0.699[bc]
28	1.576[a]	0.489[bcde]	0.894[ab]
30	1.565[a]	0.194[ef]	1.003[a]
P ≤	0.01	0.01	0.01

[z]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.
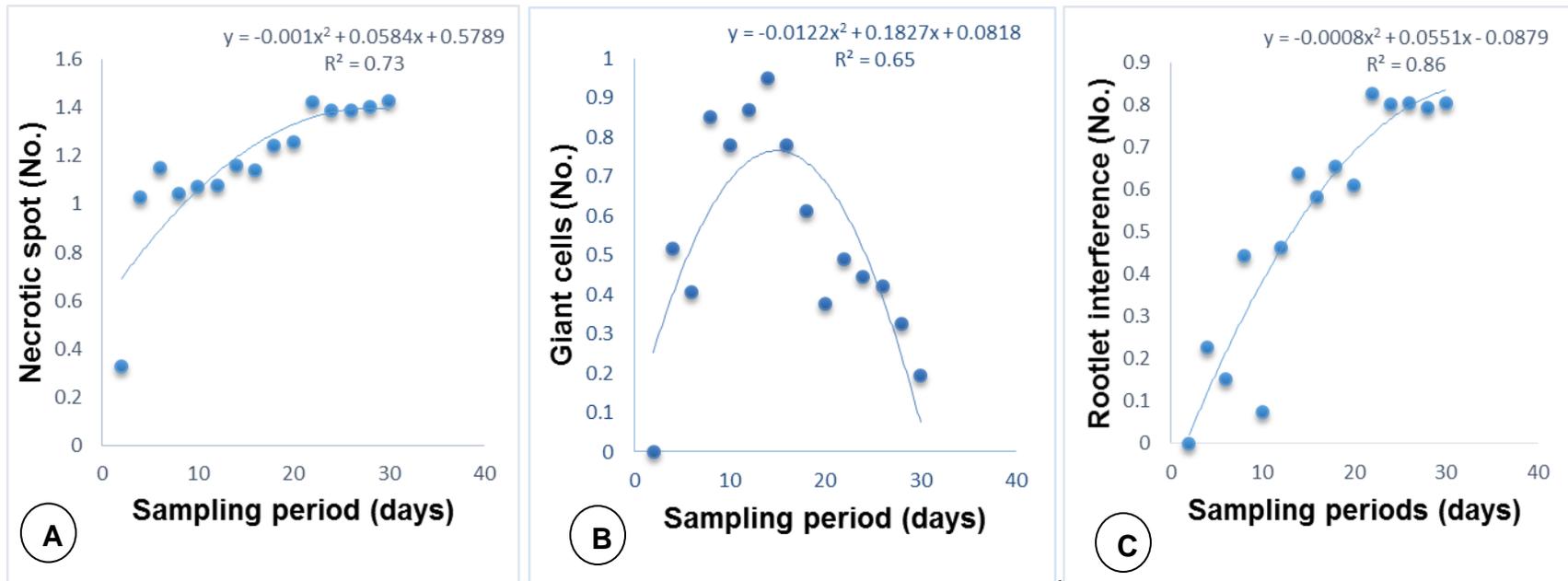
Figure 4.1 Quadratic response of (A) necrotic spots, (B) giant cells and (C) rootlet interference in cultivar 'Bosbok' to sampling periods under greenhouse conditions.
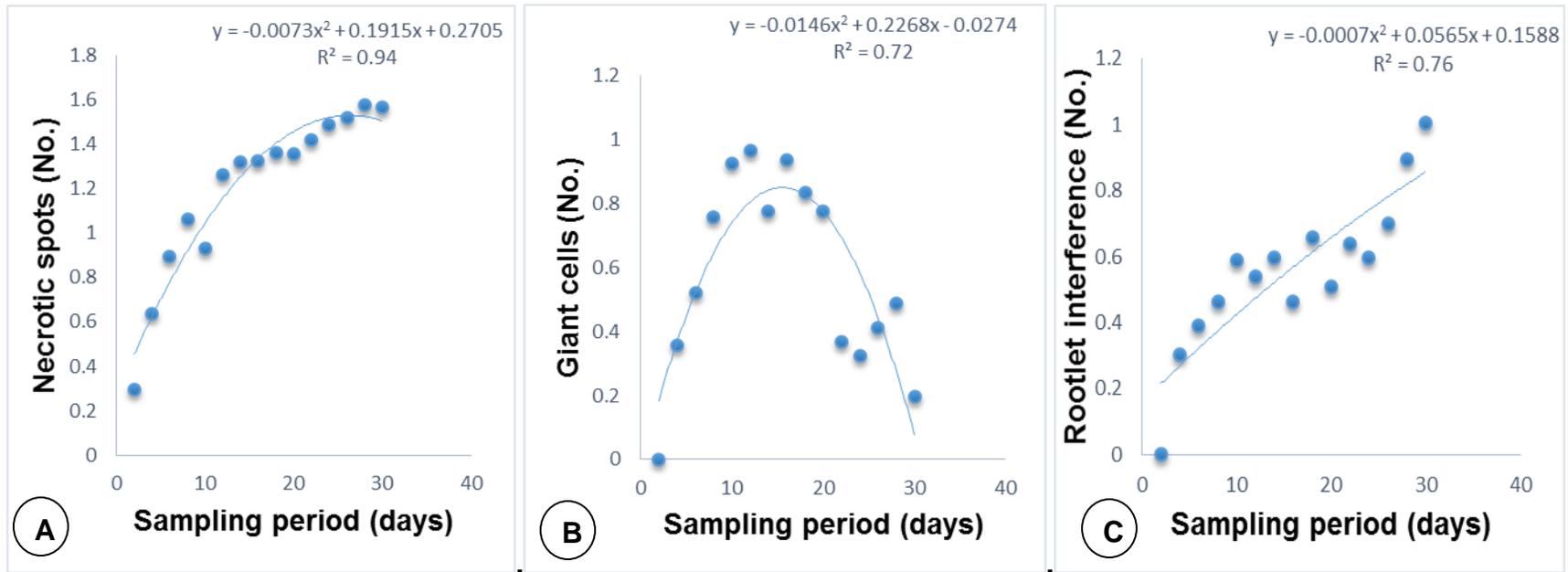
Figure 4.2 Quadratic response of (A) necrotic spots, (B) giant cells and (C) rootlet interference in cultivar 'Mvuvhelo' to sampling periods under greenhouse conditions.

Table 4.4 Quadratic relationships, coefficient of determination and computed optimum response of sampling period (days) for necrotic spot, giant cell and rootlet interference in sweet potato cultivars 'Bosbok' and 'Mvuvhelo'.

Variable	Quadratic relation	R^2	(x) Day
	Cultivar 'Bosbok'		
Necrotic spot	$y = -0.0042x^2 + 0.1168x + 0.5789$	0.73	14
Giant cell	$y = -0.0122x^2 + 0.1827x + 0.0818$	0.65	7
Rootlet interference	$y = -0.0032x^2 + 0.1102x - 0.0879$	0.86	17
	Cultivar 'Mvuvhelo'		
Necrotic spot	$y = -0.0073x^2 + 0.1915x + 0.2705$	0.94	13
Giant cell	$y = -0.0146x^2 + 0.2268x - 0.0274$	0.72	8
Rootlet interference	$y = -0.0007x^2 + 0.0565x + 0.1588$	0.76	40

$x = -b_1/2b_2$.

4.4 Discussion

4.4.1 Necrotic spots

Sampling period had highly significant effects on necrotic spots for both cultivars 'Bosbok' and 'Mvuvhelo' relations with *M. incognita* race 2*.* Necrotic spots were observed at an early stage, on Day 2 after inoculation in both cv. 'Bosbok' and 'Mvuvhelo'. For both cultivars 'Bosbok' and 'Mvuvhelo' the optimum necrotic spots were attained at Days 14 and 13 after inoculation, respectively. The common response to root-knot nematode infection in resistant crops is an early hypersensitive response, which is a reaction that results in cell death (Mashela *et al.*, 2016). Death of cells at the feeding site prevents nematode feeding formation and development, leading to nematode death (Morel and Dangl, 1997; Postnikova *et al.*, 2015). In other cases, HR-response occurs when juveniles are still moving to infection site. Upon penetration the root juveniles move to penetrate vascular bundle and in HR responses, localised necrotic spots are visible within a few days of penetration near the anterior end of the nematode (Dropkin *et al.,* 1969; Mashela *et al.*, 2016; Paulson and Webster, 1972; Ramatsitsi, 2017).

Similar results of responses to root-knot nematode infection were observed in tomato, pepper, coffee, chili pepper and myrobalan plum which had post-infection nematode resistance (Albuquerque *et al.*, 2010; Anthony *et al.*, 2005; Khallouk *et al.*, 2011; Moon *et al.*, 2010; Pegard *et al.,* 2005; Williamson and Hussey, 1996). Cabasan *et al.* (2014) observed localised necrotic spots within a few days after nematode penetration in

nematode-resistant rice genotypes. Dean and Struble (1953) observed necrotic spots in roots of nematode-resistant tomato and sweet potato, which resulted in death of *M. incognita* juveniles. *Coffea canephora* cv. '*Apoata*', resistant to *M. exigua* exhibited necrotic spots, which inhibited formation of feeding site (Silva *et al*., 2013). Kaplan (1981) observed necrotic spots on *Swingle citrumelo* seedling rootstocks that were inoculated with *Tylenchulus semipenetrans.* Moon *et al.* (2010) also observed necrotic spots in resistant *Capsicum annuum* cultivars exposed to *M. incognita.* Similar observations were reported by Riggs and Winstead (1959) on nematode-resistant tomato infected with *M. incognita.* Tissue necrosis had also been attributed for nematode resistance observed in soybean (Dropkin and Nelson, 1960), cotton (Brodie, 1960), tobacco (Powell, 1962), chrysanthemum (Wallace, 1961) and citrus (Van Gundy, 1964). The formation of necrotic spots, therefore, appeared to be a common feature in plants with post-infectional nematode resistance.

Necrotic spots in nematode-infected roots characterise hyperactive responses in nematode-resistant plants as reviewed by Mashela *et al*. (2016) in most nematode-resistant transgenic plants. In sweet potato cultivars 'Bosbok' and 'Mvuvhelo', resistance to *M. incognita* race 2 appeared to occur via a hypersensitive reaction (Komiyama *et al.,* 2006). Necrotic spots stained with acid fuschin were, in the current study, observed as a group of dead cells with thick cell walls, which agreed with observations by Komiyama *et al.* (2006), who noted that necrotic spots stained with Safranin O were a group of dead cells in roots of resistant wild sweet potato (*Ipomoea trifida*) inoculated with *M. incognita*.

According to Nicholson and Hammerschmidt (1992), the presence of necrotic spots might suggest the presence of phenols that could have played some roles in plant defense mechanisms. Generally, increased phenol production in resistant crops such as oats (*Avena sativa*), when exposed to *M. incognita*, had been characterised as being common (Marini *et al.,* 2016). Necrotic spots around J2 in roots of *A. sativa* resulted in the arrest of J2 development (*Marini et al.,* 2016). An HR-like reaction upon infection with root-knot nematodes had been observed in many nematode-resistant crops (Balhadere and Evans, 1995; Siddiqui, 1971).

4.4.2 Giant cells

Treatments significantly reduced the number of giant cells in cultivars 'Bosbok' and 'Mvuvhelo' infected with *M. incognita* race 2. Giant cells were observed from Day 4 after inoculation in both cultivars. Optimum giant cell formation in cultivars 'Bosbok' and 'Mvuvhelo' was attained at Days 7 and 8 after inoculation, respectively, and thereafter started to decline, which suggested strong resistance to the development of this variable. The presence of undeveloped giant cells in cv. 'Bosbok' and 'Mvuvhelo' at Day 4 demonstrated that *M. incognita* was able to initiate giant cell formation in roots of resistant sweet potato cultivars at early stages in the infection process. However, after the optimum periods, giant cells began to deteriorate and collapse, resulting in failure of feeding and therefore J2 development and reproduction. Similar results were observed in resistant cowpea (Das *et al.*, 2008), resistant cotton (Carneiro *et al.,* 2005; Mota *et al.,* 2013) and in resistant peppers (Bleve-Zaccheo *et al.,* 1998).

Giant cells in the current study were poorly developed, with reduced size. In nematode-resistant rice genotypes to *M. graminicola*, Cabasan *et al.* (2014) observed that some J2 that had penetrated roots at Day 3 after inoculation were already in contact with the stele and had started the formation of giant cells. In coffee, the Mex1-carrying genotype IAPAR59, did not prevent formation of a few giant cells, but J2 and feeding sites failed to develop further (Anthony *et al.*, 2005). In the review of chemicals related to nematode resistance in transgenic plants, the plant produces a wide range of plant genes referred to as antigene products, antiplant gene and RNAi (Mashela *et al.*, 2016). These plant genes counter those that are produced by nematodes, referred to as gene products (Mashela *et al.*, 2016). The interaction between gene products and plant genes, dictate whether giant cells are successfully or unsuccessfully formed (Mashela *et al.*, 2016).

According to Cabasan *et al.* (2014), giant cells were induced in the resistant rice genotypes after successful establishment of feeding sites by some J2. However, the giant cells observed in rice genotypes were poorly developed, highly vacuolated and with thin cell walls, which supported observations in the two sweet potato cv. 'Bosbok' and 'Mvuvhelo' in the current study. Similar observations had been made in cotton (McClure *et al.*, 1974), coffee (Anthony *et al.*, 2005) and cowpea (Das *et al.*, 2008). Vacuolisation of giant cells in resistant crops had been associated with the accumulation of hydrolases and toxins (Jones, 2001), which lead to cell degradation. In the current study, vacuolisation of the giant cells started at Day 8 after inoculation, reduction in size of giant cells results in a lower nutritional status of feeding sites, which

in turn leads to a delayed development of the juveniles, consequently the death of nematodes (Cabasan *et al.*, 2012; Pedrosa *et al.*, 1996).

In nematode-susceptible plant species, giant cells are formed as multinucleate structures formed when the feeding cell and those around it responds to nematode infection by undergoing repeated mitosis without cytokinesis (Huang *et al.,* 2003; Van der Eycken *et al.,* 1996). The successful establishment of feeding cells is essential for nematode development (Ferranz and Brown, 2002). *Meloidogyne* species evolved strategies that enable them to induce feeding cell formation on thousands of plant species by manipulating important factors of plant cell development (Caillaud *et al.,* 2005). The secretion of gene products is important in the formation of nematode feeding sites and nematode development to subsequent reproductive stages (Curtis, 2008; Siddique *et al.,* 2014). The giant cell serves as a source of nutrients for the developing nematodes (Bartlem *et al.*, 2013). The post-penetration compatibility in susceptible crops is usually associated with optimal development of giant cells that form a large multinucleate structure which, however, fail to develop in nematode resistant crops (Orion *et al.,* 1980).

Failure in the development of giant cells as denoted by numbers and sizes, suggested that *M. incognita* failed to establish feeding sites on the test sweet potato cultivars in the current study. Results in this study agree with those of Ramatsitsi (2017), where *M. incognita* race 2 failed to induce galls in roots of *Cucumis africanus.*

4.4.3 Rootlet interference

Periodic harvest intervals had highly significant effects also on rootlet interference for both cultivars 'Bosbok' and 'Mvuvhelo' relations with *M. incognita* race 2. Rootlet interference were observed 4 days after inoculation for both cv. 'Bosbok' and 'Mvuvhelo' inoculated with *M. incognita* race 2. The optimum rootlet interference in cv 'Bosbok' and 'Mvuvhelo' were attained at Days 17 and 40 after inoculation, respectively. Similarly, Ramatsitsi (2017) observed rootlet interference in nematode-resistant *C. africanus* inoculated with *M. incognita* race 2 and *M. javanica* and *C. myriocarpus* inoculated with *M. incognita* race 2 and *M. javanica*. The observations, confirms those in nematode-resistant *G. max* that was exposed to *M. javanica* (Doyle and Lambert, 2003) and on nematode-resistant white clover (*Trifolium repens*) that was exposed to *M. trifoliophila* (Mecer *et al*., 2004). Also, numerous rootlets on *Daucus carota* were observed when this nematode- resistant plant exposed to *M. chitwoodi* (Sone, 2010).

During the sedentary phases, for plant-nematode interactions to be compatible, most of the gene products from the dorsal gland cells of nematodes mimic plant genes by producing plant growth regulators, especially the cytokinins and the auxins (Mashela *et al*., 2016). For example, the cytokinins (Lohar *et al.,* 2004; Siddique *et al.,* 2014) and auxins (Domingo *et al.,* 1998; Duncan *et al.,* 1996; Huyangura *et al.,* 1999) produced by the dorsal gland cells in sedentary adult nematodes, are known to play a role in the initiation of lateral roots (Benkova and Bielach, 2010). During the initiation and development of the feeding sites of sedentary plant-parasitic nematodes plant growth regulator manipulation is known to be an important process (Mashela *et al*., 2016). The

root initiation, development and lateral root formation can be stimulated by use of auxin (De Smet *et al.*, 2010). In the current study, rootlets were observed originating adjacent to the poor developed giant cells, which confirmed observations of improved lateral root initiation adjacent to root galls in other studies (Goverse *et al.*, 2000; Karczmarek *et al.*, 2004). Similar rootlets were observed when various nematode-resistant *Cucumis* species were exposed to *Meloidogyne* species (Ramatsitsi, 2017).

4.4.4 Nematode juveniles

There were no nematode juveniles detected inside the roots of the two sweet potato cultivars even at Day 30 after inoculation of *M. incognita* race 2 in the current study. Similar results were observed in nematode-resistant *Cucumis* species inoculated with *M. incognita* race 2 and *M. javanica* (Ramatsitsi, 2017).

Although resistance to root-knot nematodes is usually expressed after nematode penetration of roots, several studies have indicated that some plants exhibited resistance to root-knot nematode penetration (Howard, 1965; Rohde, 1965). Sasser and Taylor (1952) suggested that resistance in plants to root-knot nematodes may be caused in part by failure of the larvae to enter the roots or entry of reduced number with little or no development. However, in resistant plants, there is no development of the feeding site. Instead, a localized tissue necrosis or hypersensitive reaction occurs at or near the site where feeding would normally be initiated. Nematodes that fail to establish feeding sites either die or leave the roots (Komiyama *et al.,* (2006), which might be the reason for the undetectable juveniles in roots of cultivars 'Bosbok' and 'Mvuvhelo'.

4.4.5 Root galls

Root galls were also not observed on roots of the two sweet potato cultivars even at Day 30 after inoculation with *M. incognita* race 2. Generally, in nematode-susceptible plant species, when root-knot J2 develop through J3, J4 and adult female stages, due to giant cells, the adjacent root cells bulge to form a root gall (Mashela *et al.*, 2016). In the current study nematode infection did not succeed in the formation of giant cells, consequently there was no development of J2 through J3, J4 and adult female stages, which resulted in absence of root galls. Similarly, there were no root galls on roots of *Vigna unguiculata* varieties and *Cucumis* species when inoculated with *Meloidogyne* species (Ramatsitsi, 2017; Williamson and Kumar, 2006).

4.5 Conclusion

Observations in the present study confirmed that post-infectional nematode resistance to *M. incognita* race 2 was in place in the two sweet potato cultivars. Responses in roots of the two sweet potato cultivars 'Bosbok' and 'Mvuvhelo' to infection by *M. incognita* race 2 were similar as depicted by (1) presence of necrotic spots, (2) poorly developed giant cell, (3) formation of rootlet interference (4) absence of root galls and (5) non detectable J2 in roots. All these responses suggested the existence of post-infectional nematode resistance in the two tested sweet potato cultivars, consequently, the cultivars 'Bosbok' and 'Mvuvhelo' could be used in areas with high nematode levels and possibly in introgression for introducing nematode resistance in susceptible sweet potato cultivars.

CHAPTER 5
SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

The study focused on (1) host-status and host-sensitivity of sweet potato (*Ipomoea batatas*) cultivars 'Bophelo', 'Bosbok' and 'Mvuvhelo' to *Meloidogyne incognita* races 2 and (2) mechanism of nematode resistance in resistant cultivars 'Bosbok' and 'Mvuvhelo' to *M. incognita* race 2. In cultivar 'Bophelo' *M. incognita* race 2 reproduced and reduced plant growth, therefore, the cultivar was a susceptible host to this *Meloidogyne* species, thereby confirming other-related observations (Pofu *et al.*, 2016). In contrast, cultivars 'Bosbok' and 'Mvuvhelo' did not allow *M. incognita* race 2 to reproduce on them and nematode infection did not reduce plant growth. Therefore, the two cultivars were resistant to *M. incognita* race 2 (Chapter 3). Mechanism of resistance to *M. incognita* race 2 on cultivars ''Bosbok' and 'Mvuvhelo' demonstrated significant hypersensitive responses, depicted by the (1) presence of necrotic spots, (2) poorly developed giant cells, (3) formation of rootlet interferences (4) absence of root galls and (5) non-detectable second-stage juveniles (J2) in roots. All the listed responses suggested the existence of post-infectional nematode resistance in the two cultivars to *M. incognita* race 2 (Chapter 4). Similar post-infectional nematode resistance were observed in *Cucumis* species indigenous to Limpopo Province, South Africa, to all tropical root-knot species (Ramatsitsi, 2017).

5.2 Significance of findings

Cultivars 'Bosbok' and 'Mvuvhelo' could be used in crop rotations systems intended to manage population densities of *M. incognita* race 2 in tropical areas of Limpopo Province. The form of nematode resistance in cultivars 'Bosbok' and 'Mvuvhelo' could be introgressed into economically important sweet potato cultivars that do not have resistance to *M. incognita* race 2 (Mashela *et al.*, 2016). Most commercially available sweet potato cultivars in South Africa are hosts to tropical *Meloidogyne* species (Pofu *et al.*, 2016). Therefore, introgression would be important since most synthetic chemical nematicides had been withdrawn from the agrochemical markets due to their environment-unfriendliness.

5.3 Recommendations

Post-infectional nematode resistance had been identified in cultivar 'Bosbok' and 'Mvuvhelo'*,* and it would be prudent to investigate the related plant genes (Mashela *et al.,* 2016) in the two sweet potato cultivars. Identified plant genes would enhance introgression of the genes into high yielding nematode susceptible cultivars using molecular approaches (Mashela *et al.,* 2016). Additionally, attempts should be made to introgress the plant genes from the two cream white-fleshed cultivars into orange-fleshed cultivars such as the susceptible cultivar 'Bophelo', which had been targeted for biofortification programmes (Pofu *et al.*, 2016). Also, the findings in the current study demonstrated the need for multidisciplinary research teams among nematologists and molecular biotechnologists in sweet potato breeding programmes.

5.4 Conclusions

Sweet potato cv. 'Bophelo' was susceptible to *M. incognita* race 2*,* whereas cultivars 'Bosbok' and 'Mvuvhelo' were resistant to the nematode species. Consequently, the two cultivars could be viewed as being suitable for use in crop rotations intended to manage population densities of *M. incognita* race 2. The hypersensitivity indicators observed in cultivars 'Bosbok' and 'Mvuvhelo' all suggested that post-infectional nematode resistance to *M. incognita* race 2 was in place, which have the plant genes with attributes for use in introgression.

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APPENDICES

Appendix 3.1 Analysis of variance for second-stage juveniles (J2) in roots of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	0.19980	0.04995		
Treatment	6	0.29970	0.04995	1.00	0.4481
Error	24	1.19881	0.04995		
Total	34	1.69831			

Appendix 3.2 Analysis of variance for second-stage juveniles (J2) in soil of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	14.8569	3.71423		
Treatment	6	9.5885	1.59808	1.47	0.2298
Error	24	26.0530	1.08554		
Total	34	50.4984			

Appendix 3.3 Analysis of variance for final nematode population (Pf) of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	12.4622	3.11556		
Treatment	6	8.6149	1.43581	1.29	0.2996
Error	24	26.7299	1.11375		
Total	34	47.8070			

Appendix 3.4 Analysis of variance for reproductive factor (RF) of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	46.512	11.6279		
Treatment	6	151.200	25.2000	4.50	0.0035
Error	24	134.486	5.6036		
Total	34	332.198			

Appendix 3.5 Analysis of variance for vine length of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	538.43	134.607		
Treatment	7	290.50	41.500	0.76	0.6232
Error	28	1524.96	54.463		
Total	39	2353.89			

Appendix 3.6 Analysis of variance for stem diameter of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	5.8990	1.47475		
Treatment	7	10.6720	1.52457	2.40	0.0470
Error	28	17.8130	0.63618		
Total	39	34.3840			

Appendix 3.7 Analysis of variance for dry shoot mass of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	542.83	135.708		
Treatment	7	236.34	33.762	0.98	0.4642
Error	28	963.32	34.404		
Total	39	1742.48			

Appendix 3.8 Analysis of variance for dry root mass of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	283.31	70.8266		
Treatment	7	440.56	62.9366	2.80	0.0241
Error	28	628.33	22.4405		
Total	39	1352.20			

Appendix 3.9 Analysis of variance for dry tuber mass of cultivar 'Bophelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	9.7932	2.44831		
Treatment	7	31.6133	4.51618	3.21	0.0126
Error	28	39.3696	1.40606		
Total	39	80.7761			

Appendix 3.10 Analysis of variance for second-stage juveniles (J2) in roots of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	0.36427	0.09107		
Treatment	6	0.54641	0.09107	1.00	0.4481
Error	24	2.18565	0.09107		
Total	34	3.09633			

Appendix 3.11 Analysis of variance for second-stage juveniles (J2) in soil of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	1.2332	0.30831		
Treatment	6	4.9330	0.82216	2.67	0.0398
Error	24	7.3994	0.30831		
Total	34	13.5656			

Appendix 3.12 Analysis of variance for final nematode population (Pf) of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	1.2570	0.31425		
Treatment	6	5.0273	0.83788	2.67	0.0398
Error	24	7.5421	0.31425		
Total	34	13.8264			

Appendix 3.13 Analysis of variance for reproductive factor (RF) of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	0.00459	0.001148		
Treatment	6	0.01826	0.003044	2.65	0.0407
Error	24	0.02755	0.001148		
Total	34	0.05041			

Appendix 3.14 Analysis of variance for vine length of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	1495.9	373.969		
Treatment	7	1853.8	264.825	0.74	0.6371
Error	28	9964.4	355.871		
Total	39	13314.0			

Appendix 3.15 Analysis of variance for stem diameter of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	5.409	1.35213		
Treatment	7	36.188	5.16971	0.96	0.4774
Error	28	150.520	5.37570		
Total	39	192.116			

Appendix 3.16 Analysis of variance for dry shoot mass of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	115.06	28.7641		
Treatment	7	642.35	91.7645	1.12	0.3809
Error	28	2302.20	82.2213		
Total	39	3059.60			

Appendix 3.17 Analysis of variance for dry root mass of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	40.44	10.1103		
Treatment	7	276.85	39.5495	1.08	0.4042
Error	28	1028.95	36.7483		
Total	39	1346.24			

Appendix 3.18 Analysis of variance for dry tuber mass of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	202.38	50.5938		
Treatment	7	159.82	22.8311	0.38	0.9041
Error	28	1666.19	59.5067		
Total	39	2028.38			

Appendix 3.19 Analysis of variance for second-stage juveniles (J2) in roots of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	2.2424	0.56060		
Treatment	6	2.7095	0.45159	1.00	0.4505
Error	24	10.8830	0.45346		
Total	34	15.8350			

Appendix 3.20 Analysis of variance for second-stage juveniles (J2) in soil of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	0.64846	0.16211		
Treatment	6	0.97269	0.16211	1.00	0.4481
Error	24	3.89075	0.16211		
Total	34	5.51189			

Appendix 3.21 Analysis of variance for final nematode population (Pf) of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	1.9461	0.48653		
Treatment	6	3.2259	0.53765	0.93	0.4915
Error	24	13.8740	0.57808		
Total	34	19.0460			

Appendix 3.22 Analysis of variance for reproductive (RF) of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 35).

Source	DF	SS	MS	F	P
Replication	4	0.10239	0.02560		
Treatment	6	0.39699	0.06616	2.03	0.1000
Error	24	0.78033	0.03251		
Total	34	1.27971			

Appendix 3.23 Analysis of variance for vine length of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	350.18	87.5446		
Treatment	7	216.00	30.8571	1.88	0.1106
Error	28	459.00	16.3929		
Total	39	1025.18			

Appendix 3.24 Analysis of variance for stem diameter of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	1.9760	0.49400		
Treatment	7	4.9998	0.71425	0.71	0.6658
Error	28	28.2640	1.00943		
Total	39	35.2398			

Appendix 3.25 Analysis of variance for dry shoot mass of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	32.252	8.0629		
Treatment	7	190.279	27.1827	1.51	0.2056
Error	28	505.095	18.0391		
Total	39	727.626			

Appendix 3.26 Analysis of variance for dry root mass of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	11.685	2.92121		
Treatment	7	23.688	3.38407	0.49	0.8350
Error	28	194.084	6.93158		
Total	39	229.458			

Appendix 3.27 Analysis of variance for dry tuber mass of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions 56 days after inoculation (n = 40).

Source	DF	SS	MS	F	P
Replication	4	236.86	59.2153		
Treatment	7	226.81	32.4013	0.70	0.6708
Error	28	1293.80	46.2070		
Total	39	1757.47			

Appendix 4.1 Analysis of variance for necrotic spots of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	0.07268	0.02423		
Treatment	14	4.25454	0.30390	12.89	0.0000
Error	42	0.99022	0.02358		
Total	59	5.31744			

Appendix 4.2 Analysis of variance for giant cells of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	0.44507	0.14836		
Treatment	14	4.07970	0.29141	2.25	0.0215
Error	42	5.43582	0.12942		
Total	59	9.96059			

Appendix 4.3 Analysis of variance for rootlet interference of cultivar 'Bosbok' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	0.00828	0.00276		
Treatment	14	4.63061	0.33076	9.40	0.0000
Error	42	1.47712	0.03517		
Total	59	6.11601			

Appendix 4.4 Analysis of variance for necrotic spots of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	0.04092	0.01364		
Treatment	14	7.65333	0.54667	30.92	0.0000
Error	42	0.74265	0.01768		
Total	59	8.43690			

Appendix 4.5 Analysis of variance for giant cells of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	0.19701	0.06567		
Treatment	14	5.01104	0.35793	4.64	0.0001
Error	42	3.24221	0.07720		
Total	59	8.45026			

Appendix 4.6 Analysis of variance for rootlet interference of cultivar 'Mvuvhelo' inoculated with *Meloidogyne incognita* race 2 under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	0.11910	0.03970		
Treatment	14	3.11203	0.22229	5.53	0.0000
Error	42	1.68875	0.04021		
Total	59	4.91988			