DEGREE OF NEMATODE RESISTANCE IN SWEET POTATO CULTIVAR 'MAFUTHA' TO TROPICAL *MELOIDOGYNE SPECIES*

BY

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MINI-DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

AGRICULTURE (AGRONOMY)

IN THE

FACULTY OF SCIENCE AND AGRICULTURE (SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES)

AT THE

UNIVERSITY OF LIMPOPO

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2018

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DECLARATION

I, Simangele Princess Nkosi, declare that this mini-dissertation hereby submitted to the University of Limpopo for the degree Master of Science in Agriculture (Agronomy) has not previously been submitted by me or anybody for a degree at this or any other University, that this is my work in design and in execution and that all materials contained herein had been acknowledged.

Candidate   : Simangele Princess Nkosi   Signature   Date

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DEDICATION

To my caring and loving aunt, Mrs Sonto Selinah Mkhabela and to my beloved

siblings Bawelile, Sphetho and Nomcebo Nkosi.

ACKNOWLEDGEMENTS

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ABSTRACT

Most sweet potato-producing regions in South Africa are heavily infested by the root-knot (*Meloidogyne* species) nematodes, which are difficult to manage since the withdrawal of the highly effective fumigant synthetic chemical nematicides. Prior to the withdrawal, the management of *Meloidogyne* species was not a priority in sweet potato *(Ipomoea batatas* L.) production since methyl bromide was highly effective in suppressing nematodes. The withdrawal resulted in the introduction of various alternative nematode management strategies, with nematode resistance being the most preferred. However, progress in the use of nematode resistance had been hindered by limited information on accurate species identification since *Meloidogyne* species have a wide host range and some biological races. The objectives of the study were (1) to determine the degree of nematode resistance in sweet potato cv. 'Mafutha' to *M. javanica*, *M. incognita* races 2 and *M. incognita* race 4 and (2) to investigate the mechanism of resistance in sweet potato cv. 'Mafutha' to *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4. A total of six Experiments were conducted. In each, treatments comprised 0, 25, 50, 125, 250, 625, 1250, 3125 and 5250 eggs and second-stage juveniles (J2), arranged in a randomised complete block design (RCBD), with six replications. Uniform rooted sweet potato cuttings were transplanted in 20-cm-diameter plastic pots filled with steam pasteurised (300˚C for 1 hour) loam soil and Hygromix-T mixed at 3:1 (v/v) ratio. At 56 days after inoculation, plant variables and nematodes in roots were collected. *Meloidogyne javanica* inoculum levels in Experiment 1 had highly significant (P ≤ 0.01) effects on dry shoot mass and, stem diameter, contributing 74% and 50% in total treatment variation (TTV) of the respective variables, whereas under *M. incognita* race 2 inoculum levels contributed 70% and 56% in TTV of dry root mass and dry shoot mass, respectively. *Meloidogyne incognita* race 4 inoculum levels contributed 65%

and 58% in TTV of stem diameter and dry shoot mass, respectively. In Experiment 2, *M. javanica* treatment levels contributed 56% in TTV of dry root mass, whereas *M. incognita* race 2 inoculum levels had no significant effect on any plant variable. In contrast, *M. incognita* race 4 contributed 51% in TTV of vine length. In Experiment 1, the nematode levels had significant effects on reproductive potential (RP) values, with treatments contributing 96%, 86% and 76% in TTV of RP values in *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4, respectively. In Experiment 2, treatments contributed 79%, 46% and 61% in TTV of RP values in the respective *Meloidogyne* species. Results of the study suggested that growth of sweet potato cv. 'Mafutha' was affected by nematode infection, whereas the test nematodes were able to reproduce and develop on the test potato cultivar. In conclusion, sweet potato cv. 'Mafutha' was susceptible to *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4 and therefore, the cultivar should not be included in crop rotation programmes intended to manage tropical *Meloidogyne* species and races in Limpopo Province, South Africa. Since the cultivar was susceptible to the test nematodes, the study did not evaluate the mechanism of resistance.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Globally, sweet potato *(Ipomoea batatas L.)* is among the seven major staple food crops and constitute an important component in food security for marginalised communities in developing countries with tropical climate (FAO, 2014). In South Africa, on average, approximately 60 000-ton sweet potato tubers per annum are being produced (DAFF, 2012). Based on its nutritional and consumer-demand attributes, sweet potatoes play a major role in ensuring national and household food security in Southern African Development Community (SADC) member states (Laurie *et al*., 2015). However, root-knot (*Meloidogyne* species) nematodes in SADC member states are the most damaging soil-borne pests of sweet potato. International estimated yield losses in crops without nematode resistance could be as high as 50% to complete crop failure (Barker *et al*., 1976; Lamberti, 1979). In South Africa, 6% losses in sweet potato had been reported prior to the 2005 withdrawal of methyl bromide and related fumigant nematicides (Kleynhans, 1991). Earlier and during the same period, sweet potato yield losses per annum due to *Meloidogyne* species had been estimated at 15% in South America, 24% in West Africa and 6% in South East Asia (Kleynhans, 1991; Sasser, 1979; Scurrah *et al*., 2005).

Highly effective synthetic fumigant nematicides such as methyl bromide were widely used to control *Meloidogyne* species. Following their withdrawal from the agrochemical markets due to their being environment-unfriendly (Mashela *et al*., 2015), alternative strategies to control the root-knot nematode population densities had been intensely researched and developed (Mashela *et al.,* 2011). The use of

nematode-resistant cultivars had since become a promising alternative method for managing root-knot nematode population densities.

1.1.1 Description of research problem

The population of South Africa which is currently estimated at 55.2 million (STATS SA, 2017), consumes mostly cereal-based staple diet. Conversely, sweet potato is grown by most small-holder farmers as cash crop and is regarded as one crop with the capacity to improve national and household food security (Fetuga *et al.*, 2013). Sweet potato had widely been used in biofortification programmes since it can combat vitamin A deficiency, especially, in the marginalised groups such as children and pregnant women (Laurie *et al.*, 2015). However, sweet potato-producing regions are infested by high populations of root-knot nematodes (Onkendi *et al.*, 2014), which are difficult to manage since the withdrawal from agrochemical markets of highly effective fumigant synthetic nematicides such methyl bromide. The withdrawal had raised the necessity to introduce alternative methods of nematode management such as the use of nematode-resistant cultivars. Progress in this regard was hindered by limited information on accurate species identification and existence of numerous races in *Meloidogyne* species.

Generally, in tropical South Africa, *M. javanica* and *M. incognita* races 2 and 4 occur in most production systems (Kleynhans *et al.*, 1996). *Meloidogyne javanica* and *M. incognita* occur as single or mixed populations. *Meloidogyne incognita* race 4 occurs mainly in cotton (*Gossypium hirsutum* L.) producing regions of South Africa (Kleynhans *et al.*, 1996). Pofu *et al.* (2016) demonstrated that most sweet potato

cultivars used in the biofortification programme in South Africa were hosts to tropical *Meloidogyne* species, whereas few were non-hosts.

Cultivar 'Mafutha', although it is orange-fleshed, had not been included in the biofortification programme (Laurie *et al.*, 2015), possibly due to its inherent low yielding capacity. However, due to its sweetness and dryness after cooking, the cultivar is in high demand among the locals, and is widely produced for subsistence purposes. Therefore, it was deemed necessary to assess its degree of resistance to *Meloidogyne* species, particularly in Limpopo Province, where the cultivar is popular in household gardens.

1.1.2 Impact of the research problem

Fumigant nematicides were withdrawn from agrochemical markets from being used in managing population densities of *Meloidogyne* species. However, the products were reported to be toxic to human health due to residues in the food chain, they contributed to environmental pollution through the depletion of the ozone layer, contributing to climate change and were also expensive to subsistence farmers and their continued use can lead to some level of resistance to the target nematode species (Onkendi *et al.*, 2014). Following the withdrawal of methyl bromide from the agrochemical markets in 2005, economic consequences like yield losses due to nematode damage skyrocketed to US$157 billion (Elling, 2013; Onkendi *et al.*, 2014) and relative to US$126 billion (Chitwood, 2003) prior to the withdrawal, the relative increase in yield losses was 37% (Mashela *et al.*, 2016).

1.1.3 Possible causes of the research problem

Root-knot nematodes are the most damaging and difficult pest to manage in agricultural crops (Taylor and Sasser, 1978). In the past years, synthetic fumigant nematicides were effective in suppressing the population densities of *Meloidogyne* species and most farmers relied on this management strategy (Onkendi *et al.*, 2014). However, Speth (2004) reported that highly effective nematicides such as methyl bromide were withdrawn from agrochemical markets due to their detrimental effects on the environment and toxicity to human health. The cut-off date of synthetic fumigant nematicides in 2005 caused serious challenges on crops due to nematode damage (Mashela *et al.*, 2015). After the withdrawal of fumigant nematicides, alternative strategies to manage nematode population densities were intensively researched and developed (Mashela *et al.*, 2015). In addition to other strategies, efforts that were directed for use as alternative nematode management strategies included the use of nematode resistant cultivars, which were considered to be cost-effective and environment-friendly (Moens *et al.*, 2009).

1.1.4 Possible solutions of research problem

The use of resistant cultivars is among the promising strategies that are being proposed as alternative to chemicals in managing nematode population densities. Nematode resistant cultivars reduce the cost of production and also protect the environment against pollution from chemical residues associated with synthetic nematicides (Onkendi *et al.*, 2014). However, progress of using resistant cultivars in this area is hindered by the wide range of the root-knot nematodes. Therefore, Elling (2013) suggested that there should be accurate identification of the targeted species in order to achieve adequate suppression of nematodes using resistant cultivars.

1.1.5 General focus of the study

The study focused on the host-status and host-sensitivity in sweet potato cv. 'Mafutha' to *M. javanica and M. incognita* race 2 and 4. Host-status is described variously using the reproductive factor (RF = Pf/Pi), which is the measure of an ability of a nematode to reproduce on a given host-plant (Windham and Williams, 1988) or the reproductive potential [RP = eggs + J2/g root]. Seinhorst (1965) described nematode resistance using host-status and host-sensitivity, through three host concepts, namely, susceptible, tolerant and resistant-hosts.

1.2 Problem statement

The emphasis on nematode management is currently on the use of nematode resistance since it is the cheapest way for managing plant-parasitic nematodes. Sweet potato cultivars are used in biofortification which is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnologies, intended for marginalised groups such as children and pregnant women (Laurie *et al.*, 2015). Laurie *et al.* (2015) reported that over 50 years the sweet potato research programme in South Africa focused on biofortification aimed at cultivars with high root yield, sweet taste, high dry matter content, high β-carotene content and the adaptation to local conditions. However, the successful implementation of biofortification programme in many countries was derailed by the destructive nature of *Meloidogyne* species. Therefore, the degree of nematode resistance should be included in biofortification research programmes.

1.3 Rationale

Sweet potato, especially the orange-fleshed cultivars, can be used to combat vitamin A deficiency among the marginalised group such as children and women in South Africa (Laurie *et al.*, 2015). However, sweet potato-producing areas are heavily infested by nematodes, particularly the root-knot nematodes (Pofu *et al.*, 2016). Nematode resistance is used as an alternative management strategy for suppressing high population densities of *Meloidogyne* species (Moens *et al.*, 2009). Nematode resistance in crops requires subjecting cultivars to known nematode species for host-status and host-sensitivity tests. Host-status measures the degree of nematode reproduction in a given plant species, whereas host-sensitivity is a measure of damage by the nematode in a given host plant (Taylor and Sasser, 1978). Generally, when the host does not allow the nematode to reproduce and plant growth or yield is not affected, the plant is said to be nematode-resistant (Seinhorst, 1965). The latter is a genotypic attribute and only when post-infectional resistance occurs, can genes be transferred from one plant to another through conventional plant breeding or through molecular technologies (Mashela *et al.*, 2017). The first step towards using nematode resistance in plant breeding is to assess the degree of nematode resistance in a given plant to know the nematode species and/or races. The second step is identifying the mechanism of nematode resistance since it is important to know whether post-infectional nematode resistance is involved or not. In South Africa, the widely distributed root-knot nematodes are *M. javanica* and *M. incognita* races 2 and 4 (Kleynhans *et al.*, 1996).

1.4 PURPOSE OF THE STUDY

1.4.1 Aim

Development of empirically-based information on host-status, host-sensitivity and mechanisms of resistance in sweet potato cv. 'Mafutha' to *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4.

1.4.2 Objectives

1. To determine the degree of nematode resistance in sweet potato cv. 'Mafutha' to *M. javanica*, *M. incognita* races 2 and *M. incognita* race 4 under greenhouse conditions.

2. To investigate the mechanism of resistance in sweet potato cv. 'Mafutha' to *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4.

1.5 Reliability, validity and objectivity

In this study, reliability of data in several experiments was ensured based on statistical analysis of data at the probability level of 5%. Validity was ensured through repeating the experiments in time (Little and Hills, 1981). 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

would be minimised by ensuring that the experimental error in each experiment was reduced through increased replications and randomisation (Little and Hills, 1981).

1.7 Scientific significance of the study

The study would provide useful information on the outcome of the host-status and host-sensitivity of sweet potato to the nematode species, *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4. The information generated could be used to enlighten the South African farmers in terms of managing *Meloidogyne* species using resistant cultivars in crop rotation and providing information on whether the cultivars could be used in plant breeding for nematode resistance.

1.8 Structure of mini-dissertation

The mini-dissertation was designed using the Senate-approved format of the University of Limpopo. Consequent to the description and detailed outlining of the research problem (Chapter 1), work done and the work not done on the research problem was reviewed (Chapter 2). Then, each of the two objectives would be addressed in separate sections of one chapter (Chapter 3). In the final chapter (Chapter 4), results in all chapters were summarised and integrated to provide the significance of the results and recommendations with respect to future research and then culminated in an overall conclusion of the study. The Harvard referencing style of using author-alphabet, as approved by the Senate of the University of Limpopo was used in this mini-dissertation.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

In sweet potato (*Ipomoea batatas* L.) production, plant-parasitic nematodes have become a major constrain in production areas (Onkendi *et al*., 2014). Nematodes are difficult soil-borne pests to manage and are of great concern to both smallholder and commercial farmers (Malungane, 2014). After the global withdrawal of the highly effective synthetic fumigant nematicides from agrochemical markets, few strategies were available for nematode management (Mashela *et al*., 2011). The use of nematode resistant cultivars had become popular as an effective nematode management alternative to reduce populations of plant-parasitic nematodes such as root-knot (*Meloidogyne* species) nematodes (Moens *et al*., 2009). Among the 12 screened biofortification and non-biofortification sweet potato cultivars, only three non-biofortified sweet potato cultivars, 'Bosbok', 'Blesbok' and 'Mvuvhelo' were non-host to *Meloidogyne* species whereas the rest were host except for the exotic cv. 'W-119' (Pofu *et al*., 2016).

Host-status is described using the reproductive factor (RF), which is a measure of the reproductive potential on a given host. Seinhorst (1967) described the concept, demonstrated that when Pf = Pi, the population is at equilibrium (E) point, beyond which nematodes are at high competition for resources, where RF is invariably less than unity. Normally, before E point, nematodes are at the lowest competition and if the plant is a host, RF is invariably greater than unity. However, the information obtained from the screening study was not sufficient due to the fact that it did not

provide information on nematode resistance or the mechanism involved. Seinhorst (1965) provided three concepts which describe nematode resistance in crops.

2.2 Work done on the problem statement

2.2.1 Nematode resistance in sweet potato

A number of studies had been conducted to assess nematode resistance in sweet potato to root-knot nematodes. According to Sun and Chen (1994), resistance of sweet potato to *Meloidogyne* species such as *M. incognita* and *M. javanica* had been conducted in Japan, the United States of America and China (Kukimura *et al*., 1989). A study that was conducted in Nigeria on pathogenicity of *M. incognita* on three cultivars of sweet potato demonstrated that all the cultivars tested were susceptible to *M. incognita* (Osunlola and Fawole, 2015). Lawrence *et al*. (1986) reported that effects of *Meloidogyne* species infection in sweet potato include formation of galls on fibrous roots and root tubers and cracking along with necrosis that reduces the quality of root tubers, hence reducing the market value of the sweet potato root.

A survey of *Meloidogyne* species and resistance to *M. incognita* on 72 sweet potato cultivars in Kenyan fields showed that there were different responses to *M. incognita* infection (Karuri *et al*., 2017). Cervantes-Flores *et al*. (2002) in USA demonstrated that sweet potato cultivars 'Excel' and 'Jewel' were resistant, whereas 'Beauregard' was highly susceptible to *Meloidogyne* species. Among all sweet potato cultivars that were assessed for nematode resistance to *M. incognita*, approximately 68% were resistant, whereas 11.1% cultivars were susceptible (Karuri *et al*., 2017). Findings on categorisation of 10 sweet potato cultivars for resistance to root-knot nematodes in

organic fields, demonstrated that among all the cultivars tested only 10% showed resistance, whereas 90% were good host and susceptible to *Meloidogyne* species (Atungwu *et al*., 2013). The susceptibility in most cultivars was probably due to unfavourable alleles that decrease the level of resistance (Cervantes-Flores *et al*., 2008).

In most sweet potato producing regions, crops are affected by either biotic or abiotic factors which include high temperatures which could result in resistant plants losing nematode resistance (Dropkin, 1969; Karuri *et al*., 2017). Gomes *et al*. (2015) demonstrated the existence of some resistance in sweet potato clones to *M. incognita* races 1 and 3. However, the response of sweet potato clones differed in resistance levels in terms of classification of reproductive factor and reproduction potential for both races. Amongst the sweet potato clones, some demonstrated a monogenic resistance which had resistance to several *Meloidogyne* species, whereas others exhibited polygenic resistance, which reflects resistance to one *Meloidogyne* species (Moens *et al*., 2009). About 78% and 79% sweet potato clones were resistant to *M. incognita* race 1 and *M. incognita* race 3, respectively, whereas 67% were resistant to both races (Gomes *et al*., 2015).

In South Africa, information on nematode resistance to sweet potato is limited. However, Pofu *et al*. (2016) screened 12 sweet potato cultivars to all *Meloidogyne* species in tropical South Africa. Among the 12 screened sweet potato cultivars, only three non-biofortified sweet potato cultivars, 'Bosbok', 'Blesbok' and 'Mvuvhelo' had non-host attributes to *Meloidogyne* species, whereas the rest were host except for

the exotic cultivar 'W-119'. However, the information obtained from the screening studies was not sufficient due to the fact that it did not provide information on nematode resistance mechanism which is either pre-infectional or post-infectional (Kaplan and Davis, 1987).

2.2.2 Nematode-resistance on other crops

Host-plant resistance development is an ideally popular alternative that is environmental-friendly and most cost effective in managing root-nematodes and in reducing crop losses (Agenbag, 2016; Onkedi *et al.*, 2014; Zasada *et al.*, 2010). Host plant resistance can be either systemic or genetically acquired. Host-plant resistance is genetically-based and could prevent the development and reproduction of root-knot nematodes, thereby lessening its negative effects on crop yield and quality (Moens *et al.*, 2009). Systemic resistance is acquired when the whole plant shows resistance after being exposed to nematodes, but when the plant retains the characteristics to avoid harm or to improve from attacks by *Meloidogyne* species it had been referred to as genetic host-plant resistance (Moens *et al.*, 2009). Genetic host-plant resistance could further be classified as monogenic which was resistant to one *Meloidogyne* species such as in coffee plants which were resistant to *M. exigua* alone (Moens *et al.*, 2009). In contrast, polygenic nematode resistance implies the resistance to several *Meloidogyne* species such as tomato cultivar with the *Mi* gene that exhibited resistance to *M. arenaria*, *M. incognita* and *M. javanica* (Moens *et al.*, 2009).

A study on responses of selected fibre hemp cultivars to *M. javanica* under greenhouse conditions was conducted and the reproductive factors of *M. javanica* on hemp cultivars were greater than one, with certain cultivars showing some high reproduction rates of tropical *Meloidogyne* species, but with limited nematode damage (Pofu *et al.*, 2010a), suggesting some degree of tolerance. Tolerant cultivars should not be recommended for use in crop rotations since they could escalate nematode build-up for the successor susceptible crops (Pofu *et al.*, 2010a). Pofu *et al.* (2009) demonstrated that two wild *Cucumis* species were highly resistant to tropical *Meloidogyne* species in South Africa. The two wild *Cucumis* species were eventually used as rootstock seedlings for intergeneric grafting with watermelon (*Citrulus lanatus*) in areas infested with *Meloidogyne* species 2 (Pofu *et al.*, 2009).

Host-status and host-sensitivity studies on *Cucumis africanus* and *C. myriocarpus* were also done in *M. javanica* and *M. incognita* races 2 and 4 which are dominant in South Africa (Pofu *et al.*, 2010b). All RF values of *M. javanica* and *M. incognita* races 2 and 4 on the two *Cucumis* species were less than unity, suggesting that the nematodes failed to reproduce on the given plant species. Interrelations between commercial beetroot cultivars and *Meloidogyne* species demonstrated that growth of cv. 'Detroit Dark Red' was significantly stimulated and inhibited at low and high nematode infection levels, respectively (Mashela, 2017). In contrast, the RF values for *M. javanica* on cultivar 'Crimson Globe' were below unity, without any significant effects on plant growth and it was concluded that cultivar 'Detroit Dark Red' was tolerant to *M. incognita*, whereas cv. 'Crimson Globe' was resistant to *M. javanica* (Mashela, 2017).

Host response of *Capsicum frutescens* cv. 'Capistrano' to *M. incognita* race 2 demonstrated that RF was less than unity, whereas nematode infection had no effect on plant growth (Pofu and Mashela, 2012). Therefore, the cultivar was resistant to *M. incognita* race 2 and could be recommended in crop rotation programmes intended to suppress nematode population densities of *M. incognita race* 2. Ngobeni *et al.* (2012) investigated the host-status of 32 maize genotypes to *M. javanica* and *M. incognita* race 2 in South Africa. Among the 32 genotypes, three varieties, namely, OBATAMPA, QPM-SR and QS-OBA, had RF values below unity, suggesting the possibility of resistance to *M. javanica* and *M. incognita* race 2 (Ngobeni *et al.*, 2012).

Host suitability of some *Solanaceous* plant cultivars to the root-knot nematodes, *Meloidogyne* species study demonstrated that there was development of great number of root galls and egg masses. Most cultivars from different *Solanaceous* plants were good hosts to root-knot nematodes (Ibrahim *et al.*, 2014). *Meloidogyne incognita* infection was investigated for its pathogenicity on pepper, where the nematode resulted in gall formation and reduced plant height, dry shoot weight and yield (Agaba *et al.*, 2015). Generally, an increase in initial nematode population density result in high nematodes and formation of galls (Udo and Ugwuoke, 2010).

Cultivars of chilli pepper (*Capsicum annuum* L.) responded to nematode infection differently. Cultivar 'Chilseongcho' was highly susceptible to *M. incognita*, whereas cv. 'CM334' was highly resistant to *M. incognita* (Moon *et al.*, 2010). *Meloidogyne incognita* J2 were able to locate and penetrate roots of both susceptible and resistant cultivars, even though more J2 penetrated roots of cv. 'Chilseongcho' than those of

cv. 'CM334' (Moon *et al.*, 2010). A host resistance study on cowpea demonstrated that all the test cowpea lines were hosts for the test Meloidogyne species, with plants and incurring high yield losses (Ibrahim and Atungwu, 2016). Susceptibility of the cowpea lines led to high root damage due to the reproduction of nematodes resulting in a large number of galls (Ibrahim and Atungwu, 2016). Response of cucurbitaceous rootstocks and bitter gourd scions to *M. incognita* suggested that three genotypes, Kumatikai, African horned cucumber and pumpkin, exhibited some high degree of nematode resistance, whereas Sponge gourd and Mithipakal were moderately resistant (Tamilselvi *et al.*, 2017).

2.2.3 Nematode-plant interactions

In plant-parasitic nematology, nematode interactions are explained using two concepts which are host-status and host-sensitivity (Seinhorst, 1967). Host-status is described by reproductive factor (RF = Pf/Pi), which is the measure of an ability of a nematode to reproduce on a given host-plant (Windham and Williams, 1988). The concept was further expounded that if all the RF values are below unity, this imply that the nematode failed to reproduce on the given host-plant whereas values greater than one indicates that the nematode were able to reproduce on the given host-plant. Seinhorst (1967) reported that when the final nematode population density (Pf) was equal to initial nematode population density (Pi), the population was at the equilibrium (E) point, beyond which intra-specific competition for resources was high, with the resultant that RF would always be below unity.

Host-sensitivity had been described using both host-status and plant response to nematode-infection (Seinhorst, 1965). Host-sensitivity was shown to be a function of (i) nematode type, (ii) inoculum level, (iii) plant type, (iv) age of plant and (v) biotic and abiotic factors (Seinhorst, 1965). According to Seinhorst (1965), when a nematode was able to reproduce on a given host-plant and inducing yield losses, the plant could be described as a susceptible host, if the plant did not incur yield losses it could be described as being a tolerant host, whereas non-host without yield loss could be denoted as resistant host.

2.2.4 Mechanism of resistance

Mechanism of resistance is described using two concepts which is pre-infectional and post-infectional resistance. Ferraz and Brown (2002) reported that when preformed chemicals that are completely expressed in the plant root tissues prior infection do not reflect an increase to complex levels in reaction to attack by penetrating nematodes, it is referred to as pre-infectional resistance. The ability of a plant to protect itself against nematode attack by releasing of chemicals existing in low levels to higher levels in the plant tissues after penetration of nematodes is referred to post-infectional resistance (Kaplan and Davis, 1987).

Pofu *et al.* (2010b) conducted a study on host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *M. incognita* 2. Reproductive factor values in both plant species were less than unity and there was no yield loss due to nematode infection and the plants were said to be resistant. The two cultivars were further investigated for mechanism of resistance to *Meloidogyne* species. *Cucumis africanus*

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was said to possess pre-infectional nematode resistance, whereas *C. myriocarpus* was post-infectional resistance (Pofu and Mashela, 2011). Failure of J2 to form at feeding sites (Ferraz and Brown, 2002), inhibition of J2 to grow past this stage, as well as conversion of J2 to males, were all observed (Pofu and Mashela, 2011; Pofu *et al.*, 2010b).

In a resistant soybean cultivar that was tested for mechanism of resistance to *M. incognita,* initially, juveniles penetrated through the soybean cultivar and were significantly lower in the roots than in soil (Fourie *et al.,* 2015). Apparently, a post-infectional mechanism of resistance was expressed in the cultivar. In another study (Ramatsitsi, 2017), mechanisms of resistance in *C. africanus* and *C. myriocarpus* to *M. incognita* race 4 were shown to be post-infectional. After penetration, J2 were significantly lower inside than outside, suggesting that the plant defended itself against nematode attack by releasing chemicals. Ramatsitsi (2017) demonstrated that post-infectional nematode resistance occurred for all three listed tropical nematodes in *C. africanus* and *C. myriocarpus.* In contrast*, C. africanus* was previously viewed as having pre-infectional nematode resistance, whereas *C. myriocarpus* had post-infectional nematode resistance (Pofu and Mashela, 2011). Consequently, the two *Cucumis* species, each with post-infectional nematode resistance, could be used in introgression during plant breeding (Thurau *et al.*, 2010). In a recent study abroad (Liu *et al.*, 2015), it was shown that *C. africanus* was highly resistant to various *Meloidogyne* species and soil-borne pathogens, whereas *C. myriocarpus* was moderately resistant.

Penetration indices proposed that open-pollinated variety OBATAMPA had post-infectional mechanism of resistance, whereas open-pollinated varieties QPM-SR and QS-OBA had pre-infectional mechanism of resistance on a study conducted on host-status of 32 maize genotypes to *M. javanica* and *M. incognita* race 2 in South Africa (Ngobeni *et al*., 2012). Mashela and Pofu (2016) reported that mechanisms of resistance on sweet stem sorghum cv. ′Ndendane-X1′ demonstrated a pre-infectional resistance, since the juveniles failed to penetrate the roots of the plant. Also, in sweet potato cv. 'Bophelo', 'Bosbok' and 'Mvuvhelo' post-infectional resistance occurred for *M. javanica* (Maseko, 2017) and for *M. incognita (*Makhwedzhana, 2017).

2.3. Work not yet done

The degree of nematode resistance to tropical *Meloidogyne* species in sweet potato cv. 'Mafutha' had not been documented. The popularity of this cultivar among the local people due to its taste dictate that assessment of host-status and host-sensitivity be conducted on the cultivar using tropical *Meloidogyne* species and races.

CHAPTER 3
CULTIVAR 'MAFUTHA'-ROOT-KNOT NEMATODE RELATIONS

3.1 Introduction

Most crop-producing regions in South Africa are heavily infested by the root-knot (*Meloidogyne* species) nematodes (Pofu *et al.*, 2016). Generally, *Meloidogyne* species are difficult to manage since the withdrawal of the highly effective fumigant synthetic nematicides (Mashela *et al.*, 2015). The withdrawal resulted in the introduction of various alternative nematode management strategies such as the use of nematode-resistant cultivars. Progress in the area had been hindered by limited information on accurate nematode species identification since *Meloidogyne* species have a wide host range and biological races (Castagnone-Sereno, 2002; Faghihi *et al.*, 1995). Biological races are nematodes with similar morphologies and could only be identified through differential host plants and molecular approaches (Taylor and Sasser, 1978). Following the withdrawal of methyl bromide from the agrochemical markets in 2005, economic consequences such as yield losses in crops due to nematode damage skyrocketed to US$157 billion (Elling, 2013) and relative to US$126 billion prior to the withdrawal (Chitwood, 2003), the relative increase in yield losses was 37%.

Alternative nematode management strategies to control root-knot nematode population densities had been intensely researched and developed in South Africa (Mashela *et al.,* 2015). The use of resistant cultivars is among the promising strategies that are being proposed as alternative to chemicals in managing nematode population densities. In a recent study (Pofu *et al.*, 2016), non-host status in sweet potato cultivars was reported among the local lines in South Africa, whereas

the exotic lines in the study were mostly hosts to the local *Meloidogyne* species. However, the host-status and host-sensitivity of sweet potato cv. 'Mafutha' are not recorded. The objective of the study was to determine the degree of nematode resistance in sweet potato cv. 'Mafutha' to *M. javanica* and *M. incognita* race 2 and *M. incognita* 4.

3.2 Materials and methods

3.2.1 Description of study area

Three parallel trials, for *M. javanica* (**Trial 1**)*, M. incognita* race 2 (**Trial 2**) and *M. incognita* race 4 (**Trial 3**) were conducted under greenhouse conditions at the Green Biotechnologies Research Centre of Excellence; University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). In turn, each trial comprised two experiments, where Experiment 1 was initiated during autumn (February-April) in 2016 and then validated as Experiment 2 in autumn 2017. Day/night temperatures were maintained at 25/21°C, with higher daily temperatures controlled using thermostat-controlled fans and with the wet wall used to retain relative humidity at 65-75%. Due to wind-blown currents during the extraction of heat, conditions inside the greenhouse were heterogeneous, thereby necessitating paying attention to the appropriate experimental designs.

3.2.2 Treatments and experimental design

Treatments (nematode levels) were arranged in a randomised complete block design, with blocking done for wind-blown currents generated by the heat-extracting fans. Treatments in *M. javanica, M. incognita* race 2 or *M. incognita* race 4 trial

comprised 0, 25, 50, 125, 250, 625, 1250, 3125 and 5250 eggs and second-stage juveniles (J2) of each nematode species and/or race.

3.2.3 Procedures

Uniform cuttings (30-cm long) of cv. 'Mafutha' were set in 20-cm-diameter plastic pots, filled with 2 700 ml steam-pasteurised (300°C for 1 h) loam soil (47% sand, 38 clay, 15% silt) and river sand with Hygromix-T (Hygrotech, Pretoria West, South Africa) at 4:2:1 (v/v) ratio. During planting, the auxiliary buds on the cuttings faced upward to enhance the establishment process (Laurie, 2004). Pots were placed on the greenhouse benches at 0.25 m inter-row and 0.30 m intra-row spacing (Figure 3.1). Sweet potato cv. ′Beauregard′ served as a nematode-susceptible standard (Cervantes-Flores et al., 2002) for verifying the viability of the inoculum.

Figure 3.1 Sweet potato cultivar 'Mafutha' rooted-cuttings

Inoculum of each nematode type were prepared by extracting eggs and J2 from the roots of greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution using maceration and blending method (Hussey and Barker, 1973). The materials were passed through a series of sieves 75-µm at the top and 25-µm at

the bottom. Eggs and second-stage juveniles were collected and counted under a light microscope. Fourteen days after planting the cuttings, levels of inoculum were dispensed using a 20-ml plastic syringe by placing in 5-cm-deep holes on cardinal points of the vines and then covering the holes with the described growing mixture.

The plants were irrigated every other day, initially with 250 ml tapwater, which was half-way to harvest increased to 500 ml tapwater. One week after transplanting, plants were fertilised with 5 g NPK 2:3:2 (22) + 5% Zn + 5% Ca and 2 g NPK 2:1:2 (43) which contained all macro- and micro-nutrient elements except Ca. Insect pests and diseases were scouted and monitored on daily basis and were observed during the two seasons.

3.2.4 Data collection

At 56 days after inoculation, nematode and plant variables for each trial were separately recorded. After measuring chlorophyll content using a chlorophyll meter, vine length using a meter stick and stem diameter at 5-cm above soil surface using a digital Vernier calibre, shoots were cut at ground level and roots removed from the pots. Roots were immersed in water to remove soil particles, pattered using paper towels to remove excess water and weighed. North Carolina differential scale index of 0 to 5 was used to determine root galling, where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 ≥ 100 galls per root system (Taylor and Sasser, 1978). Eggs and J2 were extracted from the whole root system per plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey and Barker, 1973) and then soil was separated from debris using the sugar-floatation and centrifugation method (Jenkins, 1964). The aliquot was poured through nested 150-, 75- and 25-

µm sieves. Contents of the 25-µm mesh sieve were poured into 100-ml plastic containers and brought to the mark for counting under a stereomicroscope. The reproductive potential (RP = Pf/Pi) values were computed as a proportion of the final nematode population density (Pf) in total roots per plant to the initial nematode population density (Pi). Shoots were weighed after oven-drying for 72 h at 60^0 C.

3.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) through the SAS software to determine the effects of nematode inocula on RP values and plant growth variables in each experiment for each trial (Appendix 3.1-Appendix 3.42). Mean separation for significant (P ≤ 0.05) treatments were achieved through the Waller-Duncan multiple range test (Gomez and Gomez, 1984). Reproductive potential (RP = eggs + J2/g root) was computed for host-status and analysed. The mean sums of square were partitioned to estimate the contribution of the sources in total treatment variation (TTV) of individual variables. Unless otherwise stated, data were discussed at the probability level of 5%.

3.3 Results

In each trial seasonal interactions were significant in all RP variables, therefore, data for Experiment 1 and Experiment 2 were not pooled (Gomez and Gomez, 1984).

3.3.1 Nematode variables in *Meloidogyne javanica* trial

Treatments had highly significant (P ≤ 0.01) effects on root galls (RGA) and RP values in Experiment 1 and Experiment 2, contributing 60 and 90% in TTV of RGA in the respective experiments (Table 3.1). Inoculum levels contributed 96 and 79% in

TTV of RP values in Experiment 1 and Experiment 2, respectively. The RGA numbers were equal or higher than unity in Experiment 1, whereas in Experiment 2 the RGA numbers were lower than unity at all levels of inoculation (Table 3.2). Generally, at low inoculation levels the RP values in Experiment 1 had zero values, whereas at higher inoculation levels the RP values were higher than one. In contrast to the RGA values in Experiment 2, at all inoculation levels the RP values were further above unity.

3.3.2 Plant growth variables in *Meloidogyne javanica* trial

Treatment effects were highly significant on dry shoot mass and significant on stem diameter in Experiment 1, whereas in Experiment 2 treatments were significant on dry root mass (Table 3.3). In Experiment 1, treatments contributed 74 and 50% in TTV of dry shoot mass and stem diameter, respectively, whereas in Experiment 2, the treatments contributed 56% in TTV of dry root mass (Table 3.3). In both experiments, the variables that had significant treatment effects did not have clear patterns, with a few incidents where the untreated controls had the highest mean values in both experiments (Table 3.4). In both experiments, the treatment did not have significant effects on vine length and chlorophyll content.

3.3.3 Nematode variables in *Meloidogyne incognita* race 2 trial

Treatments had no significant effects on RGA in Experiment 1, but had significant effects on the variable in Experiment 2, contributing 54% in TTV of the variable (Table 3.5). Treatment effects were highly significant on RP in Experiment 1, contributing 86% in TTV of the variable, but without having any significant effect on the variable in Experiment 2. In both experiments, the RGA values were above and

below unity, respectively, whereas the RP values were at zero at low inoculation levels, but at high inoculation level the values were above one. (Table 3.6).

3.3.4 Plant variables in *Meloidogyne incognita* race 2 trial

In Experiment 1, inoculation treatments had significant and highly significant effects on dry shoot mass and stem diameter, respectively, contributing 56 and 70% in TTV of the respective variables, without having effects on other variables (Table 3.7). Also, treatments did not have any significant effects on all plant variables in Experiment 2. Similar to observations in the *M. javanica* trial, trends related to level of nematode infection were not clear (Table 3.8).

3.3.5 Nematode variables in *Meloidogyne incognita* race 4 trial

Treatment effects had no significant effects on RGA in Experiment 1, but were highly significant on the variable in Experiment 2, contributing 76% in TTV of the variable (Table 3.9). Treatment effects had highly significant effects on RP in both experiments, contributing 61 and 61% in TTV of the RP values in the respective experiments (Table 3.9). In both experiments, high inoculation levels, particularly in Experiment 2, had RP values above one, whereas in Experiment 1, the RP values were above one in the last two highest inoculation levels (Table 3.10).

3.3.6 Plant variables in *Meloidogyne incognita* race 4 trial

Nematode treatment effects were significant and highly significant on dry root mass and stem diameter, respectively, in Experiment 1, contributing 58 and 65% in TTV of

the respective variables (Table 3.11). In Experiment 2 the treatment effects had significant effects on vine length, contributing 51% in TTV of the variable. Generally, in Experiment 1, the nematode treatment started by stimulating dry root mass, followed by inhibition and then another stimulation effect (Table 3.12). The same trend also occurred slightly on stem diameter in Experiment 1, whereas vine length in Experiment 2 increased gradually at low to high inoculation levels.

Table 3.1 Partitioning the sum of squares for root gall (RGA) and reproductive potential (RP) of *Meloidogyne javanica* on sweet potato cultivar 'Mafutha' at 56 days after application of treatments.

| | | Experiment 1 | | | | Experiment 2 | | | |
| | | RGA | | RP | | RGA | | RP | |
Source	Df	MSS	TTV (%)^x	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	0.01	20	47.57	2	0.005	6	527399	9
Treatment	7	0.03	60**	3124.45	96**	0.073	90**	4438163	79**
Error	35	0.01	20	72.90	2	0.003	4	658817	12
Total	47	0.05	100	3244.92	100	0.081	100	5624379	100

TTV (%) = total treatment variation, **Significant at P ≤ 0.01.

Table 3.2 Response of root gall (RGA) and reproductive potential (RP) of *Meloidogyne javanica* at eight levels of inoculation on sweet potato cultivar 'Mafutha' at 56 days after application of treatment.

Nematode	Experiment 1						Experiment 2					
	RGA[x]	Eggs	J2	Total	FRM (g)	RP	RGA	Eggs	J2	Total	FRM (g)	RP
25	1.50[bc]	0	0	0	20.15	0.00[c]	0.44[c]	320	17	337	28.66	11.76[c]
50	1.33[bc]	0	0	0	18.52	0.00[c]	0.48[c]	320	27	347	28.67	12.10[c]
125	1.00[c]	0	3	3	14.49	0.20[c]	0.48[c]	340	47	387	24.02	16.11[c]
250	1.00[c]	0	10	10	15.73	0.64[c]	0.45[c]	1383	43	1426	20.05	71.12[c]
625	1.00[c]	7	30	37	12.71	2.91[c]	0.60[b]	1280	40	1320	32.39	40.75[c]
1250	1.00[c]	3	3	6	11.73	0.51[c]	0.65[ab]	4507	197	4704	26.43	177.98[bc]
3125	1.67[ab]	177	73	250	18.34	13.63[b]	0.68[a]	35943	1977	37920	23.53	1611.56[a]
5250	2.17[a]	440	347	787	12.37	63.62[a]	0.70[a]	17473	1160	18633	23.60	789.53[b]

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

Table 3.3 Partitioning sum of squares for chlorophyll content (CHC), vine length (VIL), stem diameter (STD), dry shoot mass (DSM) and dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica.*

Source	Df	DSM		DRM		VIL		CHC		STD	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1											
Rep	5	1.12	3	0.09	30	601.41	26	10.91	21	0.07	6
Trt	8	21.35	74^{**}	0.15	45^{ns}	727.67	31^{ns}	23.86	45^{ns}	0.57	50^{*}
Error	40	6.53	23	0.09	28	1019.42	43	18.00	34	0.51	44
Total	53	29.00	100	0.33	100	2348.50	100	52.77	100	1.15	100
Experiment 2											
Rep	5	11.92	43	3.44	22	648.45	46	70.26	28	0.83	25
Trt	8	6.31	23^{ns}	8.78	56^{*}	293.72	21^{ns}	31.54	38^{ns}	1.48	44
Error	40	9.62	34	3.34	22	479.59	33	22.57	34	1.07	32
Total	53	27.85	100	15.56	100	1421.76	100	124.36	100	3.8	100

^{ns}Not significant at $P \leq 0.05$, *Significant at $P \leq 0.05$, **Significant at $P \leq 0.01$.

Table 3.4 Responses of chlorophyll content (CHC), vine length (VIL), stem diameter (STD), dry shoot mass (DSM) and dry root mass (DRM) of sweet potato cultivar 'Mafutha' to *Meloidogyne javanica.*

Nematode	**Experiment 1**					**Experiment 2**				
	DSM	DRM	VIL	CHC	STD	DSM	DRM	VIL	CHC	STD
0	6.23^{bc}	0.70	68.42	38.63	6.18^{a}	4.01	4.83^{a}	38.75	42.70	5.52
25	9.37^{a}	1.06	76.88	39.52	6.25^{a}	5.33	1.37^{b}	50.58	41.65	5.30
50	5.45^{bc}	0.84	91.55	37.78	5.30^{b}	5.80	1.37^{b}	45.17	46.43	6.25
125	6.39^{abc}	0.72	98.75	43.00	5.68^{ab}	4.79	1.15^{b}	43.75	43.47	5.30
250	8.16^{ab}	0.80	102.10	40.35	5.58^{ab}	4.28	0.96^{b}	33.00	46.78	5.08
625	3.85^{c}	0.63	78.00	36.87	5.68^{ab}	7.38	1.54^{b}	56.57	44.92	4.85
1250	3.80^{c}	0.58	89.97	37.77	5.60^{ab}	4.29	1.30^{b}	38.58	41.78	5.25
3125	7.72^{ab}	0.91	92.82	40.70	6.02^{ab}	4.09	1.12^{b}	45.17	42.77	4.83
5250	5.55^{bc}	0.61	83.50	41.45	5.58^{ab}	4.84	1.13^{b}	47.25	47.77	4.50
$LSD_{0.05}$	1.79	1.05	18.43	2.45	0.60	1.79	1.05	12.64	2.74	0.60

Table 3.5 Partitioning the sum of squares for root gall (RGA) and reproductive potential (RP) of *Meloidogyne incognita race 2* on sweet potato cultivar 'Mafutha' at 56 days after application of treatments.

| | | Experiment 1 | | | | | | Experiment 2 | | | |
| | | RGA | | | RP | | | RGA | | RP | |
Source	Df	MSS	TTV (%)^x		MSS	TTV (%)		MSS	TTV (%)	MSS	TTV (%)
Replication	5	0.004	40		4.91	7		0.007	25	9315.70	27
Treatment	7	0.003	30^{ns}		58.73	86^{**}		0.015	54^{**}	15740.10	46^{ns}
Error	35	0.003	30		4.96	7		0.006	21	9482.60	27
Total	47	0.01	100		68.60	100		0.028	100	34538.40	100

^{x}TTV (%) = total treatment variation, **Significant at $P \leq 0.01$.

Table 3.6 Response of root gall (RGA) and reproductive potential (RP) of *Meloidogyne incognita* race 2 at eight levels of inoculation on sweet potato cultivar 'Mafutha' at 56 days after application of treatment.

	Experiment 1						Experiment 2					
Nematode	RGA^z	Eggs	J2	Total	FRM (g)	RP	RGA	Eggs	J2	Total	FRM (g)	RP
25	1.00	0	0	0	7.76	0.00^b	0.30^b	0	0	0	23.82	0.00
50	1.17	0	0	0	5.69	0.00^b	0.30^b	0	0	0	34.55	0.00
125	1.00	0	0	0	5.58	0.00^b	0.36^{ab}	7	0	7	21.89	0.32
250	1.00	0	0	0	8.03	0.00^b	0.30^b	43	0	43	32.67	1.32
625	1.33	0	0	0	10.17	0.00^b	0.36^{ab}	97	0	97	33.99	2.85
1250	1.00	3	7	10	7.25	1.57^b	0.42^a	217	0	217	25.10	8.65
3125	1.00	30	10	40	9.85	4.06^b	0.39^{ab}	360	0	360	33.15	10.86
5250	1.17	97	40	137	9.86	13.89^a	0.41^a	1160	47	1207	29.22	41.31

^{z}Column means with the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

Table 3.7 Partitioning sum of squares for chlorophyll content (CHC), vine length (VIL), stem diameter (STD), dry shoot mass (DSM), and dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *M. incognita* race 2.

Source	Df	DSM MSS	DSM TTV (%)	DRM MSS	DRM TTV (%)	VIL MSS	VIL TTV (%)	CHC MSS	CHC TTV (%)	STD MSS	STD TTV (%)
Experiment 1											
Rep	5	1.714	16	0.036	31	852.82	33	31.73	37	0.22	6
Trt	8	5.811	56^{*}	0.044	37^{ns}	1145.83	45^{ns}	28.84	34^{ns}	2.21	70^{**}
Error	40	2.953	28	0.038	32	552.77	22	25.05	29	0.89	14
Total	53	10.478	100	0.118	100	2551.42	100	85.63	100	3.64	100
Experiment 2											
Rep	5	67.74	58	1.20	63	3742.33	69	17.90	30	1.22	45
Trt	8	28.73	25^{ns}	0.30	15^{ns}	1143.70	21^{ns}	15.94	27^{ns}	0.73	27^{ns}
Error	40	20.04	17	0.42	22	569.12	10	25.29	43	0.77	28
Total	53	116.57	100	1.92	100	5455.15	100	59.13	100	2.72	100

^{ns}Not significant at P ≤ 0.05, *Significant at P ≤ 0.05, **Significant at P ≤ 0.01.

Table 3.8 Responses of chlorophyll content (CHC), vine length (VIL), stem diameter (STD), dry shoot mass (DSM) and dry root mass (DRM) of sweet potato cultivar 'Mafutha' to *Meloidogyne incognita* race 2.

Nematode	Experiment 1					Experiment 2				
	DSM	DRM	VIL	CHC	STD	DSM	DRM	VIL	CHC	STD
0	4.88^a	0.46	90.98	44.55	5.38^b	6.10	1.38	43.83	41.83	4.98
25	3.59^{ab}	0.37	82.82	39.02	5.28^b	4.57	1.14	32.67	32.85	5.38
50	2.51^b	0.27	83.43	43.62	4.93^b	9.24	1.65	74.17	45.20	5.75
125	2.50^b	0.27	70.00	44.18	4.90^b	4.49	1.05	47.17	42.62	4.98
250	3.91^{ab}	0.38	86.38	44.03	5.62^b	8.72	1.56	52.67	43.03	4.95
625	5.19^a	0.49	107.57	39.38	5.68^{ab}	9.04	1.62	57.75	43.68	5.53
1250	3.91^{ab}	0.35	66.92	42.78	4.75^b	7.90	1.20	51.08	42.05	5.00
3125	4.76^a	0.48	76.77	42.27	6.78^a	7.31	1.58	49.58	44.07	5.58
5250	4.57^a	0.48	62.37	39.38	5.37^b	10.88	1.39	75.67	44.80	5.77
$LSD_{0.05}$	2.01	0.11	13.57	2.89	1.10	2.58	0.38	13.77	2.90	0.51

Table 3.9 Partitioning the sum of squares for root gall (RGA) and reproductive potential (RP) of *Meloidogyne incognita* race 4 on sweet potato cultivar 'Mafutha' at 56 days after application of treatments.

| | | Experiment 1 | | | | Experiment 2 | | | |
| | | RGA | | RP | | RGA | | RP | |
Source	Df	MSS	TTV (%)^x	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	0.006	50	79.96	15	0.009	14	296.83	13
Treatment	7	0.003	25^{ns}	331.34	61**	0.050	76**	1338.66	61**
Error	35	0.003	25	131.23	24	0.007	10	571.69	26
Total	47	0.012	100	542.53	100	0.066	100	2207.18	100

TTV (%) = total treatment variation, **Significant at P ≤ 0.01.

Table 3.10 Response of root gall (RGA) and reproductive potential (RP) of *Meloidogyne incognita* race 4 at eight levels of inoculation on sweet potato cultivar 'Mafutha' at 56 days after the treatment.

Nematode	Experiment 1						Experiment 2					
	RGA[x]	Eggs	J2	Total	FRM (g)	RP	RGA	Eggs	J2	Total	FRM (g)	RP
25	1.00	0	0	0	11.88	0.00^c	0.30^d	57	13	70	25.99	2.69^b
50	1.00	0	0	0	11.08	0.00^c	0.33^d	73	3	76	25.33	3.00^b
125	1.17	0	0	0	8.90	0.00^c	0.36^{cd}	20	0	20	31.50	0.63^b
250	1.17	0	3	3	9.88	0.30^c	0.36^{cd}	137	43	180	29.13	6.18^b
625	1.00	0	3	3	8.56	0.35^c	0.44^{bc}	330	40	370	27.69	13.36^b
1250	1.00	3	7	10	14.94	0.74^c	0.49^{ab}	407	67	474	21.65	21.89^{ab}
3125	1.17	50	93	143	15.84	9.03^b	0.50^{ab}	947	77	1024	36.64	27.95^{ab}
5250	1.33	137	93	230	11.65	19.74^a	0.55^a	2177	267	2444	47.04	51.96^a

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

Table 3.11 Partitioning sum of squares for chlorophyll content (CHC), vine length (VIL), stem diameter (STD), dry shoot mass (DSM), and dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4.

Source	Df	DSM MSS	DSM TTV (%)	DRM MSS	DRM TTV (%)	VIL MSS	VIL TTV (%)	CHC MSS	CHC TTV (%)	STD MSS	STD TTV (%)
Experiment 1											
Rep	5	2.62	28	0.11	12	1037.11	45	13.83	32	0.05	4
Trt	8	2.84	30^{ns}	0.54	58^{*}	667.29	29^{ns}	15.31	35^{ns}	0.76	65^{**}
Error	40	4.00	42	0.28	30	618.66	26	14.17	33	0.36	31
Total	53	9.46	100	0.93	100	2323.06	100	43.31	100	1.21	100
Experiment 2											
Rep	5	86.51	67	4.06	36	1212.42	38	18.26	28	2.12	50
Trt	8	26.95	21^{ns}	4.03	36^{ns}	1614.05	51^{*}	24.08	38^{ns}	1.27	30^{ns}
Error	40	15.81	12	3.06	27	357.69	11	21.83	34	0.82	20
Total	53	129.27	100	11.15	100	3184.16	100	64.17	100	4.21	100

^{ns}Not significant at $P \leq 0.05$, *Significant at $P \leq 0.05$, **Significant at $P \leq 0.01$.

Table 3.12 Responses of chlorophyll content (CHC), vine length (VIL), stem diameter (STD), dry shoot mass (DSM) and dry root mass (DRM) of sweet potato cultivar 'Mafutha' to *Meloidogyne incognita* race 4.

Nematode	Experiment 1					Experiment 2				
	DSM	DRM[y]	VIL	CHC	STD	DSM	DRM	VIL	CHC	STD
0	4.82	0.78^c	81.62	41.33	5.73^{abcd}	7.45	3.67	34.42^c	40.30	5.52
25	6.13	1.11^{abc}	100.57	45.98	5.95^{abc}	5.71	1.24	34.00^c	42.27	4.77
50	5.49	1.12^{abc}	72.48	43.78	5.42^{bcd}	4.32	1.21	38.50^c	41.85	5.20
125	5.04	0.89^{bc}	86.12	43.87	5.63^{abcd}	6.68	1.50	35.42^c	44.73	5.07
250	4.59	0.76^c	86.47	43.02	5.20^d	6.18	1.39	50.00^{bc}	43.18	5.45
625	5.07	0.76^c	97.22	44.20	5.28^{cd}	5.10	1.32	41.67^{bc}	37.77	5.60
1250	5.32	0.75^c	93.25	41.90	5.30^{cd}	4.83	1.03	38.42^c	41.25	5.43
3125	6.31	1.41^{ab}	69.80	40.92	6.17^a	8.64	1.75	83.50^a	43.40	5.68
5250	6.53	1.55^a	79.59	43.90	6.03^{ab}	11.01	2.24	60.83^b	41.92	6.42
$LSD_{0.05}$	1.79	-	12.64	2.74	-	2.30	1.01	-	2.70	0.52

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

3.4 Discussion

3.4.1 Nematode variables in all three trials

The presence of root galls (RGA) had historically been used as an indicator that the penetrating second-stage juveniles (J2) had successfully established a feeding site, which allowed J2 to feed and develop to the subsequent stages through moulting (Ferraz and Brown, 2002). In the early 1970s, Fassuliotis (1970) in histopathology studies 26 days after infection of *Cucumis ficifolia* and *Cucumis metuliferus*, observed that roots had noticeable giant cells that developed in regions of roots associated with adult females. However, in *Cucumis metuliferus*, the immature female nematodes were associated with formation of small giant cells which were limited to a few cells near the head of the nematode. In the current study, seasonal interactions were highly significant, and the data could not be pooled. In all nematode variables, particularly the RGA, the seasonal effects were advanced, with limited RGA in the validated experiments.

Fourie *et al.* (2015) listed a number of assessment tools, for example, the use of root gall indices, which might not be entirely accurate in assessment of non-host status. For instance, in most cases, lack of pronounced root galls did in no way imply that nematode reproduction and juvenile hatch did not occur (Fourie *et al.*, 2015). In certain instances, although root galls were negligent, the susceptible cultivars of sweet potato like cv. 'EM7' had the highest number of eggs (Karuri *et al.*, 2017). Generally, the formation of root galls could range from few, as observed in the current study, to many as observed in another study on sweet potato roots (Okechalu and Wonang, 2015). Additionally, in highly nematode resistant plant species, root galls could remain small and undeveloped, with failure to produce

eggs, whereas J2 could also be limited. In support of the RP values used in the current study, when using RP values, Osunlola and Fawole (2015) demonstrated that a large number of sweet potato cultivars were susceptible to *Meloidogyne* species. In another study, where RP values were used, Karuri *et al.* (2017) also noted that among the test sweet potato cultivars, 11% were susceptible to *Meloidogyne* species.

In all experiments, regardless of *Meloidogyne* species, the reproductive potential (RP) values were primarily low at low inoculation and high at high inoculation level. The RP should however not be confounded with the reproductive factor as expounded by Seinhorst (1965). The current findings supported those on screening tests of various sweet potato cultivars in South Africa, where RP was used (Pofu *et al.*, 2016), with non-host and host-status established on various cultivars. However, in the original study (Pofu *et al.*, 2016) and subsequent nematode resistance studies (Makhwedzhana, 2017; Maseko, 2017), cv. ′Mafutha′ was not included as a test cultivar. The latter had been excluded in sweet potato cultivar development due to its low yield when compared to other commercially available cultivars (Laurie *et al.*, 2015). Notwithstanding the yield, the cultivar has excellent eating attributes and it is in high demand in marginalised communities of South Africa (Laurie *et al.*, 2015).

Findings related to RP above unity suggested that the test cv. ′Mafutha′ was a host to all three tropical *Meloidogyne* species used in the study, which confirmed both local (Pofu *et al.*, 2016) and international (Cervantes-Flores *et al.*, 2002; Scurrah *et al.*, 2005) sweet potato-*Meloidogyne* interaction trials. Internationally, *M. incognita* is

widely distributed and had been considered the most aggressive when compared with *M. javanica* (Kleynhans *et al.*, 1996). In contrast, in South Africa, *M. javanica* is more aggressive than *M. incognita*, but occur as singly or mixed population densities (Kleynhans *et al.,* 1996). Another challenge in assessing host-status in plant-nematode relations, is the existence of biological races, which are nematode species that have similar morphological structures and could only be identified using differential host tests and molecular approaches (Mashela *et al.*, 2017). In South Africa, *M. incognita* race 4 had been restricted to the cotton-producing regions and the race was originally believed to have limited hosts (Kleynhans *et al.*, 1996). The current observations suggested all existing tropical *M. incognita* races in South Africa could infect and reproduce on the test cultivar. Maseko (2017) and Makhwedzhana (2017) demonstrated that cv. 'Bosbok' and cv. 'Mvuvhelo', which are indigenous to South Africa, were non-hosts to *M. javanica* and *M. incognita* and the local biological races of the latter. However, another promising local sweet potato cultivar, cv. 'Bophelo' was non-host to all three test nematodes in the current study.

3.4.2 Plant variables in all three trials

The three test nematodes each damaged the sweet potato cv. 'Mafutha', which supported observations on various sweet potato lines that were tested in the USA (Cervantes-Flores *et al.*, 2002) and in sub-Sahara Africa (Okechalu and Wonang, 2015; Osunlola and Fawole, 2015) against *Meloidogyne* species. Generally, root galls, as observed in other studies (Khan, 2009), increase root mass, which should not be mistaken for the stimulated root growth. Limited evidence existed in the current study that certain plant variables were stimulated, which is common in cases where nematode numbers are below the damage threshold density (Mashela *et al.*,

2017). In the current study, it was clearly evident that all test tropical *Meloidogyne* species could induce yield loss in cv. ′Mafutha′.

3.4.3 Cultivar ′Mafutha′-*Meloidogyne* species relations

In plant nematology, when the plant allows nematode reproduction as shown by the RP or RF values that are greater than unity, and the plant suffered damage due to nematode infection, the plant could be considered to be susceptible to the test nematode (Seinhorst, 1965). In contrast, when the nematode reproduced but did not cause plant damage, the plant could be described as being tolerant to the test nematode; and when reproduction could not be allowed as well as plant damage, the plant is resistant to the test nematode. The first descriptor would be appropriate for the associated relations of cv. ′Mafutha′ and the three test *Meloidogyne* species, which imply that the cultivar was susceptible to the test nematodes.

3.5 Conclusion

The cv. 'Mafutha' was shown to be susceptible to *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4, which are widely spread in tropical areas of South Africa, where the cultivar had been shown to be the most preferred. Due to the current observation of being susceptible to all the test nematodes, it was not necessary to assess the mechanism of nematode resistance against any of the test nematodes in the test cultivar. Consequently, the production of the cultivar in rural areas due to its preferred eating attributes, implied that there could be the need to develop management strategies of the test nematode on the test cultivar.

CHAPTER 4
SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS
AND CONCLUSIONS

4.1 Summary of findings

The study focused on two objectives, namely, to (1) determine the degree of nematode resistance in sweet potato cv 'Mafutha' to *Meloidogyne javanica* and *Meloidogyne incognita* races 2 and 4 under greenhouse conditions and (2) investigate the mechanism of resistance in sweet potato cv. 'Mafutha' to *M. javanica* and *M. incognita* races 2 and 4. Generally, there are two forms of nematode-resistant mechanisms, namely, pre-infectional and post-infectional nematode resistance (Kaplan and Davis, 1987). Only the latter could be used in introgression for nematode resistance during plant breeding (Thurau *et al*., 2010) and therefore, when nematode resistance occurs, it is imperative to assess the mechanism involved. Cervantes-Flores *et al*. (2008) had since detected the existence of unfavourable alleles that reduce the degree of resistance in certain sweet potato cultivars. Results from the current study demonstrated that sweet potato cv. 'Mafutha' was susceptible to the test *Meloidogyne* species. Consequently, the mechanism of resistance (Objective 2) was not investigated. However, due to its favourable eating properties (Laurie, 2004), cv. 'Mafutha' could be used as the recipient candidate for introgression using plant genes during plant breeding for nematode resistance (Mashela *et al*., 2017; Thurau *et al*., 2010).

4.2 Significance of findings

Sweet potato cv. 'Mafutha' was identified as being susceptible to *M. javanica* and *M. incognita* races 2 and 4 and therefore, although the cultivar had high local

preference, its production should be accompanied by the inclusion of nematode management strategies. Also, due to its susceptibility to tropical *Meloidogyne* species, the cultivar should not be included in crop rotation systems, but the cultivar could be used as a candidate for introgression of nematode resistant plant genes in breeding programmes. Additionally, because the cultivar was highly favoured in most subsistence farming systems in Limpopo Province due to its eating qualities, there could be a community-driven need for developing nematode management intervention systems during the production of the cultivar in household gardens.

4.3 Recommendations

Currently, most smallholder farmers who had historically been cultivating sweet potato cv. 'Mafutha' could not afford the costly synthetic nematicides such as Velum. Thus, the suitability of using cucurbitacin-containing phytonematicides, which had been researched and developed for various crops (Mashela *et al.*, 2017), should be investigated the test cultivar and nematodes. However, since the cucurbitacins would come into contact with the sweet potato roots by harvest time, the cucurbitacin residue trials should be conducted to ensure that consumers were not unnecessarily exposed to this group of potent chemicals (Shadung, 2016). Also, it would be imperative that the cultivar be tested for host-status against temperate *Meloidogyne* species since sweet potato could be grown for food security in some of the regions of South Africa that have temperate climates or alternatively, higher altitudes.

4.4 Conclusions

Results of the current study suggested that cv. 'Mafutha' was susceptible to all the tropical *Meloidogyne* species in South Africa, namely, *M. javanica* and *M. incognita*

races 2 and 4. Consequently, cv. 'Mafutha' should not be included in crop rotation programmes for suppressing tropical *Meloidogyne* species population densities, since they would result in crop damage and nematode build-up for subsequent crops.

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APPENDICES

Appendix 3.1 Analysis of variance for root galls (RGA) sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 1).

Source	DF	SS	MSS	F	P ≤
Replication	5	0.02585	0.00517		
Treatment	7	0.18110	0.02587	3.81	0.01
Error	35	0.23795	0.00680		
Total	47	0.44490	0.03784		

Appendix 3.2 Analysis of variance for root galls (RGA) sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 2).

Source	DF	SS	MSS	F	P ≤
Replication	5	0.02276	0.00455		
Treatment	7	0.51162	0.07309	27.21	0.01
Error	35	0.9401	0.00269		
Total	47	0.628403	0.08033		

Appendix 3.3 Analysis of variance for reproductive potential (RP) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 1).

Source	DF	SS	MSS	F	P ≤
Replication	5	237.8	47.57		
Treatment	7	21871.1	3124.45	42.86	0.01
Error	35	2551.5	72.90		
Total	47	24660.5	3244.92		

Appendix 3.4 Analysis of variance for reproductive potential (RP) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 2).

Source	DF	SS	MSS	F	P ≤
Replication	5	2636995	527399		
Treatment	7	31070000	4438163	6.74	0.01
Error	35	23060000	658817		
Total	47	56760000	5624379		

Appendix 3.5 Analysis of variance for dry shoot mass (DSM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 1)*.*

Source	DF	SS	MSS	F	P ≤
Replication	5	5.610	1.1221		
Treatment	7	170.788	21.3485	3.27	0.05
Error	35	261.000	6.5250		
Total	47	437.399	28.9956		

Appendix 3.6 Analysis of variance for dry shoot mass (DSM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 2)*.*

Source	DF	SS	MSS	F	P
Replication	5	59.598	11.9195		
Treatment	7	50.485	6.3106	0.66	0.7260
Error	35	384.704	9.6176		
Total	47	494.787	27.8477		

Appendix 3.7 Analysis of variance for dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	0.43857	0.08771		
Treatment	7	1.17090	0.14636	1.60	0.1569
Error	35	3.66881	0.09172		
Total	47	5.27828	0.32579		

Appendix 3.8 Analysis of variance for dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 2).

Source	DF	SS	MSS	F	P ≤
Replication	5	17.188	3.43759		
Treatment	7	70.250	8.78123	2.63	0.05
Error	35	133.500	3.33749		
Total	47	220.937	15.55631		

Appendix 3.9 Analysis of variance for vine length (VIL) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	3007.1	601.41		
Treatment	7	5821.4	727.67	0.71	0.6779
Error	35	40776.9	1019.42		
Total	47	49605.3	2348.50		

Appendix 3.10 Analysis of variance for vine length (VIL) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	3242.2	648.449		
Treatment	7	2349.8	293.722	0.61	0.7620
Error	35	19183.6	479.590		
Total	47	24775.6	1421.761		

Appendix 3.11 Analysis of variance for chlorophyll content (CHC) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 1)*.*

Source	DF	SS	MSS	F	P
Replication	5	54.561	10.9123		
Treatment	7	190.899	23.8624	1.33	0.2590
Error	35	719.845	17.9961		
Total	47	965.306	52.7708		

Appendix 3.12 Analysis of variance for chlorophyll content (CHC) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 2)*.*

Source	DF	SS	MSS	F	P
Replication	5	351.29	70.2576		
Treatment	7	252.34	31.5427	1.40	0.2272
Error	35	902.61	22.5651		
Total	47	1506.23	124.3654		

Appendix 3.13 Analysis of variance for stem diameter (STD) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	0.00332	0.0006641		
Treatment	7	0.04560	0.0057	1.12	0.3721
Error	35	0.20393	0.005098		
Total	47	0.25285	0.0068739		

Appendix 3.14 Analysis of variance for stem diameter (STD) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne javanica* (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	4.1459	0.82919		
Treatment	7	11.8693	1.48366	1.39	0.3341
Error	35	42.6641	1.0660		
Total	47	58.6793	3.37885		

Appendix 3.15 Analysis of variance for root galls (RGA) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	0.01873	0.003747		
Treatment	7	0.02003	0.002861	1.00	0.4478
Error	35	0.10013	0.002861		
Total	47	0.13889	0.009469		

Appendix 3.16 Analysis of variance for root galls (RGA) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 2).

Source	DF	SS	MSS	F	P ≤
Replication	5	0.03372	0.00674		
Treatment	7	0.10188	0.01455	2.33	0.05
Error	35	0.21835	0.00624		
Total	47	0.35395	0.02753		

Appendix 3.17 Analysis of variance for reproductive potential (RP) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 1*).*

Source	DF	SS	MSS	F	P≤
Replication	5	24.537	4.9075		
Treatment	7	411.096	58.7279	11.85	0.01
Error	35	173.473	4.9564		
Total	47	609.105	68.5918		

Appendix 3.18 Analysis of variance for reproductive potential (RP) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	46579	9315.7		
Treatment	7	110181	15740.1	1.66	0.1514
Error	35	331891	9482.6		
Total	47	488650	34538.40		

Appendix 3.19 Analysis of variance for dry shoot mass of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	8.570	1.71404		
Treatment	7	46.487	5.81092	1.97	0.0761
Error	35	118.101	2.95252		
Total	47	173.159	10.47748		

Appendix 3.20 Analysis of variance for dry shoot mass of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	338.71	67.7418		
Treatment	7	229.86	28.7324	1.43	0.2126
Error	35	801.52	20.0381		
Total	47	1370.09	116.5123		

Appendix 3.21 Analysis of variance for dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	0.18300	0.03660		
Treatment	7	0.35217	0.04402	1.14	0.3558
Error	35	1.53823	0.03846		
Total	47	2.07340	0.1190		

Appendix 3.22 Analysis of variance for dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	5.9873	1.19745		
Treatment	7	2.4349	0.30436	0.72	0.6744
Error	35	16.9578	0.42394		
Total	47	25.3799	1.651826		

Appendix 3.23 Analysis of variance for vine length (VIL) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 1)*.*

Source	DF	SS	MSS	F	P
Replication	5	4261.1	852.82		
Treatment	7	9166.6	1145.83	2.07	0.0619
Error	35	22110.8	552.77		
Total	47	35541.6	2551.42		

Appendix 3.24 Analysis of variance for vine length (VIL) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 2)*.*

Source	DF	SS	MSS	F	P
Replication	5	89.49	17.8985		
Treatment	7	127.51	15.9382	0.63	0.7475
Error	35	1011.71	25.2929		
Total	47	1228.71	59.1296		

Appendix 3.25 Analysis of variance for chlorophyll content (CHC) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	158.70	31.7394		
Treatment	7	230.70	28.8371	1.15	0.3520
Error	35	1002.07	25.0519		
Total	47	1391.47	85.6284		

Appendix 3.26 Analysis of variance for chlorophyll content (CHC) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	89.49	17.8985		
Treatment	7	127.51	15.9382	0.63	0.7475
Error	35	1011.71	25.2929		
Total	47	1228.71	59.1296		

Appendix 3.27 Analysis of variance for stem diameter (STD) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 1).

Source	DF	SS	MSS	F	P ≤
Replication	5	1.1156	0.22311		
Treatment	7	17.6700	2.20875	2.47	0.05
Error	35	35.7678	0.89419		
Total	47	54.5533	3.32605		

Appendix 3.28 Analysis of variance for stem diameter (STD) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 2 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	6.0948	1.21896		
Treatment	7	5.8137	0.72671	0.94	0.4931
Error	35	30.8352	0.77088		
Total	47	42.7437	2.062511		

Appendix 3.29 Analysis of variance for root galls (RGA) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 1)*.*

Source	DF	SS	MSS	F	P
Replication	5	0.00323	0.0006460		
Treatment	7	0.02003	0.0002861	0.87	0.5425
Error	35	0.11563	0.00304		
Total	47	0.13889	0.0039721		

Appendix 3.30 Analysis of variance for root galls (RGA) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 2)*.*

Source	DF	SS	MSS	F	P
Replication	5	0.00323	0.0006460		
Treatment	7	0.02003	0.0002861	0.87	0.5425
Error	35	0.11563	0.00304		
Total	47	0.13889	0.0039721		

Appendix 3.31 Analysis of variance for reproductive potential (RP) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 1).

Source	DF	SS	MSS	F	P ≤
Replication	5	399.81	79.961		
Treatment	7	2319.39	331.341	2.52	0.05
Error	35	4593.07	131.231		
Total	47	7312.26	542.533		

Appendix 3.32 Analysis of variance for reproductive potential (RP) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 2).

Source	DF	SS	MSS	F	P ≤
Replication	5	1484.2	296.83		
Treatment	7	9370.6	1338.66	2.34	0.05
Error	35	20009.2	571.69		
Total	47	30863.9	2207.18		

Appendix 3.33 Analysis of variance for dry shoot mass (DSM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	13.104	2.62073		
Treatment	7	22.718	2.83980	0.71	0.6814
Error	35	160.075	4.00189		
Total	47	195.897	9.46242		

Appendix 3.34 Analysis of variance for dry shoot mass (DSM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	432.55	86.5092		
Treatment	7	215.58	26.9479	1.70	0.1273
Error	35	632.40	15.8100		
Total	47	1280.53	129.2671		

Appendix 3.35 Analysis of variance for dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	0.5369	0.10739		
Treatment	7	4.3050	0.53813	1.90	0.0862
Error	35	11.3029	0.28257		
Total	47	16.1448	0.92809		

Appendix 3.36 Analysis of variance for dry root mass (DRM) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	20.315	4.06292		
Treatment	7	32.246	4.03073	1.32	0.2621
Error	35	122.211	3.05528		
Total	47	174.771	11.14893		

Appendix 3.37 Analysis of variance for vine length (VIL) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	5185.5	1037.11		
Treatment	7	5338.3	667.29	1.08	0.3974
Error	35	24746.4	618.66		
Total	47	12998.2	2323.06		

Appendix 3.38 Analysis of variance for vine length (VIL) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 2).

Source	DF	SS	MSS	F	P ≤
Replication	5	6062.1	1212.42		
Treatment	7	12912.4	1614.05	4.51	0.01
Error	35	14307.7	357.69		
Total	47	33282.2	1982.888931		

Appendix 3.39 Analysis of variance for chlorophyll content (CHC) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	69.138	13.8276		
Treatment	7	122.516	15.3145	1.08	0.3960
Error	35	566.855	14.1714		
Total	47	758.509	43.3135		

Appendix 3.40 Analysis of variance for chlorophyll content (CHC) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	91.30	18.2607		
Treatment	7	192.65	24.0810	1.10	0.3816
Error	35	873.24	21.8311		
Total	47	1157.19	64.1728		

Appendix 3.41 Analysis of variance for stem diameter (STD) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 1).

Source	DF	SS	MSS	F	P
Replication	5	2.4210	0.48421		
Treatment	7	6.1121	0.76402	2.09	0.0602
Error	35	14.6440	0.36610		
Total	47	23.1771	1.61433		

Appendix 3.42 Analysis of variance for stem diameter (STD) of sweet potato cultivar 'Mafutha' inoculated with *Meloidogyne incognita* race 4 (Experiment 2).

Source	DF	SS	MSS	F	P
Replication	5	10.6015	2.12030		
Treatment	7	10.1504	1.26880	1.55	0.1725
Error	35	32.8385	0.82096		
Total	47	53.5904	4.21276		