HOST-STATUS AND SENSITIVITY OF TWENTY-TWO SWEET POTATO LINES TO *MELOIDOGYNE INCOGNITA* AND *MELOIDOGYNE JAVANICA* AND POTENTIAL MECHANISMS OF RESISTANCE

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[redacted]

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DECLARATION

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

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DEDICATION

This work serves as a tribute to the memory of my late beloved grandmother, Mrs Phuti Jackinah Morifi.

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ABSTRACT

Most thermophilic crop-producing regions are replete with root-knot *(Meloidogyne* species) nematodes especially *M. incognita*, *M. javanica* and *M. enterolobii*. *Meloidogyne* species are a serious threat on various sweet potato (*Ipomoea batatas* L.) cultivars. Previously, internationally *M. incognita* was viewed as being a more aggressive *Meloidogyne* species than *M. javanica,* whereas in South Africa the opposite had been correct. Additionally, in most cropping systems, *M. incognita* and *M. javanica* occur as mixed population densities and were previously managed using fumigant nematicides, which were later withdrawn from the agrochemical markets due to their negative impact on human and environmental health. Following their withdrawal, the use of resistant plant genotypes served as an alternative strategy in managing high nematode population densities. However, after the withdrawal of fumigant nematicides, another root-knot nematode, namely, *M. enterolobii*, emerged as a major threat in various crops, breaking the renowned Mi resistant genes in various crops. The objectives of this study were to: (1) determine whether the reproductive potential (RP) and relative susceptibility (r_{sa}) values of *M. incognita, M. javanica* and *M. enterolobii* each on sweet potato test lines and cultivars would be below unity and then (2) investigate whether non-host status in sweet potato lines would have post-infectional nematode resistance mechanism to *M. incognita, M. javanica* and *M. enterolobii*. To achieve Objective 1, three greenhouse experiments were conducted, and all 19 sweet potato line cuttings were inoculated at 14 days after transplanting with the same level of 250 eggs and second stage-juveniles (J2) of *M. incognita*, *M. javanica* and *M. enterolobii* in each trial. In each trial, nematode-plant relations were assessed using RP and r_{sa}, each indicator suggesting that the lines were expressing either host-status or non-host-status. In Objective 2, two sweet potato lines, namely, nematode resistance, namely, pre- or post-infectional nematode resistance mechanism. The two lines had each features which suggested the existence of post-infectional nematode resistance mechanism, .consistently characterised by the existence of (1) necrotic spots, (2) poorly developed giant cells, (3) proliferation of rootlet interference, (4) absence of root galls and (5) non-detectable J2 in roots. In conclusion, the two sweet potato lines were added to the existing sweet potato cultivars with post-infectional nematode resistance to thermophilic *Meloidogyne* species.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

In most thermophilic regions, sweet potato (*Ipomoea batatas* L.) serves a major role in promoting food security, human nutrition and poverty alleviation (Amagloh *et al*., 2021; Karuri *et al*., 2017). In the past, prior to the 2005 withdrawal deadline, fumigant nematicides had commonly been used to manage population densities of various plant nematodes. However, the substances have been withdrawn from the agrochemical markets due to widespread concerns about their harmful effects on the environment, particularly their toxicity to humans and animals. Additionally, their restricted availability in developing countries restricted their use in smallholder farming systems (Peiris *et al*., 2021; Saad *et al*., 2019). Following the withdrawal of the products, *Meloidogyne* species were observed to be a major limiting factor in the production of different crops, including sweet potato, resulting in crop losses estimated at 15% per annum. However, during the era of nematicides, *Meloidogyne* species caused yield losses in sweet potato production with the estimation of 6% for South Africa, South America (15%), West Africa (24%) and Southeast Asia (6%) (Kleynhans, 1991; Sasser, 1979). Globally, in thermophilic regions of the undeveloped countries, plant parasitic nematodes had been responsible for billions of USA dollars in sweet potato crop losses annually (Oloka *et al*., 2021).

Following the scale-down uses of the highly effective fumigant nematicides, the subsequent uses of non-fumigant nematicides exhibited challenges of not killing nematodes, resulted in high accumulation of chemical residues in certain plant organs, which were ultimately poisonous to consumers. The latter was due to limited information on acropetal (from roots to shoots) and basipetal (from shoots to roots) movements of the substitute synthetic products (Oka, 2020). Consequently, dependable alternative nematode management strategies were necessary to improve the productivity of various crops, including sweet potato, with the most cost-efficient and environment-friendly approach being the use of resistant plant genotypes (Karuri *et al*., 2017). Additionally, nematodes as biological entities have developed various survival strategies, collectively termed cryptobiosis (Vlaar *et al*., 2021), which made it impossible to eliminate this group of pests (Mashela, 2007).

1.1.1 Description of the research problem

Globally, sweet potato serves as a main food crop and therefore provides nutritional advantage to rural and urban regions where its production and consumption are common. The produce is a rich source of β-carotene (pro-vitamin A), along with vitamins B1, B6 and C) and the essential nutrient elements Ca, K, Cu, Mn and Mg (Alam, 2021). Therefore, sweet potato could serve as an excellent source of essential dietary nutrients to prevent and manage vitamin A and other nutrients associated with malnutrition in various communities (Laurie *et al*., 2024). Most crop-producing regions are replete with root-knot *(Meloidogyne* species) nematodes, with sweet-potato producing regions having high population densities of *M. incognita, M. javanica* and *M. enterolobii (*Piedra-Buena *et al*., 2011)*.* These species produce specific symptoms, namely, root galls, knobbling, clumping, deformed roots and cracking, collectively decreasing water and nutrient intake, as well as lowering both yield and quality (Karuri *et al*., 2017). Plant nematodes, as a result, have a severe negative impact on food security and the ability to maintain the rising global human populations as expected to rise as high as 35% by 2050 (Karuri *et al*., 2017; Kim and Yang, 2019). The suspension of the ozone-depleting fumigant nematicides since 2005 has led to increasing farmer-attention on the use of nematode resistant crops as the most cost- effective tool and environment-friendly approach of managing *Meloidogyne* species in sweet potato production (Karuri *et al*., 2017; Mashela *et al*., 2015). Generally, nematode plant resistance is compatible with other nematode management strategies, which may include sanitation, phytonematicides, intercropping, solarisation, crop rotations and biological agents (Tapia-Vázquez *et al*., 2019). However, there is a challenge in using nematode-resistant plants strategy to manage nematode population densities in sweet potato as *Meloidogyne* species have a wide distribution, wide host-range, multiple races and limited empirically based information on the degree of nematode resistance in certain crops (Makhwedzhana, 2018). The use of nematode resistant varieties is still considered the most effective and environment-friendly method for managing *Meloidogyne* species, as it also reduces the production costs, while being compatible with other strategies (Karuri *et al.,* 2017).

1.1.2 Impact of the research problem

Previously, fumigant nematicides were used to manage population densities of *Meloidogyne* species in various crops (Meza and Sagredo, 2021). However, due to widespread concerns about their negative impact on soil deterioration and ozone layer depletion, along with their toxicity to human and animal health, the products were eventually withdrawn from the agrochemical markets (Onkendi *et al*., 2014; Peiris *et* *al*., 2021). However, the withdrawal posed a significant threat on sweet potato production, with producers being in desperate need of trustworthy alternative nematode management strategies (Karuri *et al*., 2017). Following the withdrawal of methyl bromide from the agrochemical markets in 2005, severe reduction in sweet potato production due to *Meloidogyne* species occurred, resulting in global losses of up to 12.3% (Khan and Khan, 2021). According to Molinari (2011), in the agricultural industry, plant-parasitic nematodes globally cause severe economic losses of more than US$ 100 billion crop losses, whereas prior to the withdrawal of fumigant nematicides the total expense of using chemical nematicides was less than 1 billion US$ billion/year. Globally, nematodes damage is the major contributing factor on tuber rejection and revenue loss in markets (Kolombia and Fabiyi, 2023).

1.1.3 Possible causes of the research problem

Prior to the withdrawal of fumigant nematicides, it was believed that most nematodes were not of economic importance. However, after the withdrawal, the economic importance of most plant nematodes was realised (Dasaeger *et al*., 2020; Karuri *et al*., 2017; Parsons *et al*., 2015). The use of nematode resistance requires screening of various genotypes for non-host status to specific *Meloidogyne* species that occur in a particular field, which is time-consuming. Additionally, certain *Meloidogyne* species, such as *M. enterolobii*, emerged as a major threat after the withdrawal of fumigant nematicides.

1.1.4 Proposed solution(s)

Non-time-consuming tests of resistance, such as relative susceptibilities test could be compared with the time-consuming tests such as the reproductive potential (RP = Pf/Pi). However, after using r_{sa} test, it would still be necessary to identify the mechanism of nematode resistance in genotypes with nematode resistance in order to allow its future use in plant breeding if it has post-infectional mechanism of resistance.

1.1.5 General focus of the study

The study focused on establishing the host-status and rs_a of 18 Agricultural Research Council (ARC) sweet potato lines over susceptible sweet potato cv. ′Beauregard' to three *Meloidogyne* species, together with the mechanism of nematode resistance to any of the test species.

1.2 Problem statement

Meloidogyne incognita, M. javanica and *M. enterolobii* s are thermophilic and widely distributed in sweet potato-producing regions of South Africa. In nematode susceptible sweet potato cultivars, high nematode population densities induce plant wilting, decrease water absorption and nutrient intake, along with vertical cracking of roots, thereby reducing both yield and market value of the produce (Karuri *et al*., 2017; Khanam, 2016). Some widely used sweet potato cultivars in South Africa were shown to exhibit some resistance to the test *Meloidogyne* species (Makhwedzhana, 2018;

Nkosi, 2019). The ARC is breeding high-yielding sweet potato cultivars using molecular breeding technologies to introgress essential nutrition attributes through the biofortification technology (Thurau *et al*., 2010). Information on the host-status of recently introduced sweet potato lines to the three-test root-knot nematodes had not been documented.

1.3 Rationale of the study

The ARC sweet potato cultivars and lines are being bred for their high nutritional values through the biofortification technology (Laurie *et al*., 2015). Biofortification is the process of producing crops with increased nutritional value, and it can be accomplished using traditional selective breeding or molecular approaches (Malik and Maqbool, 2020). The selected ARC sweet potato lines have primarily completed the biofortification process and are being assessed for various attributes including their host-status to *Meloidogyne* species. Internationally, *M. incognita* was previously viewed as the most aggressive thermophilic *Meloidogyne* species than *M. javanica* (Asamizu *et al*., 2020). However, in South Africa *M. javanica* was viewed as being more aggressive than *M. incognita* (Kleynhans *et al*., 1996). Currently, *M. enterolobii* is viewed as the most aggressive thermophilic *Meloidogyne* species and was, after the approval of the title, included in the study on the insistence of the funders. Generally, the identification of the degree of nematode resistance is a lengthy process, starting with the identification of the nematode species to be managed, followed by screening using several cultivars with an identified nematode species. Additionally, in most cropping systems, *M. incognita* and *M. javanica* co-exist as mixed population densities (Pofu *et al*., 2016). Most importantly, it is necessary that the three *Meloidogyne* species be tested individually in separate trials rather than combining the populations since only one species could exist in some environments. Two tests are available to assess host-status, namely reproductive potential (RP = Pf/Pi, where Pf is the final nematode population density and Pi is the initial nematode population density (Seinhorst, 1967). The second test uses the concept of relative susceptibility (rs_a), where test lines were compared with the sweet potato that has known nematode susceptibility to the test nematode species (Seinhorst, 1995). Once the host-status has been established in certain lines using any or both tests, the mechanism of nematode resistance should further be investigated. In plant nematology, there are two mechanisms of nematode resistance, namely, pre-infectional and post-infectional nematode resistance mechanisms (Shigueoka *et al*., 2019). Plants with post-infectional nematode resistance mechanism can be used in plant breeding for introgression of desired attributes (Thurau *et al*., 2010). Introgression is defined as the transfer of a small fraction of the genome from one parental taxon (often species) to another through hybridisation and continuous backcrossing (Suarez-Gonzalez *et al*., 2018; Thurau *et al*., 2010).

1.4 Purpose of the study

1.4.1 Aim

Investigation of the relative susceptibility of 18 ARC sweet potato lines *to M. incognita M. javanica* and *M. enterolobii* nematode species compared to a susceptible sweet potato cv. ′Beauregard′ and the related mechanisms of resistance.

1.4.2 Objectives

I. To determine whether RP and rs_a values of *M. incognita, M. javanica* and *M. enterolobii* each on test lines and cultivars would be below unity.

II. To investigate whether non-host status in sweet potato lines would have post-infectional nematode resistance to *M. incognita, M. javanica* and *M. enterolobii*.

1.4.3 Null hypotheses

I. Reproduction potential and rs_a values of *M. incognita, M. javanica* and *M. enterolobii* each on test sweet potato cultivars and lines would each be below unity.

II. Non-host sweet potato lines would not have post-infectional nematode resistance to *M. incognita,* and *M. javanica* and *M. enterolobii.*

1.5 Reliability, validity and objectivity

In this study, reliability of data was ensured by the use of appropriate statistical levels of significance ($P \leq 0.05$). Validity was ensured by conducting the experiments at the same location with the same replications during different times (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 defined as any influence or series of conditions that affects the data, individually or collectively (Leedy and Ormrod, 2005). In the current study, experimental error in each experiment was minimised through the use of replications and randomisation as a way to avoid bias.

1.7 Scientific significance of the study

Outcomes of the current study would provide useful information on the host-status, relative susceptibility and mechanism of nematode resistance of the 18 sweet potato lines against selected thermophilic *Meloidogyne* species. The data obtained might be utilised to enlighten South African sweet potato farmers on management of *Meloidogyne* species utilising resistant cultivars/lines as a way to reduce their financial costs, environment preservation against chemicals and maintain food security, as well as to provide transparency on whether the lines could be exploited in plant breeding for introgression as this would help increase sweet potato production yields and market quality.

1.8 Structure of the dissertation

The dissertation was designed using the Senate-approved format of the University of Limpopo. Consequent to the description and detailed outlining of the research problem (would be in Chapter 1, with Chapter 2 having the literature review of the work done and the work not done on the research problem. Then, Objective 1 and Objective 2 constituted the research Chapter 3 and Chapter 4, respectively. Objective 2 was reviewed (Chapter 4). In the final Chapter 5, the two research chapters would be summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in conclusions that would provide the entire study. The Harvard author-alphabet format was adopted for all the in-text citations and final reference list. In the next chapter, literature was reviewed in detail on the work done on the research problem and the knowledge gaps clearly identified.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Numerous plant parasitic nematode species have been discovered in plant production sites across the world, with root-knot (*Meloidogyne* species) nematodes causing an estimated 14% yield loss in agricultural produce each year (Asamizu *et al*., 2020). Sweet potato (Scientific name) ranks seventh among agricultural crops, and it is the world's third-largest important root tuber crop faces a tremendous threat from root-knot (*Meloidogyne* species) nematodes (Kim and Yang, 2019). Sweet potato is highly susceptible to *Meloidogyne* species, which cause severe damage on root tubers (Wendimu, 2021). This genus can induce cracks on root tubers, which facilitate the entrance of fungi and bacteria, thereby promoting postharvest rots during storage (Makhado, 2020; Nnadi and Carter, 2021).

According to Oka (2020), several fumigant nematicides including ethylene dibromide (EDB), methyl bromide, a combination of 1,3-dichloropropene and 1,2-dichloropropane (D-D) and chloropicrin (tear gas) have been used in the past to population densities of the test nematode. However, the products were withdrawn from the agrochemical markets due to their environment-unfriendly attributes (Pofu and Mashela, 2019). The withdrawal of methyl bromide has led to increased interest in the development of alternative nematode management strategies The most effective and environment-friendly strategy for managing population densities of *Meloidogyne* species had since the fumigant nematicide-withdrawal deadline of 2005 the use of nematode resistant cultivars (Fosu-Nyarko and Jones, 2015; Karuri *et al*., 2017).

2.2 Work done on the research problem.

The research problem was previously expounded in detail earlier (Chapter 1) and would not be repeated.

2.2.1 Screening *Meloidogyne* species in sweet potato

Sweet potato is a warm-loving (thermophilic) crop, where *M. incognita, M. javanica*, *M. enterolobii* and *M. arenaria* are widely distributed (Wendimu, 2021). The pest causes severe root tuber damage, at times rendering the produce unmarketable (Lee *et al*., 2019).

In order to establish the degree of nematode resistance, it is important to identify the test nematode to a species level. Generally, the latter is achieved using molecular techniques or differential host plants, whereas the former is achieved through various tests, including screening (Chapter 1). Screening constitutes using several cultivars inoculated with one level of the test nematode, with the indicator being (Pofu *et al*., 2016).

Piedra-Buena *et al*. (2011) screened three sweet potato cultivars 'C4', 'TIS 3290' and 'TIS 9162' against 156 isolates of *Meloidogyne* species. The isolates included *M.*

incognita races 1, 2, 3, 4, 5 and 6, *M. arenaria* and *M. javanica*. In the study, sweet potato cv. 'TIS 3290' was resistant to *M. arenaria*, cv. 'TIS 9162' to *M. javanica,* whereas *M. incognita* infected all test sweet potato cultivars. According to Asamizu *et al*. (2020), *M. incognita* races in most regions infect sweet potato and induce huge crop losses. In another study (Pofu *et al*. 2016), 12 sweet potato cultivars were screened against *Meloidogyne* species and races, with most cultivars being host to *M. javanica* and *M. incognita* races 2 and 4. In South Korea, cv. 'Juhwangmi' was highly resistant to *M. incognita*, whereas cv. 'Yulmi' was highly sensitive to *M. incognita* (Lee *et al*., 2019). In Japan, 96% of sweet potato cultivars were shown to be susceptible to *M. incognita* under field conditions (Yigezu, 2021). In general terms, Wendimu (2021) demonstrated that *M. incognita* could pose a greater threat to sweet potato root tubers than other *Meloidogyne* species.

In Limpopo Province, South Africa, cultivars 'Bosbok', 'Mvuvhelo and 'Bophelo' were shown to be non-host to all test *Meloidogyne* species and races (Pofu *et al*., 2016). However, that test did not include the recent thermophilic M. *enterolobii* (Scientific name), which considered a major threat on various crops. In the USA, *M. enterolobii* reproduced profusely on sweet potato cultivars 'Covington′, 'Evangeline′, ′Beauregard′, ′Orleans′ and 'Hernandez′ (Rutter *et al*., 2019). Except for cultivars ′Beauregard′ and ′Orleans′ all cultivars were later reported as being moderately resistant to *M. incognita* (Brito *et al*., 2020). In South Carolina (United States), *M. enterolobii* infected root-knot nematode resistant sweet potato cultivar 'Covington′ (Rutter *et al*., 2019). In July 2020, sweet potatoes with galled roots were collected from a commercial Asian vegetable farm located in Wimauma (USA), the morphological characteristics of second-stage juveniles (J2) and perineal patterns of mature females being consistent with those of

M. enterolobii, which was the first report on sweet potato in Florida (Gu *et al*., 2021). Twenty sweet potato lines from the breeding programme of The Agricultural Research Council (ARC) of South Africa were screened against *M. incognita* race 2, *M. incognita* race 4 and *M. javanica* at the University of Limpopo, with only line 1990-10-2 being a non-host to all the test *Meloidogyne* species and races (Pofu *et al*., 2020). Additionally, at the same university, In Limpopo Province, Makhwedzhana (2018) demonstrated that cv. 'Bophelo′ was susceptible to *M. incognita* race 2, whereas cultivars 'Bosbok' and 'Mvuvhelo' were resistant hosts. In Kenya, Karuri *et al*. (2017) screened 72 sweet potato cultivars against *M. incognita*, where susceptible cv. 'Beauregard' and resistant cv. 'Tanzania' were included as positive and negative controls, respectively. In that study, 68.0% cultivars were non-host, whereas 11.1% were host. A screening study was carried out to investigate the resistance of 91 sweet potato genotypes against *M. enterolobii*, the results demonstrated 19 sweet potato genotypes as resistant to the test species, the genotypes 'Bwanjule', 'Dimbuka-Bukulula', 'Tanzania', 'Jewel', 'Centennial' and 'Murasaki-29' were some of the resistant sweet potatoes (Schwarz *et al*., 2021). Pinto *et al*. (2023) observed resistance of three test sweet potato clones 'BGBD 1399', 'MD 1610036', and 'MD 1609024' against *M. enterolobii*. Another study was carried out to assess the resistance of 44 sweet potato genotypes to *M. enterolobii*, *M. javanica* and *M. incognita* race 1 under greenhouse conditions in Brazil. The results of the study indicated that *M. javanica, M. incognita* and *M. enterolobii* had reproduced only 10% (less aggressive), 48% and 80%, respectively (Carmona *et al*., 2020). However, six sweet potato genotypes, namely, CNPH 1292, CNPH 1392, CNPH 1200, CNPH 1219, CNPH 60, and cv. 'Coquinho' exhibited the highest levels of resistance to the three test *Meloidogyne* species (Carmona *et al*., 2020). Twenty-seven sweet potato genotypes were evaluated for resistance to three North Carolina root-knot nematode populations namely, *M. arenaria* races 1 and 2, *M. incognita* races 1, 2 and 3 and *M. javanica.* Among the 27 sweet potato genotypes only two African landraces 'Tanzania' and 'Wagabolige' were resistant to all test nematode species (Cervantes-Flores *et al*., 2002).

2.2.2 Relative susceptibility in crops

Relative susceptibility (rs_a) is the ratio between the maximum rates of production (a), comparing the initial nematode population densities (Pi) and final nematode population densities (Pf) at the end of the growing season of the test plants to the standard susceptible control cultivar (Seinhorst *et al*., 1995). The latter is one of the approaches used in plant-nematology to evaluate a plant's resistance and susceptibility to a test nematode by comparing a susceptibility of a standard susceptible cultivar with other test plants, using a fixed criteria (rs_a = Pf test plant/ Pf susceptible standard cv. ′Beauregard′), indicating a plant as resistant when less than one and susceptible when greater than one (Kort *et al*., 1977). The approach has been widely adopted in the Netherlands since the year 1999 and has since been implemented throughout in plant-nematology (Norshie *et al*., 2011).

Several studies have been conducted on agricultural crops to evaluate their relative susceptibilities against nematode species using rs_a approach. However, there is no reported information on r_{sa} of sweet potato to *Meloidogyne* species. As a result, the work done was reviewed using rs_a of other agricultural crops against nematode species. A study was conducted to determine the relative susceptibilities of eleven potato cultivars and breeders’ clones to the standard cultivars 'Bintje' and 'Irene' to

Globodera pallida (Stone) Behrens.] pathotype *Pa* 3, the results showed all potato cultivars as resistant to *G. pallida* pathotype Pa 3 as their rs_a values ranged 0.15 to 0.50 (Seinhorst *et al*., 1995). Norshie *et al*. (2011) conducted a study to determine r_{sa} of three potato genotypes, namely, AR 04-4096, AR 04-4098 and AR 04-4107 compared to the susceptible cv. 'Desiree' against *M, chitwoodi* Golden (O′Bannon, Santo and Finley, 1980*),* with rs_a of all potato cultivars were 0%, 2.8% and 1.7%, respectively. Under greenhouse conditions, a study was carried out to measure r_{sa} of 13 Egyptian wheat cultivars to the cereal cyst nematode (*Heterodera avenue* Wollenweber*)* compared to the susceptible standard cv. 'Sakha-93'*,* the results suggested that all test wheat cultivars were susceptible as *H. avenue* infected and produced successfully on all of them as their rs_a ranked over 92% (Korayem and Mohammed, 2019). A recent study was established to determine r_{sa} of seven potato genotypes compared to the susceptible cv. 'Desiree', with the results demonstrating that all genotypes were resistant to *M. chitwoodi* as their rs_a were all below 0.29% (Teklu *et al*., 2014). Another study was carried out to investigate r_{sa} of potato genotypes with a single resistant gene for *M. chitwoodi* and all test genotypes were shown to be highly resistant compared to the susceptible cv. 'Desiree' (Teklu *et al*., 2012).

2.2.3 Mechanisms of nematode resistance in sweet potato

Even though various sources of genetic resistance to *Meloidogyne* species have been discovered and reported on many crops, the biochemical, physiological, and genetic mechanisms of *Meloidogyne* resistance in sweet potato are poorly understood (Lee *et al*., 2019). According to reports, the gene expression patterns in sweet potato during

Meloidogyne infection have not yet been studied (Lee *et al*., 2019). Sweet potato has a significant number of potential *Meloidogyne* resistance-related genes, and this has been used as evidence to suggest that *Meloidogyne* resistance in this crop may be inherited as multiple molecular genetic factors (Lee *et al*., 2019). Nematode resistance mechanisms could play a role in the application of nematode resistance in crop rotation systems (Mashela and Pofu, 2016). There are two mechanisms of nematode resistance, namely, pre-infectional and post-infectional nematode resistance (Kaplan and Davis, 1987).

The same level of inoculation is used to identify the mechanism of nematode resistance, and stained roots are observed for the presence of necrotic spots (suberised cells), root galls, giant cell number and proliferation of rootlet interference number every second day as indicators of successful or unsuccessful nematode penetration for a period of 30 days. Nematode bodies are covered with sensory organs that are utilised to detect substances in small quantities and so control the nematode's movement. Nematodes are attracted to and repelled by chemo-attractants and chemo-repellents, respectively, hence nematode chemical interaction is the basis for nematode resistance mechanisms (Wuyts *et al*., 2006; Zhao *et al*., 2000). Basically, it is important to study the type of nematode resistance mechanism involved in nematode-plant relations as this helps to opt for the mechanism that lowers nematode population densities in the soil, promoting the soil from being free from nematodes (Chemo-attractants).

2.2.4 Pre-infectional nematode resistance

Pre-infectional nematode resistance occurs when nematodes are unable to penetrate the plant roots due to the presence of antagonistic substance chemicals found in the plat root tissues (Sujatha *et al*., 2017). Generally, in pre-infectional nematode resistance, mechanism occurs when plants release pre-formed root metabolites that repel or reduce mobility of plant-parasitic nematode in the rhizosphere, which, before infection are completely expressed in root tissues and do not rise in levels in response to nematode invasion (Chiuta, 2021; Ferraz and Brown, 2002). Root exudates such as alpha-terthienyl, dhurrin, glucosinolate, monocrotaline and sorgoleone play a major role in repelling and attracting nematodes in pre-infectional nematode resistance and were shown to have nematicidal effects on *Meloidogyne* species (Jatala and Russel, 1972).

Pepper: Pepper has been shown to be resistant to a number of *Meloidogyne* species, particularly *M. incognita* due to its microbial agents, as a result it can be used to manage plant-parasitic nematode population densities (Ahmed *et al*., 2019). Using the resistant pepper rootstocks with a susceptible tomato, the virulent *M. incognita* did not penetrate the pepper rootstock (Fullana *et al*., 2023). In order to compete with the host, bacteria (*Pseudomonas fluorescens* Migula, 1980) bind the root surface with carbohydrates and lectin, making it a suitable biocontrol agent against root-knot nematodes (Sharma *et al*., 2021). According to Liu *et al*. (2021), the fungus (*Arthrobotrys oligospora* Fresen. 1850) activity in the soil causes a decrease in nematode population, minimising crop nematode damage.

Sorghum: It was discovered that using sorghum varieties Sudan grass cv. 'Piper' or Sudan grass hybrid (*Sorghum bicolor* × *Sorghum bicolor* var. 'Sudanense') cv. '27091' as summer or winter cover crops was a successful in reducing *Meloidogyne* infestation in the soil for susceptible crops (chard, lettuce or lemon) with low and high dhurrin secretions in respective of each variety (Djian-Caporalino *et al*., 2019). These cover crops act as both trap and biofumigant plants releasing hydrogen cyanide. However, for best nematode suppression the sorghum cover should be cultivated in one month or less and biofumigated a month before crop planting (Djian-Caporalino *et al*., 2005; Djian-Caporalino *et al*., 2019). In pre-infectional nematode resistant sorghum-potato rotation system, efficiently reduced *Meloidogyne* species population densities in the soil, resulting in less nematode harm to the potato crop, increased shoot mass, enhanced nutrient accumulation in potato leaf tissues, improved soil health and higher tuber production (Chiuta, 2021).

Tomato: Exudates from resistant tomato roots consist of chemicals that can either attract or repel nematodes to the roots, become immobile, or even die. Following inoculation, levels of four substances 2, 6-Di-tert-butyl-p-cresol, L-ascorbyl 2, 6-dipalmitate, dibutyl phthalate, and dimethyl phthalate significantly increased. Each component prevented *M. incognita* eggs from hatching. The greatest effect was demonstrated in a concentration-dependent manner by L-ascorbyl 2, 6-dipalmitate (Yang *et al*., 2016). There was an interaction between the four compounds and higher J2 mortality. At a concentration of 2 $mmol \cdot L^{-1}$, dimethyl phthalate showed the strongest resistance effects to the test species. The only substance that was found to repel *M. incognita* J2 was dibutyl phthalate; the other compounds had little to no impact detected (Yang *et al*., 2016).

2.2.5 Post-infectional nematode resistance

Plants with post-infectional resistance can protect themselves against nematode damage by releasing chemicals in low to higher levels in the host tissues after penetration of nematodes (Kaplan and Davis, 1987). Their resistance is triggered once J2 are inside the root system during movement to infection sites or upon arrival at the feeding site, the feeding site fails to develop (Chiuta, 2021; Kaplan and Davis, 1987). According to Navarrete *et al*. (2016), the resistant plants have been used as pre-plant trap cover crops. Post-infectional nematode resistance is typically related with an early hypersensitive reaction (HR), in which spontaneous localized cell death in root tissue surrounding the nematode inhibits the growth of a developed feeding site, leading to plant resistance (Sujatha *et al*., 2017). Plants with post-infectional nematode resistance could only be utilized in plant breeding programs to transfer nematode resistant genes into host plants, through a process known as introgression (Thurau *et al*., 2010). Post-infectional nematode resistance genes found in crop species and their relatives have long been utilised to transmit requisite features to commercially valuable crops that lack effective resistance, also with advanced biotechnology helping to find and combine the greatest nematode resistance genes (Fosu-Nyarko and Jones, 2015). However, failure of post-infectional nematode resistance may result from high soil temperatures, high salinity and being attacked by insects that produce honeydew, such as aphids, mealybugs and greenhouse whiteflies (Mashela and Nthangeni, 2002; Pofu *et al*., 2012).

Wild watermelon and wild cucumber: A study was conducted under greenhouse conditions to investigate the form of nematode resistance to the highly resistant *Cucumis* species to *M. incognita* and *M. javanica*, namely wild watermelon (*Cucumis africanus* L.) and wild cucumber (*Cucumis myriocarpus* Naude), the results of the study demonstrated the mechanism of nematode resistance involved in both *Cucumis* species as post-infectional nematode resistance (Ramatsitsi, 2017). Chiuta (2021) suggested that *Meloidogyne* species population densities in potato-based cropping systems might be efficiently managed by using the *C. africanus*-potato sequence.

Pepper: Conventional breeding of black pepper is considered a safe and effective method in managing plant parasitic nematodes due to its microbial control that contains fungi and bacteria (Saad *et al*., 2019). Watermelon was grafted with resistant pepper to manage *M. incognita* population densities, however, in comparison to *Citrullus amarus* Schrad., there were no variations in the number of giant cells per feeding site in watermelon, but they were more voluminous and contained more nuclei per giant cell and per feeding site (Fullana *et al*., 2023). Fungi (*Pochonia halamydosporia* Goddard, 1913) reduced the egg hatching of *Meloidogyne* species in pepper by 41.4% in a period of five days (Ghahremani *et al*., 2019), whereas bacteria (*Bacillus thuringiensis* Ishiwata, 1902) killed 80% of freshly hatched J2 of *M. javanica* (Leong *et al*., 2021).

Sweet potato: Resistance mechanisms to *M. incognita* race 2 on sweet potato cultivars 'Bosbok' and 'Mvuvhelo' were tested with post-infectional mechanism observed characterised by poorly developed giant cells, undetectable J2 in roots, noticeable necrotic patches, rootlet interference formation and the absence of root galls (Makhwedzhana, 2018).

2.3 Work not done on problem statement

The 18 ARC sweet potato lines have not been assessed for their host status to *M. incognita*, *M. javanica* and *M. enterolobii*, along with their relative susceptibility as compared to the susceptible standard cv. ′Beauregard′ and the mechanism of nematode resistance involved on the resistant lines. The latter is important since the findings will demonstrate cultivars that can be utilised during introgression using molecular techniques.

2.4 Addressing the identified gaps

To address the identified gaps, this research study focused on establishing the host-status, relative susceptibilities, as well as the mechanism of resistance involved in the 18 sweet potato lines against *M. incognita*, *M. javanica* and *M. enterolobii*. The effectiveness of sweet potato lines with post-infectional nematode resistance could be used to transfer nematode resistance genes into host plants in plant breeding programs. Following the provided literature review, the next chapter provides in detail the work not yet done on the research problem, highlighting the host-status and relative susceptibilities of 18 sweet potato lines against *M. incognita, M. javanica* and *M. enterolobii*.

CHAPTER 3

REPRODUCTIVE POTENTIAL AND RELATIVE SUSCEPTIBILITY OF ARC-VIMP SWEET POTATO LINES TO *MELOIDOGYNE INCOGNITA, MELOIDOGYNE JAVANICA* AND *MELOIDOGYNE ENTEROLOBII*

3.1 Introduction

Globally, root-knot (*Meloidogyne* species) nematodes are the most tremendous yield-reducing nematode pathogens in crop production and are posing a serious risk in the productivity of a wide range of crops (Karuri *et al*., 2017). Although there are more than 50 *Meloidogyne* species, *M. incognita*, *M. javanica* and *M. enterolobii* are widely distributed in the tropical and subtropical regions, where sweet potatoes (*Ipomoea batatas* L.) are produced (Cervantes-Flores *et al*., 2002; Seid *et al*., 2015). Prior to the withdrawal of fumigant nematicides from the agrochemical markets, *Meloidogyne* species were hardly perceived as economic pests in the production of most crops. However, due to widespread concerns about their negative impact on the environment such as ozone layer depletion and soil deterioration, including their toxicity to humans and animals, the products were withdrawn from the agrochemical markets (Peiris *et l*., 2021). Post the withdrawal, severe yield reduction in various crops, including sweet potatoes, were ascribed to the existence of *Meloidogyne* species (Asamizu *et al*., 2020; Khan and Khan, 2021).

The suspension of the ozone-depleting fumigant nematicides, with the cut-off date of 2005, has led to increasing attention on nematode plant resistance as the most cost-effective and environment-sustainable approach of managing population densities of plant nematodes in various crops, including sweet potato cultivars (Karuri *et al*., 2017; Mashela *et al*., 2015). Due to the existence of wide host range and biological races in the genus *Meloidogyne,* progress in the field of study had been hindered by limited information on accurate nematode species identification (Mitiku, 2018). As a result, there is limited information on sweet potato cultivars with resistant genotypes to thermophilic *Meloidogyne* species in South Africa, namely, *M. incognita*, *M. javanica* and *M. enterolobii.* A previous host-status study (Pofu *et al*., 2016) on sweet potato cultivars in South Africa indicated that non-host status was reported among the local lines, whereas the exotic lines were mostly host to thermophilic *Meloidogyne* species.

In screening for host-status, in addition to RP as an indicator, another indicator, namely, relative susceptibility (rs_a) is used. The latter was defined as the ratio between the maximum rate of reproduction, comparing the initial nematode population densities (Pi) and the final nematode population density (Pf) at the end of the growing season. The maximum rate of reproduction (*a*) of the test plants is assessed relative to that of the standard with known susceptibility to the test nematode (Seinhorst *et al*., 1995). The inherent ability of a plant to restrict or prevent nematode development is regarded as resistance. In contrast, when the nematode is capable of reproducing in the test plant, the plant is considered a host plant (Korayem and Mohammed, 2019). Generally, when RP or rs_a value is below and above one, the test plant is considered resistant and susceptible, respectively (Kort *et al*., 1977). In the current study, rs_a values of 18 sweet potato lines from the germplasm of the Agricultural Research Council were investigated in relation to the standard susceptible cv. 'Beauregard' from the USA (Cervantes-Flores *et al*., 2002). The objective of the study was to determine whether RP and rs_a values of *M. incognita, M. javanica* and *M. enterolobii* on 18 ARC sweet potato lines would be below unity.

3.2 Materials and methods

3.2.1 Description of the study site

At the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'10S, 29°44'15E), three parallel trials were conducted under the greenhouse conditions. Trial 1, Trial 2 and Trial 3 for *M. incognita*, *M. javanica* and *M. enterolobii,* respectively. The greenhouse size was 20 m × 100 m, with *M. enterolobii* experiments (Experiment 1 and Experiment 2) were initiated during spring (August-November) of 2019 then *M. incognita* and *M. javanica* experiments (Experiment 1 and Experiment 2) initiated during spring (August-November) of 2022, then both validated in autumn (January-March) of 2023. Maximum temperatures were regulated by thermostatically activated fans on the northern side wall with ambient day/night temperatures averaging 28/21°C, with the relative humidity maintained at 65-75% using a wet wall on the southern side wall.

3.2.2 Treatments and research design

In each trial, 18 sweet potato lines together with control cv. ′Beauregard′ served as treatments, which were arranged in a randomised complete block design (RCBD), with

6 replications (Figure 3.1). Due to the wind streams created by fans during the heat extraction process, the conditions within the greenhouse were heterogeneous, necessitating the use of RCBD. For inoculation, each nematode type was prepared by extracting eggs and second-stage juveniles (J2) from the roots of greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution using maceration and blending methods (Marais *et al.,* 2017), with each test plant inoculated with 250 eggs + J2.

3.2.3 Procedures

In each Experiment consisting of three trials for *M. incognita*, *M. javanica* and *M. enterolobii*: Eighteen disease-free sweet potato cuttings and the control cv. ′Beauregard′ obtained from the ARC-Vegetable, Industrial and Medicinal Plants (ARC-VIMP) were set in plastic pots containing the growing mixture described hereunder. Twenty-diameter plastic pots were each filled with a 3:1:1 (v/v) mixture of steam-pasteurised river sand, loam soil (65% sand, 30% silt, and 5% clay) and Hygromix (Hygrotech, Pretoria West, South Africa). Pots were placed on the greenhouse benches at 0.25 m inter-row and 0.30 m intra-row spacing and irrigated to field capacity. Uniform cuttings (30-cm-long), with auxiliary buds facing upward, were each inserted in the mixture to enhance the establishment process (Laurie, 2004). Cultivar 'Beauregard' was used to verify the viability of the inoculum (Cervantes-Flores *et al*., 2002). Cuttings were inoculated at 14 days after setting by dispensing 250 eggs +J2 into 5-cm-deep holes on the cardinal points of the stems, then holes were filled with the growing medium. After extraction of the nematodes from the susceptible tomato cultivar 'Floradade', the materials were passed through a top-down nested 75-µm and 25-µm mesh sieves, contents of the 25-µm-opening sieve was poured into 10 ml plastic containers for nematode counting under a stereomicroscope. One week after setting, plants were fertilised with 5 g NPK 2:3:2 (19) + 5% Zn + 5% Ca to provide a total of 155 mg N, 105 mg P and 130 mg K. Addition to that, 5 g of 2:3:2 (19) + 0.5% ZN + 5% S + 5% Ca and 2 g Multifeed NPK 2:1:2 (43) (Nulandies, Johannesburg) 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. Every other day, the plants were irrigated with 250 ml of tap water, then 500 ml when harvest time approached. Throughout the two seasons, insect pests and diseases were scouted and monitored daily.

Figure 3.1 *Meloidogyne incognita* inoculated sweet potato lines root cuttings under greenhouse conditions.

3.2.4 Data collection

At harvest, fifty-six days after treatment application, shoots were chopped at ground level and fresh roots were collected from the pots. The roots were immersed in tap water to wash away dirt particles, patted dry using laboratory paper towels, and then weighed on a weighing balance. The fresh root mass was determined using a weighing balance and North Carolina differential scale index of 0 to 5 was used to determine root galling (Figure 3.2), 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). Nematodes were extracted from 5 g roots per plant by maceration and blending for 30 seconds in 1% $NaOCl_2$ (Marais *et al*., 2017). The material was passed through top-down nested 75-µm and 25-µm mesh sieves. Contents of the 25-µm was poured into 10 ml plastic containers for nematode counting under a stereomicroscope. Roots in each pot were collected for nematode extraction using the sugar-floatation and centrifugation method (Marais *et al*., 2017). To make a sugar stock solution, 624 g of sugar was dissolved in 1 L of tap water, 45 ml of the sugar stock solution was then added to centrifuge containers, stirred once before centrifugation, and centrifuged for 3 minutes at 1 800 rpm (Figure 3.3). The aliquot was then washed off with running water onto a 25-µm sieve to separate the nematodes from the sugar. The residual water was then collected into a 100-ml plastic container for further analysis. Nematodes were counted out of 10 ml aliquot with the use of stereomicroscope (Figure 3.4). The reproductive potential (RP = eggs + J2/g root) values were computed as a proportion of final nematode population in roots per g fresh root.

Figure 3.2 Sweet potato line 'FS1-1' fresh roots infected with *Meloidogyne incognita* accessed for root galling then rated using North Carolina differential scale index of 0 to 5.

Figure 3.3 Nematode extraction using the sugar-floatation and centrifugation method.

Figure 3.4 Eggs and second-stage juveniles (J2) counted out of 10 ml aliquot in a petri dish with the use of a stereomicroscope.

3.2.5 Data analysis

Reproductive potential: Using Statistix 10.0 software, analysis of variance (ANOVA) was used on the variable data to analyse the effects of nematode inocula on RP levels in each experiment and trial. The host-status was computed and the reproductive potential (RP = eggs + J2/g root) was analysed. The sum of squares were partitioned to determine the contribution of the sources of variation to the total treatment variation (TTV) in plant and nematode variables (Gomez and Gomez, 1984). Mean separation for significant ($P \leq 0.05$) treatments were achieved through the Tukey test and significant variables were assessed using Relative impact (RI) index. Unless otherwise stated, data were discussed at the probability level of 5%.

Relative susceptibility: Each test plant's Pf was divided by the Pf of a susceptible standard control Cv. 'Beauregard' to compute the rs_a of the sweet potato lines, (rs_a = Pf test plant/ Pf susceptible standard cv. 'Beauregard').

3.3 Results

3.3.1 Host-status of sweet potato lines using reproductive potential

Analysis of variance for the three *Meloidogyne* species: Eighteen sweet potato lines treatment effects had significant (Appendix 3.1) and highly significant (Appendix 3.2, $P \leq 0.01$) effects on RP of *M. incognita* in Experiment 1 and Experiment 2 respectively, contributing 40 and 78% in TTV of the variable in the respective experiments (Table 3.1). However, treatments had no significant effects on RP of *M. javanica* in Experiment 1 (Appendix 3.3) but had significant effects on the variable in Experiment 2 (Appendix 3.4), contributing 50% in TTV of the variable (Table 3.1). In *M. enterolobii* trial, treatments had highly significant effects on RP in Experiment 1 (Appendix 3.5) but had significant effects on the variable in Experiment 2 (Appendix 3.6), contributing 45 and 86% in TTV of the variable in the two respective experiments (Table 3.1).

Host-status in *Meloidogyne incognita* trials: According to Pofu *et al*. (2019), in plant nematology, RP values less than 1 indicate non-host status, while RP values greater than 1 indicate a host. In Experiment 1, four sweet potato lines '2015-1-6', 'FS5-2', 'FS1-1' and 'Khumo' were categorized as *M. incognita* non-hosts based on their RP values falling below unity (Table 3.2). However, '2014-7-3', 'FS10-8', '2013-26-5', '2014-17-6', '2014-13-1', '2014-14-5', '2015-7-1', '2015-2-1', 'FS10-21', 'FS10-25', '2015-9-2', '2014-14-2', '2015-19-1', '2013-28-5' and cv. 'Beauregard' had RP values above unity, suggesting that they were host, but on the basis of Tukey test, they were also clustered with those lines with RP values below unity, which was the sub-category of non-host. In contrast, in Experiment 2, four sweet potato lines '2013-26-5', 'FS5-2', '2015-2-1' and 'Khumo' were categorised as non-hosts based on their RP values falling below unity (Table 3.2). However, '2014-7-3', 'FS10-8', '2014-17-6', '2014-13-1', '2014-14-5', '2015-7-1', '2015-19-1', 'FS10-21', 'FS10-25', 'FS1-1', '2015-1-6', '2015-9-2', '2014-14-2', '2015-19-1', '2013-28-5' and ′Beauregard′ had RP values above unity, suggesting that they were host.

Host-status in *Meloidogyne javanica* trials: In experiment 1, two sweet potato lines '2013-26-5' and '2014-17-6' were categorized as being non-host to *M. javanica* based on RP values that were less than unity (Table 3.3). However, '2014-7-3', 'FS10-8', '2014-13-1', '2014-14-5', '2015-7-1', '2015-19-1', 'FS10-21', 'FS10-25', 'FS1-1', '2015-1-6', '2015-9-2', '2014-14-2', '2013-28-5', 'FS5-2', '2015-2-1', 'Khumo' and 'Beauregard' had RP values above unity, suggesting that they were host (Table 3.3). In Experiment 2, only line 'FS10-25' was categorized as a non-host based on its RP value less than unity (Table 3.3). However, all other lines had RP values greater than unity, suggesting that they were host.

Host-status in *Meloidogyne enterolobii* trials: In Experiment 1, '2015-1-6' was the only non-host sweet potato line to *M. enterolobii* as indicated by its RP value less than unity (Table 3.4), all other 18 test sweet potato lines were grouped as hosts to *M. enterolobii* namely, '2014-7-3', 'FS10-8', '2014-13-1', '2014-14-5', '2015-7-1', '2015-19-1', 'FS10-21', 'FS10-25', 'FS1-1', '2013-26-5', '2015-9-2', '2014-14-2', '2013-28-5', 'FS5-2', '2015-2-1', '2017-14-6', 'Khumo' and ′Beauregard′ based on their RP values above unity (Table 3.4). However, in Experiment 2, all 18 sweet potato lines were classified hosts to *M. enterolobii* based on having RP values of above unity.

Table 3.1 Partitioning mean sum of squares (MSS) into total treatment variation (TTV) of reproductive potential (RP) of *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne enterolobii* on ARC sweet potato lines at 56 days after inoculation.

EXPERIMENT 1							
		M. incognita		*M. javanica*		*M. enterolobii*	
Source	DF	MSS	TTV%	MSS	TTV%	MSS	TTV%
Replication	5	2643.86	39	5070.23	40	3464.0	5.43
Treatment	18	2700.40	40**	4813.96	38ns	55017.5	86***
Error	90	1389.92	21	2774.45	19	5328.7	8.35
Total	113	6734.18	100	12658.64	100	63810.2	100
EXPERIMENT 2							
		M. incognita		*M. javanica*		*M. enterolobii*	
Source	DF	MSS	TTV%	MSS	TTV%	MSS	TTV%
Replication	5	346.05	8	57201	25	118497	27.05
Treatment	18	3509.60	78***	114919	50**	198134	45**
Error	90	631.20	14	55834	25	121381	27.71
Total	113	4486.85	100	197957	100	438012	99.99

nsNot significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

Total treatment variation (TTV) = MSS/Total × 100.

Table 3.2 Responses for fresh root mass (FRM), eggs, second-stage juveniles (J2), total nematodes (PF), reproductive potential (RP) and relative impact (RI) of *Meloidogyne incognita* on ARC sweet potato lines at 56 days after inoculation.

EXPERIMENT 1

Lines	FRM (g)	[x]RI (%)	Eggs	[x]RI (%)	J2	[x]RI (%)	PF	[x]RI (%)	[y]RP	[x]RI (%)
1.[z]'Beauregard'	16.65	–	520	–	583	–	583	–	72.80^{a}	–
2. '2014-7-3'	35.68	114	107	–79	103	–82	210	–63	7.25^{a}	–90
3. 'FS10-8'	32.50	95	27	–94	6	–98	33	–94	1.10^{a}	–98
4. '2013-26-5'	19.00	35	13	–98	10	–98	23	–96	1.67^{a}	–97
5. '2014-17-6'	14.78	–11	57	–89	40	–93	97	–83	8.66^{a}	–88
6. '2014-13-1'	18.27	9	40	–92	90	–84	130	–77	6.30^{a}	–91
7. '2014-14-5'	19.45	16	170	–67	30	–95	200	–65	14.37^{a}	–80
8. '2015-7-1'	17.18	3	40	–92	13	–98	53	–90	3.26^{a}	–95
9. '2015-1-6'	9.27	–44	13	–98	0	–99	13	–97	0.66^{a}	–99
10. 'FS5-2'	19.37	16	13	–98	0	–99	13	–97	0.71^{a}	–99
11. '2015-2-1'	2.40	–85	7	–99	3	–99	10	–98	5.66^{a}	–93
12. 'FS10-21'	11.48	–38	43	–91	47	–91	90	–84	11.40^{a}	–84
13. 'FS10-25'	26.37	58.37	173	–66	180	–69	353	–39	12.15^{a}	–83
14. '2015-9-2'	3.28	–80	150	–71	73	–87	193	– 61	69.60^{a}	–4
15. '2014-14-2'	19.57	17	260	–50	50	–91	310	–45	17.16^{a}	–76
16. '2015-19-1'	29.57	77	43	–92	200	–5	243	–58	8.88^{a}	–88
17. '2013-28-5'	25.03	50	30	–94	3	–99	33	–94	1.25^{a}	–98
18. 'FS1-1'	2.58	–84	3	–96	7	–98	10	–98	0.46^{a}	–99
19. 'Khumo'	18.28	9	0	–99	0	–99	0	–99	0.00^{a}	–99

EXPERIMENT 2

Lines	FRM (g)	[x]RI (%)	Eggs	[x]RI (%)	J2	[x]RI (%)	PF	[x]RI (%)	[y]RP	[x]RI (%)
1.[z]'Beauregard'	27.67	–	935	–	92	–	2397	–	98.11^{a}	–
2. '2014-7-3'	45.35	66	230	–75	15	–83	245	–89	5.84^{b}	–94
3. 'FS10-8'	48.17	77	117	–87	12	–86	128	–94	2.85^{b}	–97
4. '2013-26-5'	42.72	55	8	–99	5	–94	13	–99	0.28^{b}	–99
5. '2014-17-6'	32.13	18	212	–77	0	–99	218	–90	4.27^{b}	–96
6. '2014-13-1'	35.48	31	27	–97	2	–97	28	–98	3.95^{b}	–97
7. '2014-14-5'	21.83	–19	128	–86	10	–89	138	–94	6.28^{b}	–94
8. '2015-7-1'	21.83	–19	55	–94	7	–92	62	–97	2.52^{b}	–98
9. '2015-1-6'	21.47	–19	87	–90	15	–83	102	–95	5.81^{b}	–95
10. 'FS5-2'	32.56	18	20	–97	0	–99	20	–99	0.41^{b}	–99
11. '2015-2-1'	4.63	–85	12	–99	0	–99	18	–99	0.42^{b}	–99
12. 'FS10-21'	27.65	0	50	–97	7	–92	57	–97	3.07^{b}	–97
13. 'FS10-25'	33.80	19	145	–94	20	–78	165	–93	16.95^{b}	–85
14. '2015-9-2'	13.98	51	60	–93	0	–99	60	–97	2.47^{b}	–98
15. '2014-14-2'	26.23	–3	17	–98	13	–85	30	–98	1.06^{b}	–99
16. '2015-19-1'	20.78	–25	97	–95	2	–97	98	–95	3.61^{b}	–97
17. '2013-28-5'	30.50	11	197	–89	10	–89	207	–91	6.58^{b}	94
18. 'FS1-1'	30.93	11	103	–88	15	–83	118	–95	4.18^{b}	–96
19. 'Khumo'	23.57	–14	8	–99	2	–97	10	–99	0.28^{b}	–99

[x] Relative impact (RI) (%) = [(treatment/control) – 1] x 100.

[y] RP = eggs + J2/g root and [z]'Beauregard' served as a susceptible standard.

Table 3.3 Responses for fresh root mass (FRM), eggs, second-stage juveniles (J2), total nematodes (PF), reproductive potential (RP) and relative impact (RI) of *Meloidogyne javanica* on ARC sweet potato lines at 56 days after inoculation.

EXPERIMENT 1

Lines	FRM (g)	[x]RI (%)	Eggs	[x]RI (%)	J2	[x]RI (%)	PF	[x]RI (%)	[y]RP	[x]RI (%)
1.[z]′Beauregard′	13.867	–	120	–	17	–	137	–	17.63	–
2. '2014-7-3'	27.267	66	50	–58	23	35	73	–46	3.05	–82
3. 'FS10-8'	26.800	–66	10	–92	10	–41	20	–85	1.08	–93
4. '2013-26-5'	30.500	0	3	–98	13	–23	16	–88	0.52	–96
5. '2014-17-6'	23.500	–89	4	–96	3	–82	7	–94	0.27	–98
6. '2014-13-1'	6.600	–16	130	8	40	55	170	24	54.99	77
7. '2014-14-5'	7.750	–83	33	–73	14	–17	47	–65	8.35	–50
8. '2015-7-1'	13.867	–33	26	–78	27	58	53	–61	3.70	–78
9. '2015-1-6'	6.867	–66	13	–89	20	17	33	–75	5.34	–68
10. 'FS5-2'	21.117	–66	10	91	10	–41	20	–85	1.28	–92
11. '2015-2-1'	6.183	–66	14	–88	13	–23	27	–80	9.38	–44
12. 'FS10-21'	14.567	–16	137	14	133	82	270	97	20.12	18
13. 'FS10-25'	14.433	–19	457	80	120	65	577	321	76.68	37
14. '2015-9-2'	3.133	–16	276	89	297	67	573	318	102.68	50
15. '2014-14-2'	7.767	–16	32	–73	123	62	157	14	26.28	52
16. '2015-19-1'	20.600	39	70	–41	397	76	467	240	20.35	–88
17. '2013-28-5'	16.358	–50	7	–94	0	–99	7	–94	3.17	–81
18. 'FS1-1'	1.402	–99	7	–94	3	–82	10	–92	1.56	–90
19. 'Khumo'	5.392	–66	37	69	53	76	90	–34	13.01	–23

EXPERIMENT 2

Lines	FRM (g)	[x]RI (%)	Eggs	[x]RI (%)	J2	[x]RI (%)	PF	[x]RI (%)	[y]RP	[x]RI (%)
1.[z]′Beauregard′	24.80	–	1572	–	85	–	1657	–	62.14^{a}	–
2. '2014-7-3'	33.02	–99	48	–96	3	–96	52	–96	1.13^{b}	–98
3. 'FS10-8'	49.92	–66	70	–97	13	–84	83	–94	1.64^{b}	–97
4. '2013-26-5'	37.50	–33	1447	–7	85	0	1531	–7	41.47^{b}	–33
5. '2014-17-6'	25.05	–44	272	–82	10	–88	282	–82	18.53^{b}	–70
6. '2014-13-1'	12.03	–72	110	–93	15	–82	125	–92	13.70^{b}	–77
7. '2014-14-5'	19.66	–61	155	–90	28	–67	183	–88	13.31^{b}	–78
8. '2015-7-1'	24.42	–33	52	–96	12	–85	63	–96	2.18^{b}	–96
9. '2015-1-6'	24.27	–19	518	–67	48	–43	567	–65	23.53^{b}	62
10. 'FS5-2'	14.60	–39	433	–72	43	–49	477	–71	17.63^{b}	–72
11. '2015-2-1'	30.97	–11	498	–68	38	–55	537	–67	25.40^{b}	–59
12. 'FS10-21'	26.63	–89	275	–82	57	–32	332	–79	12.57^{b}	–80
13. 'FS10-25'	13.87	–89	8	–99	0	–32	8	–99	0.98^{b}	–98
14. '2015-9-2'	15.12	–5	188	–88	19	–74	210	–87	16.05^{b}	–74
15. '2014-14-2'	26.15	–39	1075	–31	38	–55	1113	–32	28.66^{b}	–54
16. '2015-19-1'	20.93	–27	347	–77	32	–62	378	–77	39.54^{b}	–37
17. '2013-28-5'	34.08	–11	1015	–35	67	–21	1082	–34	35.45^{b}	–43
18. 'FS1-1'	32.02	5	1037	–34	38	–55	1075	–35	32.57^{b}	–48
19. 'Khumo'	30.75	–39	608	–61	53	–37	662	–60	25.66^{b}	–59

[x]Relative impact (RI) (%) = [(treatment/control) – 1] x 100.

[y]RP = eggs + J2/g root and [z]′Beauregard′ served as a susceptible standard.

Table 3.4 Responses for fresh root mass (FRM), eggs, second-stage juveniles (J2), total nematodes (PF), reproductive potential (RP) and relative impact (RI) of *Meloidogyne enterolobii* on ARC sweet potato lines at 56 days after inoculation

EXPERIMENT 1

Lines	FRM (g)	[x]RI (%)	Eggs	[x]RI (%)	J2	[x]RI (%)	PF	[x]RI (%)	[y]RP	[x]RI (%)
1.[z]'Beauregard'	2.997	–	1190	–	173.33	–	130.00	–	455.13[a]	–
2. '2014-7-3'	38.990	18	100.00	–91	30.000	–82	180.00	38	3.8217[b]	–99
3. 'FS10-8'	80.405	39	143.33	–88	36.667	–79	160.00	23	2.2517[b]	–99
4. '2013-26-5'	48.810	23	123.33	–89	36.667	–79	123.33	–5	3.1917[b]	–99
5. '2014-17-6'	31.547	15	96.667	–92	26.667	–84	310.00	38	6.5533[b]	–98
6. '2014-13-1'	27.165	13	256.67	–79	53.333	–69	783.33	52	29.948[b]	–93
7. '2014-14-5'	15.780	7	690.00	–43	93.333	–46	183.33	19	65.213[b]	–85
8. '2015-7-1'	81.418	40	150.00	–87	33.333	–80	26.667	–80	2.3367[b]	–99
9. '2015-1-6'	27.517	13	20.000	–98	6.6667	–96	470.00	26	0.8950[b]	–99
10. 'FS5-2'	27.862	13	443.33	–63	26.667	–84	163.33	25	10.667[b]	–97
11. '2015-2-1'	24.238	11	133.33	–89	30.000	–82	143.3	10	6.7367[b]	–98
12. 'FS10-21'	44.202	21	1273.3	4	160.00	–7	540.00	58	34.675[b]	–92
13. 'FS10-25'	54.605	26	463.33	–62	76.667	–56	1429.0	99	14.548[b]	–96
14. '2015-9-2'	23.868	11	1266.7	3	163.33	–5	370.00	34	92.079[b]	–79
15. '2014-14-2'	31.463	16	293.33	–75	76.667	–56	163.3	–51	16.600[b]	–96
16. '2015-19-1'	29.955	14	1436.7	17	196.67	13	340.00	–73	56.537[b]	–87
17. '2013-28-5'	32.213	15	290.00	–76	50.000	–71	776.67	–26	10.337[b]	–97
18. 'FS1-1'	34.252	16	673.33	–44	103.33	–40	496.67	–41	41.140[b]	–90
19. 'Khumo'	29.732	14	400.00	–76	96.667	–44	1393.3	–84	13.460[b]	–97

EXPERIMENT 2

Lines	FRM (g)	[x]RI (%)	Eggs	[x]RI (%)	J2	[x]RI (%)	PF	[x]RI (%)	[y]RP	[x]RI (%)
1.[z]'Beauregard'	18.697	–	193.33	–	2480.0	–	2703.3	–	859.93[a]	–
2. '2014-7-3'	69.075	28	20.00	–91	76.7	–96	96.7	–96	1.36[b]	–99
3. 'FS10-8'	84.807	36	100.00	–57	596.7	–75	696.7	–74	9.13[b]	–98
4. '2013-26-5'	40.000	12	20.00	–91	50.0	–97	70.0	–97	1.68[b]	–99
5. '2014-17-6'	33.255	83	40.00	–82	113.3	–95	153.3	–94	4.49[b]	–99
6. '2014-13-1'	55.553	25	70.00	–69	260.0	–89	330.0	–87	6.81[b]	–99
7. '2014-14-5'	39.862	11	23.33	–90	170.0	–93	193.3	–92	5.69[b]	–99
8. '2015-7-1'	74.127	31	36.67	–84	143.3	–94	180.0	–93	3.75[b]	–99
9. '2015-1-6'	52.395	18	66.67	–71	263.3	–89	330.0	–87	9.59[b]	–98
10. 'FS5-2'	48.218	16	63.33	–72	190.0	–92	253.3	–90	5.97[b]	–99
11. '2015-2-1'	29.178	61	326.67	39	156.7	–93	483.3	–82	16.40[b]	–98
12. 'FS10-21'	52.698	18	80.00	–65	276.7	–88	356.7	–86	6.74[b]	–99
13. 'FS10-25'	77.467	32	116.67	–50	656.7	–73	773.3	–71	10.78[b]	–98
14. '2015-9-2'	24.572	63	53.33	–77	163.3	–93	216.7	–90	8.28[b]	–99
15. '2014-14-2'	51.737	18	86.67	–63	356.7	–85	443.3	–83	9.86[b]	–98
16. '2015-19-1'	58.085	19	180.00	–19	689.7	–72	866.7	–67	21.57[b]	–97
17. '2013-28-5'	44.268	14	66.67	–71	136.7	–94	203.3	–92	4.66[b]	–99
18. 'FS1-1'	57.492	21	106.67	–54	736.7	–70	843.3	–68	13.75[b]	–98
19. 'Khumo'	55.797	25	106.67	–54	400.0	–83	506.7	–81	9.42[b]	–98

[x]Relative impact (RI) (%) = [(treatment/control) – 1] x 100.

[y]RP = eggs + J2/g root and [z]'Beauregard' served as a susceptible standard.

3.3.2 Relative susceptibility of three *Meloidogyne* species

***Meloidogyne incognita* trials**: The rs_a values in Experiment 1 and Experiment 2 were below unity (Table 3.5).

***Meloidogyne javanica* trials**: In Experiment 1, rs_a values of 14 sweet potato lines were below unity. In contrast, four lines, namely, '2014-13-1', 'FS10-21', 'FS10-25' and '2015-9-2' had rs_a values 1.08, 1.14, 3.81 and 2.3, respectively (Table 3.5). However, in Experiment 2, all lines expressed rs_a values less than one (Table 3.5).

***Meloidogyne enterolobii* trials:** In Experiments 1 and Experiment 2, two sweet potato lines, namely, '2014-13-1' and '2015-9-2' and six lines, namely, '2013-26-5', '2014-13-1', 'FS5-2', '2014-14-2', '2013-28-5' and 'FS1-1' in respective of each Experiment had rs_a values greater than unity (Table 3.5). However, other test lines had rs_a values below unity in both experiments (Table 3.5).

Table 3.5 Final population densities (Pf) and relative susceptibility (rs_a) of ARC sweet potato lines inoculated with 250 eggs and second stage juveniles (J2) of *Meloidogyne incognita, Meloidogyne javanica* and *Meloidogyne enterolobii* at 56 days after inoculation.

	M. incognita				*M. javanica*				*M. enterolobii*			
	Experiment 1		**Experiment 2**		**Experiment 1**		**Experiment 2**		**Experiment 1**		**Experiment 2**	
Line	Pf (eggs)	rs_a	Pf (eggs)	rs_a	Pf (eggs)	rs_a	Pf (eggs)	rs_a	Pf (eggs)	rs_a	Pf (eggs)	rs_a
'2014-7-3'	500	0.21	1380	0.10	300	0.42	290	0.03	780	0.05	4810	0.67
'FS10-8'	80	0.03	700	0.05	60	0.08	420	0.05	1080	0.07	460	0.06
'2013-26-5'	60	0.03	50	0.00	20	0.03	8680	0.92	1540	0.11	7350	1.20
'2014-17-6'	190	0.10	1270	0.09	20	0.03	1630	0.17	968	0.07	765	0.01
'2014-13-1'	240	0.10	160	0.01	780	1.08	660	0.07	14860	1.08	8650	1.20
'2014-14-5'	1020	0.42	770	0.06	200	0.28	930	0.09	4698	0.34	965	0.13
'2015-7-1'	240	0.10	330	0.02	160	0.19	310	0.03	1090	0.13	900	0.12
'2015-1-6'	80	0.03	520	0.04	80	0.11	3310	0.33	669	0.07	2640	0.36
'FS5-2'	80	0.03	120	0.01	60	0.08	2600	0.28	470	0.34	7347	1.01
'2015-2-1'	40	0.02	70	0.01	80	0.11	2990	0.32	2820	0.03	3586	0.49
'FS10-21'	260	0.11	300	0.02	820	1.14	1650	0.17	1433	0.02	2136	0.29
'FS10-25'	1000	0.41	870	0.06	2740	3.81	50	0.01	3240	0.38	6184	0.85
'2015-9-2'	900	0.37	360	0.03	1660	2.31	1130	0.12	14308	1.03	1080	0.15
'2014-14-2'	1460	0.60	100	0.01	200	0.28	6450	0.68	869	0.27	8987	1.24
'2015-19-1'	260	0.11	580	0.04	420	0.58	2080	0.19	3635	0.26	6928	0.96
'2013-28-5'	160	0.07	1180	0.09	40	0.06	6090	0.64	2040	0.24	8438	1.17
'FS1-1'	20	0.01	620	0.05	40	0.06	6190	0.66	1776	0.14	9058	1.26
'Khumo'	0	0.00	50	0.00	190	0.31	3650	0.39	496	0.03	4047	0.56
′Beauregard′	2440	–	13830	–	720	–	9430	–	13693	–	7214	–

rs_a= Pf test plant/ Pf susceptible standard (′Beauregard′)

3.4 Discussion

3.4.1 Reproductive potential of three *Meloidogyne* species

According to Seinhorst (1965), nematode resistance is interpreted using host-status and host-sensitivity, through three host concepts, namely, susceptible, tolerant and resistant-hosts. The host-status, which is a measure of a nematode's potential to reproduce in a host plant, is described using the reproductive potential [RP = eggs + J2/g root] (Mashela *et al.,* 2016). According to Seinhorst (1967), RP serves as an indicator of whether a plant is a host or non-host for the test nematode. In the current study, one inoculation level of 250 eggs + J2 for all the experiments was used and RP values ranged from less than unity to greater than unity. The inocula were viable for all *Meloidogyne* species, based on the highest RP values for the control cv. ′Beauregard′ in all three experiments, this confirms previous observations where cv. ′Beauregard′ was the most susceptible among other sweet potato cultivars to the test *Meloidogyne* species (Cervantes-Flores *et al*., 2002). The test nematode failed to establish feeding sites and reproduce on the test plants, according to the RP values below unity, while RP values above unity indicated that the nematodes established feeding sites and reproduced on the test plants (Mashela *et al.,* 2016).

In plant nematology, the host-status of a plant may also be indicated by root galls through visual diagnosis of the root-knot, this can be achieved by looking at the galls, but only molecular methods can be used in a diagnostics laboratory to determine the species. The level of root galling depends on the host's susceptibility, the nematodes population density and the aggressiveness of the specific nematode that is responsible for the root gall (Quesada-Ocampo, 2018). In the current study, some sweet potato lines had no appearance of root galls, but some had limited root galls as time towards harvest progressed. However, most test lines supported the development and reproduction of *Meloidogyne* species, toward harvest time, but without the formation of noticeable root galls. According to Fourie *et al*. (2015), the absence of root galls does not rule out the possibility of nematode reproduction, juvenile hatch and development.

For all three *Meloidogyne* species, namely, *M. incognita*, *M. javanica* and *M. enterolobii*, treatment effects had significant and highly significant effects on RP variables, with high percentage contributions to TTV in all two experiments, with the exception of no significant effects on RP for *M. javanica* only in Experiment 1. This had similar observations with previous results from a screening study of sweet potato genotypes against *M. enterolobii* (Schwarz *et al*., 2021), which showed that the genotypes had significant and highly significant effects on the RP variables. Due to RP values below unity to the three test *Meloidogyne* species, the results have demonstrated some existence of non-host status among the 18 ARC sweet potato lines. However, in the current study, *M. enterolobii* is suggested to have infested and reproduced on almost all the test sweet potato lines based on their RP values above unity, except for sweet potato line '2015-1-6' in Experiment 1 with RP value below unity. The latter confirms that *M. enterolobii* is the highly virulent *Meloidogyne* species when compared to all the test *Meloidogyne* species (Rutter *et al.,* 2021). Furthermore, the host-status inconsistency of most sweet potato lines may be due to different seasons as Experiment 1 and 2 were conducted during spring and autumn, respectively. The result supported the view that inconsistent results in biological entities like nematodes might be due to plant genotype and environmental interactions

(Phillips *et al*., 1979). Similar results were observed on a screening study of 91 sweet potato genotypes against *M. enterolobii*, where 19 out of 91 sweet potato genotypes were non-hosts, with majority of the sweet potato genotypes reported susceptible (Schwarz *et al*., 2021). Consequently, no line was found to be a non-host to all three test *Meloidogyne* species. In contrast, sweet potato cultivars 'Bosbok', 'Mvuvhelo' and 'Bophelo' were previously shown to be non-host to all thermophilic *Meloidogyne* species (Pofu *et al*., 2016). Another study (Carmona *et al*., 2020), showed that sweet potato genotypes, namely, CNPH 1200, CNPH 1219, CNPH 129, CNPH 1392, CNPH 60 and 'Coquinho' were non-host to three thermophilic *Meloidogyne* species (Carmona *et al*., 2020). Therefore, lines with non-host status in the current study should join sweet potato line '1990-10-2' which was identified as non-host *to M. incognita*, along with cv. 'W-119.' as non-host to *M. incognita* race 4 and cultivars 'Bosbok' and 'Mvuvhelo’ as non-host to *M. incognita* race 2 (Makhwedzhana, 2018; Pofu *et al*., 2016). Moreover, it was reported that sweet potato cultivars 'Excel' and 'Hernandez' were resistant to the test *M. incognita* (race 3), *M. arenaria* (race 2) and *M. javanica* species (Cervantes-Flores *et al*., 2002). Another host-status study reported cv. ′Blesbok' as tolerant to the test *M. javanica* (Makhado, 2020)*.* In the current study, none of the lines had a consistent non-host status against the three test *Meloidogyne* species in both experiments. However, the results in the current study illustrated that sweet potato might contain plant genes that interact with gene products in test *Meloidogyne species*, to produce various degrees of nematode resistance (Mashela *et al.*, 2016).

Clearly, the host status of sweet potato root-knot nematode depends on the sweet potato genotype, and *Meloidogyne* population densities (Cervantes-Flores *et al*.,

2002) and the environmental conditions. Since mixed populations of *Meloidogyne* species often co-exist, a nematode-resistant cultivar should therefore be resistant to all *Meloidogyne* species and races in a specific region (Pofu *et al*., 2016). Although screening is an effective method for host status, it is insufficient for determining host sensitivity. Thus, it is crucial to discover the degree of nematode resistance and mechanism of nematode resistance involved (Mashela *et al*., 2015). Therefore, it is necessary to determine the degree of nematode resistance together with nematode resistance mechanisms for lines that were identified during screening as non-hosts. The latter states that, due to the lack of knowledge on nematode resistance mechanisms, the results of this study would not be useful for breeding purposes. The adoption of nematode resistance plants in crop rotation systems is dependent on the type of nematode resistance mechanism involved. According to Mashela and Pofu (2016), plants have two mechanisms of nematode resistance, namely, pre-infectional and post-infectional nematode resistance mechanisms. Second-stage juveniles (J2) are not allowed to penetrate the root systems and are repelled back to the soil in pre-infectional nematode resistance, however, in post-infectional nematode resistance, J2 enter the root system but are unable to move through the root system or establish feeding sites, preventing them from maturing and reproducing (Shigueoka *et al*., 2019). Between the two mechanisms, only post-infectional nematode resistance can be used in plant breeding for introgression of resistant plant genes in susceptible cultivars (Thurau *et al*., 2010).

3.4.2 Relative susceptibility of three *Meloidogyne* species

The relative susceptibility (rs_a) is one of the approaches used in plant-nematology to evaluate a plant's resistance and susceptibility to a test nematode, relative susceptibility is the ratios between the maximum rates of reproduction (*a*), comparing the initial nematode population densities (Pi) and final nematode population density (Pf) at the end of the growing season of the test plants to the standard susceptible control cultivar (Seinhorst *et al*., 1995). The Pf/Pi, when less than one indicates that a plant is a non-host, when greater than one it is a host (Kort *et al*., 1977). The relative susceptibilities are said to be independent of the initial nematode population density where Pf increases with Pi and *vice versa*. However, some studies re-confirmed that the reproduction rate of the nematode could be affected by the initial nematode population density (Phillips, 1984; Seinhorst *et al*., 1995). In the current study, the same level of inoculation Pi (250 eggs and J2) was used to determine the host-status of the 18 ARC sweet potato lines against three *Meloidogyne* species, as a result, the differences between a rise in Pi and Pf were hindered. The susceptibility and resistance of sweet potato lines in the current study were explored using rs_a in comparison to the highly susceptible standard control cv. 'Beauregard'. Therefore, high Pf levels in all three experiments to the highly susceptible cv. 'Beauregard' demonstrated that the Pi used was viable and infective.

In the current study, the results demonstrated that three test *Meloidogyne* species failed to reproduce on majority of sweet potato lines and were regarded as resistant compared to the susceptible cv. 'Beauregard' based on their rs_a values below unity. However, some sweet potato lines’ relative susceptibility values were inconsistent as some were resistant, susceptible and highly susceptible to the three *Meloidogyne* species in all experiments. As a result, the inconsistency of rs_a values across all experiments may be due to different seasons of the experiments, as Experiment 1 was conducted in spring and Experiment 2 in autumn. This study draws the conclusion that Pf/Pi values are too sensitive to change in environmental conditions as this had been observed and supported by previous published results (Phillips, 1984). Phillips *et al*. (1979) on the other hand, discovered that plant genotype and environmental interactions are the cause of the variations between experiments. According to Seinhorst *et al*. (1995), the reduction in the size and quality of the root system of the plants due to high competition of resources by the nematodes and its impact on final nematode densities may vary across experiments and between different cultivars. This observation is related to a previous study where the relative susceptibility of 25 soybean cultivars against reniform nematode (*Rotylenchulus reniformis* Robinson. 1997*)* varied depending on the level of inoculation, 10 cultivars were found to be resistant, 11 susceptible and 4 highly susceptible to the test nematode (Gupta and Jain, 2009). The findings of the current study are however in contrast with few rs_a study findings, where a previous study showed that all test wheat cultivars were susceptible to the test cereal cyst nematode (*Heterodera avenae* Wollenweber) with the susceptible control cv. 'Sakha-93' (Korayem and Mohammed, 2019). Another study was conducted on 93 accessions obtained from Department of Agriculture in the United States to identify lines with strong resistance against two *M. enterolobii* isolates, namely, SC.1 and NC.1, however, 19 out of 93 accessions, namely, Cordner, Hayman White, Carver, Southern Delight, Travis, Tanzania, W-353, Regal, White Bunch, Georgia Red, Heart gold, Pelican Processo, Sc-1149-19, Resisto, Porto Rico, Red Reristo, Apache Topaz and La 11-94 displayed statistically consistent high levels of resistance against the two *M. enterolobii* isolates (Rutter, 2021). Additionally, another study was carried out to investigate the relative susceptibility of 5 fodder radish varieties, namely, 'Anaconda', 'Defender', 'Contra', 'Terranova' and 'Doublet', compared to the susceptible standard cv. ′Radical' against *M. chitwoodi,* with all five suggesting the existence of nematode resistance since rs_a values were below one (Teklu *et al*., 2014). Moreover, investigations were conducted on a new orange flesh sweet potato cultivar ′CIP BRS Nuti' (CIP 106902.1) crossed between the elite orange flesh sweet potato (OFSP) clone (CIP101048.1) and the advanced OFSP clone (CIP194583.2), to establish its relative susceptibility against *M. incognita*, *M. javanica* and *M. enterolobii* in comparison with the susceptible cv. ′Beauregard' and the results indicated resistance to all the three *Meloidogyne* species (Mello *et al*., 2019). The latter suggests, the difference between the studies could be environmental conditions, as all studies were conducted at different site locations, the harvesting time, for instance, the roots of the studies in Brazil were harvested 157 days after inoculation, while in the current study roots were harvested at 56 days after inoculation and plant genotype and environmental interactions (Phillips,1984; Phillips *et al*.,1979).

Even though genetic plant resistance is an essential part to manage high nematode population densities, there are few conducted and published results on the relative susceptibility and resistance of sweet potato cultivars to *Meloidogyne* species (Pinto *et al*., 2023). However, almost all the test lines of sweet potato were highly susceptible to *M. enterolobii* including those cultivars that have been reported resistant to other test *Meloidogyne* species (Schwarz *et al*., 2021). The results of this study are however not beneficial in plant breeding since the mechanism of resistance in the resistant sweet potato lines is still unknown, therefore, further investigation is needed to determine whether the mechanism of resistance involved is pre-infectional or post-infectional. Only sweet potato lines with post-infectional nematode resistance could be used for plant-introgression as this mechanism only activates its resistance after allowing the nematodes to enter the root system then fails their development, resulting in lower nematode population densities in the soil (Kaplan and Davis, 1987).

3.5 Conclusion

Both indicators for non-host were below unity. Therefore, the null hypothesis which suggested that RP values of *M. incognita, M. javanica* and *M. enterolobii* on 18 ARC sweet potato lines along with their associated relative susceptibilities to cv. ′Beauregard' would separately not be below unity was therefore rejected.

CHAPTER 4

MECHANISM OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* AND *MELOIDOGYNE JAVANICA* IN TWO NON-HOST SWEET POTATO LINES

4.1 Introduction

Nematodes attack thousands of plant species; however, some plants harbour resistant genes referred to as plant genes (Williamson and Kumar, 2006). In contrast, certain nematodes have corresponding resistant genes referred to as gene products (Mashela *et al*., 2016). Plant genes and gene products interact to confer either non-host or host status. In plant nematodes, non-host status could be pre-infectional or post-infectional. Pre-infectional nematode resistance mechanisms prevent nematode second-stage juveniles (J2) from penetrating the root systems, thus, leaving a large number of residual nematodes in the soil for the next crop (Chiuta, 2021). In contrast, post-infectional nematode resistance allows J2 to penetrate the root system, with gene products in nematodes interacting with plant genes in plants to confer non-host status, with most nematodes trapped to death inside the root systems. Consequently, such plants are suitable to manage high nematode population densities as opposed to those that contain pre-infectional nematode resistance. Roots of infected plants where post-infectional nematode resistance mechanism have manifested depict clearly defined descriptive symptoms (Makhwedzhana *et al*., 2018; Ramatsitsi, 2017) Thus, the effective mechanism of nematode resistance in crop rotation systems since such crops leave limited population densities in the soil to serve as inoculum for the crops that follow the non-host (Kaplan and Davis, 1987). Additionally, only plant genes with post-infectional nematode resistance can be transferred from one plant to another through introgression in plant breeding (Thurau *et al.*, 2010). Hence, it is important to establish the mechanism of nematode resistance involved in any nematode resistant plant before it could be considered for its practical utility.

The results of the host-status and relative susceptibility tests suggested that two sweet potato lines '2015-2-1' and 'Khumo' were non-host and resistant to *Meloidogyne incognita* and *M. javanica* (Chapter 3). However, the mechanism of nematode resistance in the two sweet potato lines had not been documented. The objective of the study was, therefore, to investigate whether sweet potato lines '2015-2-1' and 'Khumo' would depict post-infectional nematode resistance mechanism characteristics to *M. incognita* and *M. javanica*. Thus, the null hypothesis suggested that sweet potato lines '2015-2-1' and 'Khumo' would not depict post-infectional nematode resistance mechanism characteristics to *M. incognita* and *M. javanica*.

4.2 Materials and methods

4.2.1 Description of the study site

The study was carried out under greenhouse conditions at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53′10″S. 29°44′15″E), with the structure being as described previously (Chapter 3). The mechanism of resistance test was conducted during summer (November-December) 2023. The average daytime and nighttime temperatures were 28°C and 21°C, respectively, with thermostatically activated fans used to control the maximum temperatures.

4.2.2 Treatments and research design

Treatments were arranged in a randomised complete block design (RCBD) with two experiments for sweet potato lines '2015-2-1' and 'Khumo', each separately inoculated with *M. incognita* and *M. javanica*, replicated four times with 15 harvest times (Figure 4.1).

Figure 4.1 Mechanisms of resistance studies of sweet potato line '2015-2-1' inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions.

4.2.3 Procedures

The sterilised cuttings of two resistant sweet potato lines '2015-2-1' and 'Khumo' were set in seedling trays containing pasteurised (300 ºC for one hour) fine sand and raised for six weeks. Established uniform cuttings were then transplanted into 250 ml polystyrene cups each containing 200 ml pasteurised sand. Cups were placed on greenhouse benches at 40-cm × 10-cm inter and intra row spacing. Nematodes and sweet potato cuttings were obtained from Green Biotechnologies Research Centre of Excellence (GBRCE) and the Agricultural Research Council-Vegetable, Industrial and Medicinal Plants (ARC-VIMP), respectively. Isolates of *M. incognita* and *M. javanica* were each previously raised on nematode-susceptible tomato cv. 'Floradade′ seedlings and roots collected for egg mass. Uniform egg masses were hand-picked using a toothpick and put in tapwater in Petri dish for over 72 hours to allow for the development of all eggs to J2 and then juvenile hatch (Powers *et al*., 1992). Each sweet potato line was infested with *M. incognita* J2 or *M. javanica* J2 in separate trials, starting from a day after transplanting by dispensing approximately 100 J2 of each species into 5-cm-deep holes on the cardinal points of the stem of each cutting for using a 20-ml plastic syringe (Figure 4.2), with holes thereafter covered with the growing medium. Every other day, 40-ml tapwater was used to irrigate each line. Prior to inoculation each cutting was fertilised by applying approximately 5 g NPK 2:3:2 (26) + 5% S, + 5% Ca + 0.5% Zn was dissolved in 5 -L tapwater and substitute irrigation

Figure 4.2 Resistant sweet potato line 'Khumo' cuttings inoculated with *Meloidogyne incognita* a day after transplanting under greenhouse conditions.

4.2.4 Data collection

During each harvest, four sweet potato cuttings were harvested every other day for a period of thirty days, shoots were separated from roots and the roots rinsed in tapwater to remove growing medium particles, with excess water removed using a laboratory paper towel and stained in 3.5% acid fuchsin (Byrd *et al*., 1983). Briefly, total roots were soaked in 1.5% NaOCl solution for four minutes to remove any associated microbe (Figure 4.3), followed by a 15 minute immersion in tapwater to remove excess NaOCl. The roots sample were stained in 30 ml tapwater mixed with 1 ml acid fuchsin (Figure 4.4), which was then heated to boiling point in a microwave for 1 minute and then cooled to room temperature. Excess stains were 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 (5 N HCL) and boiled for 30 s and then cooled to room temperature (Figure 4.5). Total distained root system was placed in a 9 cm Petri dish and assessed under a microscope at 45 × magnification for necrotic spots, giant cell number, rootlet interference and root gall number (Makhwedzhana, 2018; Ramatsitsi, 2017).

Figure 4.3 Harvest 3 root samples of resistant sweet potato lines '2015-2-1' and 'Khumo' each infected with *Meloidogyne incognita* or *Meloidogyne javanica* soaked in 1.5% NaOCl solution for four minutes.

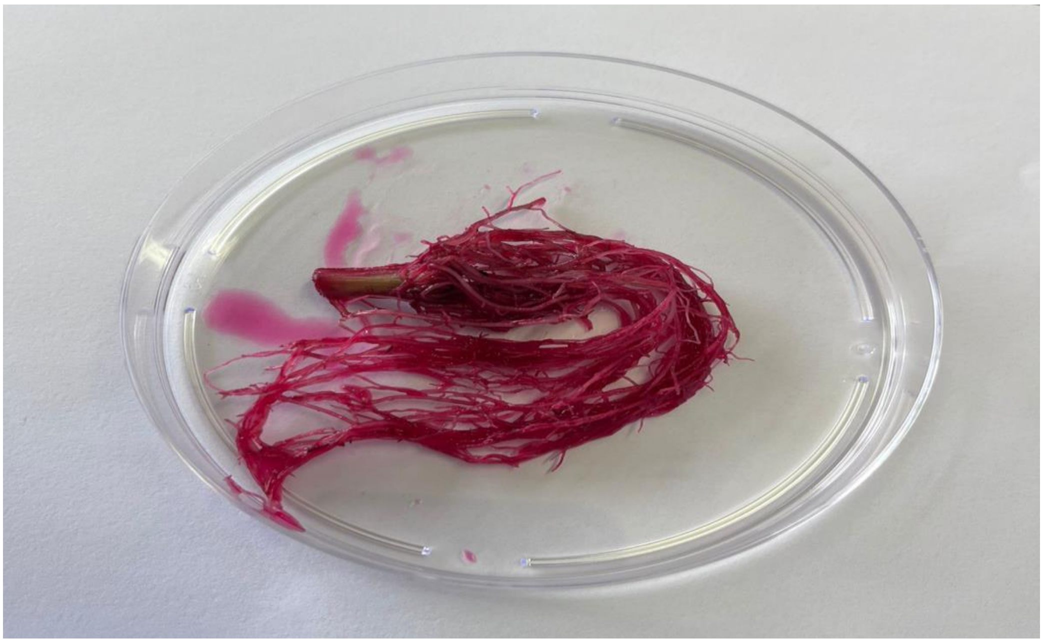

Figure 4.4 Harvest 15 *Meloidogyne javanica* infected root sample of resistant sweet potato line 'Khumo' stained with acid fuchsin.

Figure 4.5 Harvest 15 distained root sample of resistant sweet potato line 'Khumo' infected with *Meloidogyne javanica*.

4.2.5 Data analysis

Data were subjected to analysis of variance using SAS software (Chapter 3), with mean separation achieved using Duncan multiple range test. Unless otherwise stated, results were discussed at the probability level of 5%.

4.3 Results

4.3.1 Sweet potato line '2015-2-1' to *Meloidogyne incognita* relations

In sweet potato line '2015-2-1' harvest time (days) had highly significant effects on necrotic spots, giant cell number and rootlet interference (Table 4.2). The treatments contributed 69, 74 and 50% 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 30 (Table 4.2). Necrotic spots, giant cell and rootlet interference over sampling period each exhibited positive quadratic relations, with the model having 96, 98 and 97% associations, respectively (Figure 4.6). Using the relation $x = -b1/2b_2$ (Gomez and Gomez, 1984), the optimum necrotic spots, giant cells and rootlet interference were attained at Days 19, 18 and 27 after inoculation, respectively (Table 4.5).

4.3.2 Sweet potato line '2015-2-1' to *Meloidogyne javanica* relations

In sweet potato line '2015-2-1', periodic harvest time (days) had highly significant effects on necrotic spots, giant cell number and rootlet interference, respectively (Table 4.2). The treatments contributed 75, 58 and 50% in TTV for necrotic spots, 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 Day 4 to Day 30 (Table 4.2). Necrotic spots, giant cells and rootlet interference over sampling period each exhibited positive quadratic relations, with the models having 98, 98 and 97% associations, respectively (Figure 4.7). Using the relation $x = -b1/2b_2$, the optimum necrotic spots, giant cells and rootlet interference were attained at Day 19, 16 and 53 after inoculation, respectively (Table 4.5).

4.3.3 Sweet potato line 'Khumo' to *Meloidogyne incognita* relations

In sweet potato line 'Khumo', harvest time (days) had highly significant on necrotic spots and giant cell number, except for non-significant effects on rootlet interference (Table 4.4). The treatments contributed 67% in TTV for both necrotic spots and giant cells number and 50% in TTV for rootlet interference (Table 4.3). 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 30 (Table 4.4). Necrotic spots, giant cell and rootlet interference over sampling period each exhibited positive quadratic relations, with the model having 96, 98 and 85% associations, respectively (Figure 4.8). Using the relation $x = -b1/2b_2$, the optimum necrotic spots, giant cells and rootlet interference were attained respectively at Day 19, 16 and 48 after inoculation (Table 4.6).

4.3.4 Sweet potato line 'Khumo' to *Meloidogyne javanica* relations

In sweet potato line 'Khumo', periodic harvest time (days) had highly significant effects on necrotic spot, giant cell number and rootlet interference, respectively (Table 4.4). The treatments contributed 75, 93 and 54% in TTV for necrotic spots, giant cell number and rootlet interference, respectively (Table 4.3). Also, starting from Day 2 after inoculation, only necrotic spots were noticeable on the first harvest, whereas giant cell number and rootlet interference 50 were noticeable from Days 4 to 30 (Table 4.4) Also, necrotic spots, giant cells and rootlet interference over sampling period each exhibited positive quadratic relations, with the models having 86, 98 and 99% associations, respectively (Figure 4.9). Using the relation $x = -b1/2b_2$, the optimum necrotic spots, giant cells and rootlet interference were attained respectively at Day 17, 16 and 45 after inoculation (Table 4.6).

4.3.5 Root galls in the two resistant sweet potato lines

In the current study, root galls were not observed on roots of the two resistant sweet potato lines '2015-2-1' and 'Khumo' infected with *M. incognita* and *M. javanica* even at day 30 after inoculation.

4.3.6 Nematode juveniles in the two resistant sweet potato lines

There were no nematode juveniles detected inside the roots of the two sweet potato lines '2015-2-1' and 'Khumo' at various developmental stages even at day 30 after inoculation of *M. incognita* and *M. javanica* in all experiments, even under higher magnification (× 100), when using oil emersion, nematode juveniles in stained roots were still undetectable.

Table 4.1 Total treatment variation (TTV) on necrotic spot, giant cell number and rootlet interference in resistant sweet potato line '2015-2-1' infected by *Meloidogyne incognita* and *Meloidogyne javanica* 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 (%)
				Meloidogyne incognita			
Rep	3	248.93	16	19.728	2	1640.36	28
Treatment	14	1080.74	69^{***}	694.602	74^{***}	2961.38	50^{***}
Error	42	237.20	15	194.275	24	1267.78	19
Total	59	1566.87	100	941.605	100	5869.52	100
				Meloidogyne incognita			
Rep	3	86.283	11	155.511	23	1779.67	29
Treatment	14	563.293	75^{***}	386.731	58^{***}	3010.78	50^{***}
Error	42	105.617	14	125.118	19	1250.10	21
Total	59	755.193	100	667.36	100	6040.55	100

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

Table 4.2 Mean separation for necrotic spot (NS). giant cell number (GC), rootlet interference (RIN) and the relative impacts (RI) in resistant sweet potato line '2015-2-1' infected by *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 30 days after inoculation (n = 60).

	Meloidogyne incognita						*Meloidogyne javanica*					
Days	Necrotic spot[z]	[y]RI	Giant cell number[z]	[y]RI (%)	Rootlet interference[z]	[y]RI (%)	Necrotic spot[z]	[y]RI (%)	Giant cell number[z]	[y]RI (%)	Rootlet interference[z]	[y]RI (%)
2	19.000^{cd}	–	0.001^{d}	–	0.000^{e}	–	9.750^{bc}	–	0.001^{c}	–	0.000^{c}	–
4	24.005^{cd}	0	4.500^{d}	39	32.750^{cde}	60	17.000^{bc}	88	6.000^{b}	59	17.250^{b}	2166
6	32.250^{bcd}	45	13.500^{bcd}	129	48.250^{bcde}	140	19.000^{abc}	144	13.500^{b}	129	25.250^{b}	3233
8	35.750^{bcd}	59	19.250^{abcd}	219	59.250^{abcde}	195	27.250^{abc}	200	17.890^{ab}	169	32.500^{b}	4166
10	40.250^{ab}	81	24.750^{abcd}	239	69.650^{abcd}	245	33.750^{ab}	266	21.750^{ab}	209	39.550^{b}	5100
12	46.290^{ab}	109	29.750^{abc}	289	70.140^{abc}	250	37.000^{ab}	311	23.250^{ab}	199	45.500^{ab}	5900
14	49.000^{abc}	119	29.750^{abc}	289	70.750^{abc}	250	36.500^{ab}	300	25.750^{ab}	249	48.250^{ab}	6300
16	50.000^{ab}	127	30.780^{abc}	299	82.500^{ab}	310	38.500^{ab}	319	25.500^{ab}	249	50.750^{ab}	6566
18	50.560^{a}	127	30.250^{abc}	299	83.250^{ab}	315	38.750^{ab}	319	24.750^{ab}	239	57.750^{ab}	7500
20	50.120^{a}	127	33.250^{a}	329	82.500^{ab}	310	40.000^{a}	344	24.500^{ab}	239	62.500^{ab}	8166
19	52.150^{a}	136	31.750^{a}	309	87.500^{ab}	335	40.250^{a}	344	19.250^{ab}	219	67.500^{ab}	8833
24	49.250^{abc}	119	27.750^{ab}	269	89.500^{ab}	345	38.000^{ab}	319	19.000^{ab}	189	68.500^{ab}	8966
26	45.750^{bc}	104	23.250^{abcd}	199	90.860^{a}	350	36.000^{ab}	300	14.750^{ab}	139	69.500^{ab}	9100
28	38.000^{bcd}	72	19.780^{bcd}	189	95.250^{a}	375	33.500^{ab}	266	12.500^{b}	119	80.250^{ab}	1056
30	35.750^{bcd}	59	12.500^{bcd}	119	94.750^{a}	370	28.900^{abc}	211	1.500^{b}	90	92.000^{a}	1299
P ≤	0.01		0.01		0.02		0.01		0.01		0.01	

[y] Relative impact (RI) (%) = [(treatment/control) – 1] x 100.

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

Table 4.3 Total treatment variation (TTV) on necrotic spot, giant cell number and rootlet interference in resistant sweet potato line 'Khumo' infected by *Meloidogyne incognita* and *Meloidogyne javanica* 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 (%)
				Meloidogyne incognita			
Rep	3	26.444	4	65.972	10	1547.66	20
Treatment	14	418.410	67^{***}	466.243	67^{***}	3876.07	50^{ns}
Error	42	182.397	29	159.151	23	1970.58	30
Total	59	627.251	100	691.366	100	7694.31	100
				Meloidogyne javanica			
Rep	3	143.794	16	2.444	1	6331.87	39
Treatment	14	692.393	75^{***}	492.552	93^{***}	8910.17	54^{***}
Error	42	85.294	9	31.397	6	1128.87	7
Total	59	921.481	100	526.393	100	16370.91	100

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

Table 4.4 Mean separation for necrotic spot (NS), giant cell number (GC), rootlet interference (RIN) and the relative impacts (RI) in resistant sweet potato line 'Khumo' infected by *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 30 days after inoculation (n = 60).

	Meloidogyne incognita						*Meloidogyne javanica*					
Days	Necrotic spot^{z}	RI^{y}	Giant cell number^{z}	RI^{y}	Rootlet interference^{z}	RI^{y}	Necrotic spot^{z}	RI^{y}	Giant cell number^{z}	RI^{y}	Rootlet interference^{z}	RI^{y}
2	14.502^{bcd}	–	0.001^{d}	–	0.000	–	20.250^{abc}	–	0.001^{f}	–	0.000^{d}	–
4	19.000^{bcd}	35	6.258^{cd}	59	40.250	233	19.840^{bc}	10	5.750^{ef}	49	25.500^{c}	150
6	23.750^{bcd}	64	11.879^{ab}	109	34.000	183	24.500^{bc}	20	10.250^{def}	99	29.750^{c}	190
8	24.620^{abc}	71	17.566^{bcd}	169	26.250	116	26.750^{bcd}	30	15.500^{bcde}	149	35.330^{c}	250
10	26.250^{abc}	85	20.547^{abc}	199	29.500	141	26.005^{bcd}	30	17.800^{cde}	169	42.550^{b}	320
12	28.750^{abc}	100	20.235^{abc}	199	29.250	141	28.560^{bcd}	40	21.750^{a}	209	51.750^{bc}	410
14	29.240^{abc}	107	19.325^{a}	219	66.000	450	29.750^{abcd}	45	21.500^{a}	209	56.500^{bc}	460
16	31.890^{cd}	121	21.250^{abc}	209	65.750	441	30.990^{a}	50	21.000^{ab}	209	63.700^{bc}	530
18	33.750^{de}	135	23.358^{a}	199	72.500	500	31.320^{a}	55	19.970^{cde}	189	64.000^{bc}	540
20	35.750^{de}	150	21.250^{abc}	209	77.000	541	32.750^{a}	60	20.750^{abc}	199	67.500^{bc}	570
19	85.250^{a}	507	20.000^{abc}	199	70.250	483	30.140^{a}	50	17.500^{cde}	169	75.000^{bc}	650
24	85.090^{a}	507	16.259^{bcd}	159	73.500	508	29.860^{abcd}	45	12.740^{bcde}	119	78.900^{bc}	680
26	34.270^{cde}	142	11.259^{ab}	109	81.000	575	29.230^{abcd}	45	9.500^{de}	89	86.750^{ab}	760
28	30.530^{abc}	114	6.675^{cd}	59	86.250	616	26.500^{bcd}	30	5.500^{ef}	49	83.500^{ab}	730
30	30.250^{abc}	114	0.507^{d}	400	83.250	591	19.250^{c}	– 5	2.760^{ef}	19	93.250^{a}	830
P ≤	0.02		0.01		0.09		0.01		0.01		0.01	

^{y}Relative impact (RI) (%) = [(treatment/control) – 1] x 100.

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

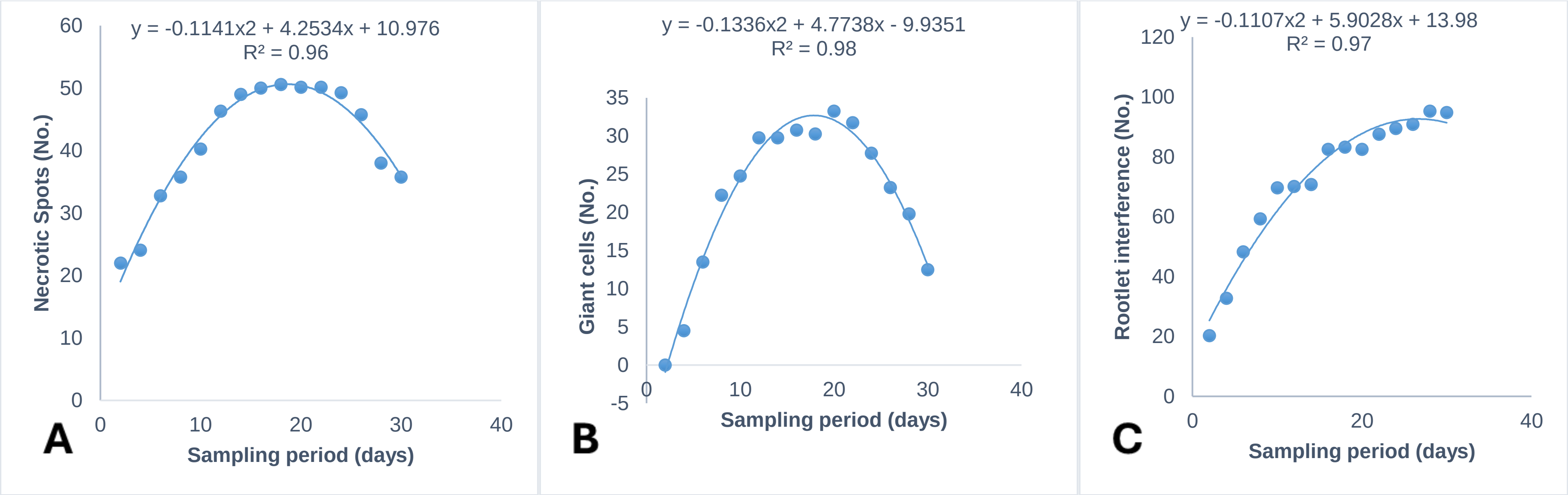

Figure 4.6 Quadratic response of (A) necrotic spots, (B) giant cells and (C) rootlet interference in sweet potato line '2015-2-1' to sampling periods infected with *Meloidogyne incognita* under greenhouse conditions.

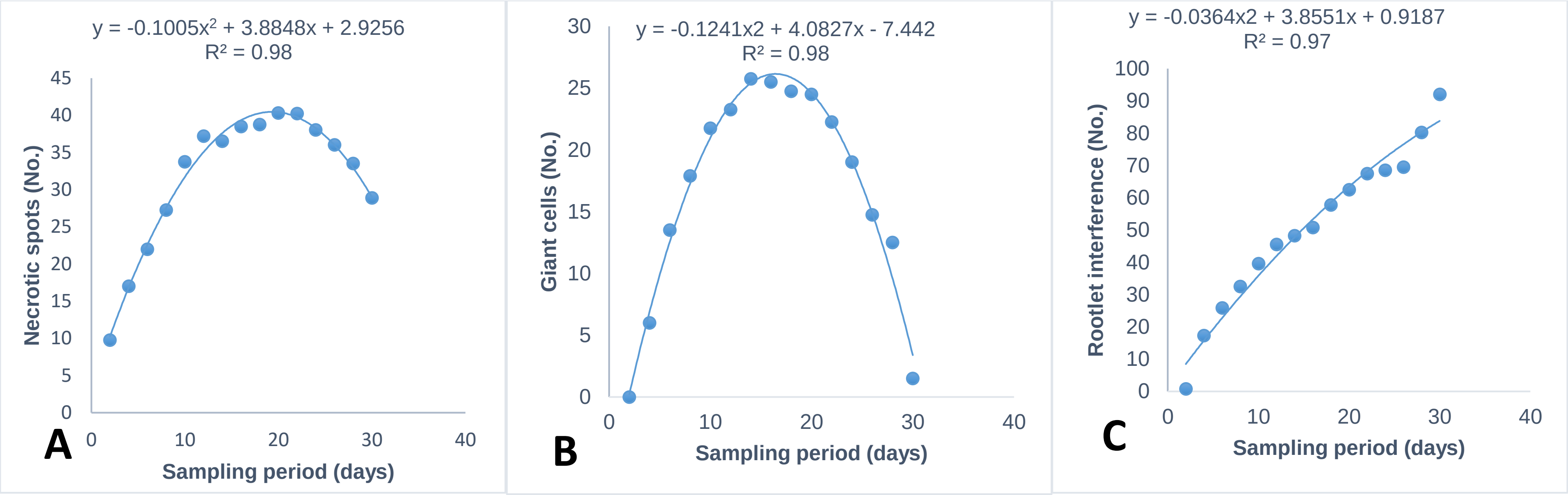

Figure 4.7 Quadratic response of (A) necrotic spots, (B) giant cells and (C) rootlet interference in sweet potato line '2015-2-1' to sampling periods infected with *Meloidogyne javanica* under greenhouse conditions.

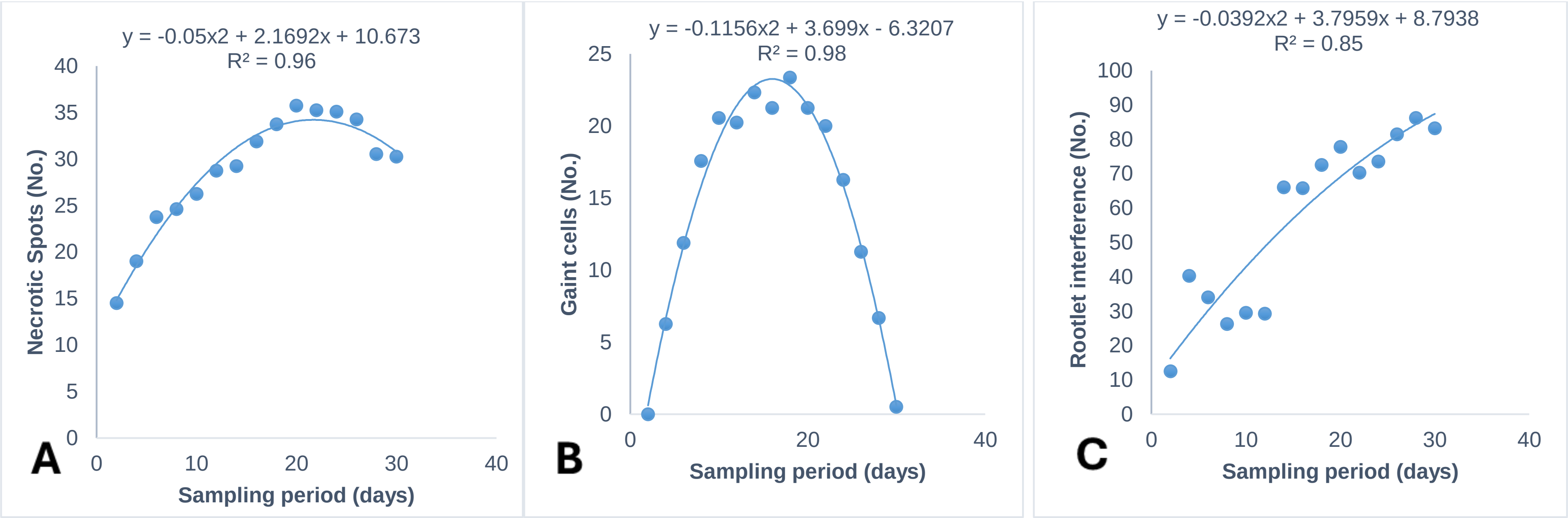

Figure 4.8 Quadratic response of (A) necrotic spots, (B) giant cells and (C) rootlet interference in sweet potato line 'Khumo' to sampling periods infected with *Meloidogyne incognita* under greenhouse conditions.

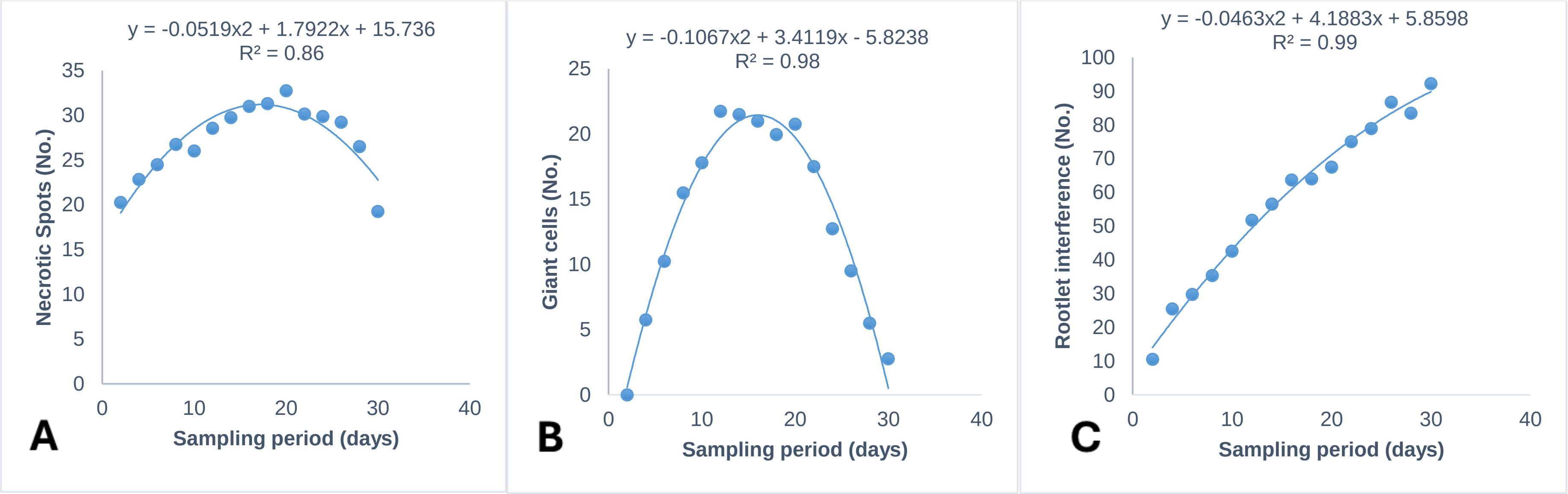

Figure 4.9 Quadratic response of (A) necrotic spots, (B) giant cells and (C) rootlet interference in sweet potato line 'Khumo' to sampling periods infected with *Meloidogyne javanica* under greenhouse conditions.

Table 4.5 Quadratic relationships, coefficient of determination and computed optimum response of sampling period (days) for necrotic spot, giant cell and rootlet interference in sweet potato line '2015-2-1' infected with *Meloidogyne incognita* and *Meloidogyne javanica*.

Variable	Quadratic relation	R^2	(x) Day
	M. incognita		
Necrotic spot	$y = -0.1141x^2 + 4.2534x + 10.976$	0.96	19
Giant cell	$y = -0.1336x^2 + 4.7738x - 9.9351$	0.98	18
Rootlet interference	$y = -0.1107x^2 + 5.9028x + 13.98$	0.97	27
	M. javanica		
Necrotic spot	$y = -0.1005x^2 + 3.8848x + 2.9256$	0.98	19
Giant cell	$y = -0.1241x^2 + 4.0827x - 7.442$	0.98	16
Rootlet interference	$y = -0.0364x^2 + 3.8551x + 0.9187$	0.97	53

$x = -b_1/2b_2$.

Table 4.6 Quadratic relationships, coefficient of determination and computed optimum response of sampling period (days) for necrotic spot, giant cell and rootlet interference in sweet potato 'Khumo' infected with *Meloidogyne incognita* and *Meloidogyne javanica*.

Variable	Quadratic relation	R^2	(x) Day
	M. incognita		
Necrotic spot	$y = -0.05x^2 + 2.1692x + 10.673$	0.96	19
Giant cell	$y = -0.1156x^2 + 3.699x - 6.3207$	0.98	16
Rootlet interference	$y = -0.0392x^2 + 3.7959x + 8.7938$	0.85	48
	M. javanica		
Necrotic spot	$y = -0.0519x^2 + 1.7919x + 15.736$	0.86	17
Giant cell	$y = -0.1067x^2 + 3.4119x - 5.8238$	0.98	16
Rootlet interference	$y = -0.0463x^2 + 4.1883x + 5.8598$	0.99	45

$x = -b_1/2b_2$.

4.4 Discussion

4.4.1 Necrotic spots

Harvest period had highly significant effects on necrotic spots for both sweet potato lines '2015-2-1' and 'Khumo' relations infected with *M. incognita* and *M. javanica*. Necrotic spots were detected at an early stage (Days 2 after inoculation) in the roots of the test sweet potato lines '2015-2-1' and 'Khumo' to *M. incognita* and *M. javanica*. Optimum giant cell formation in the two sweet potato lines '2015-2-1' and 'Khumo' were attained at Days 18 and 21, respectively after infection with *M. incognita*. However, for *M. javanica*, optimum giant cell formation was attained at Days 20 for both sweet potato lines '2015-2-1' and 'Khumo', and thereafter began to drop in all experiments. According to Mashela *et al*. (2016), infection of root-knot nematodes to resistant crops results to an early hypersensitive response (HR), which is a reaction that causes plant cell death, prevents feeding sites, hinders nematode development and consequently nematode death. Makhwedzhana (2018) had similar observations of early HR to root-knot nematode (*M. incognita* race 2) infection to two resistant sweet potato cultivars 'Bosbok' and 'Mvuvhelo', which was denoted by the presence of necrotic spots in roots of the two resistant sweet potato cultivars stained with acid fuchsin at Days 2 after inoculation, which probably resulted in arresting of J2 development. In contrast, for resistant *Cucumis* species, necrotic spots were observed in acid fuchsin stained roots at Days 20 and 26, respectively for *C. africanus-M. incognita* and *C. africanus-M. javanica* relations (Ramatsitsi, 2017), this observation suggested a delayed HR. The latter may be due to high humidity as it has also been reported to delay or suppress HR in some cases, this could be due to delayed dehydration of plant cells and consequently delayed plant cell death (Wang *et al*., 2005).

The existence of necrotic spots in the majority of nematode resistant transgenic plants suggest the presence of phenols that could have potentially been involved in plant defense mechanisms as reviewed by Nicholson and Hammerschmidt (1992). Generally, phenolic compounds act as the first line of defence for plant resistance and often increase during a plethora of stress conditions caused by pathogens and pests' infection (Pratyusha, 2019). The latter is confirmed by the reported observations on toluidine blue stained root cross sections of resistant banana (*Musa*) to burrowing nematode *Radopholus similis* infection compared to non-infected *Musa* root cells three weeks post infection, phenols were the main constituents of the nematode-infected necrotic cells, contrarily, there were no detected phenolic cells and necrotic cells in the roots of non-infected *Musa* (Dhakshinamoorthy *et al*., 2014).

In plant nematology, necrotic spots are viewed as a common feature of HR and suggest the plants to have post-infectional nematode resistance as they ultimately result to nematode death in most cases. According to Saucet *et al*. (2016), plants with post-infectional nematode resistance often form necrotic spots, which are observed as a group of dead cells with thick cell walls under a microscope. Similarly, in the current study, necrotic spots have been observed under a microscope as a group of dead cell walls with thick cell walls in acid fuchsin stained roots of resistant sweet potato lines '2015-2-1' and 'Khumo'. Several other studies have reported the presence of necrotic spots in the roots of different resistant crops infected with *Meloidogyne* species. For example, Cabasan *et al*. (2014) observed localised necrotic spots in 58 nematode-resistant rice genotypes within few days post nematode penetration. Similarly, seven weeks after penetration of *M. incognita* race 1 juveniles, necrotic spots were observed in the roots of 61 resistant carrot lines stained with stained toluidine blue O (Seo *et al*., 2014). Necrotic spots were also observed in Safranin O-stained roots of nematode-resistant wild sweet potato after *M. incognita* J2 penetration, which ultimately resulted in death of the infected nematodes (Komiyama *et al*., 2006). Another study has reported similar results in roots of resistant oats (*Avena sativa*) infested with *M. incognita* at 15 days post penetration, the necrotic spots were observed inside the acid fuchsin stained roots of *A. sativa* which resulted in failure of J2 development and therefore nematode death (Marini *et al*., 2016).

4.4.2 Failure of giant cell development

Harvest period had highly significant effects on undeveloped giant cell number for the two sweet potato lines '2015-2-1' and 'Khumo' to both *M. incognita* and *M. javanica* relations. Giant cells were observed from Day 4 after inoculation for the two sweet potato lines to *M. incognita* and *M. javanica*. Optimum giant cell formation in the sweet potato lines '2015-2-1' and 'Khumo' were attained at Days 20 and 18, respectively after infection with *M. incognita*. However, for *M. javanica*, optimum giant cell formation was attained at Days 10 and 12 respectively for the two sweet potato lines '2015-2-1' and 'Khumo', and thereafter began to drop, indicating a strong resistance to the development of this variable. In the current study, *M. incognita* and *M. javanica* were able to initiate giant cell formation in roots of two resistant sweet potato lines '2015-2-1' and 'Khumo' at early stages in the infection process, this is based on the detection of undeveloped giant cells in roots of the two sweet potato lines at Day 4 post inoculation. However, after the optimum periods, giant cells began to deteriorate and collapse, resulting in failure of feeding sites and consequently J2 development and reproduction. Observations on early detection of giant cells in the current study also confirm the reported observations in resistant roots of two sweet potato cv. 'Bosbok' list iand 'Mvuvhelo' at Day 4 and giant cells deteriorating post optimum periods and therefore resulting into failure of feeding sites, J2 development and reproduction (Makhwedzhana, 2018). Similarly, giant cells were observed at late stages in the roots of resistant *Cucumis* species, *C. africanus* and *C. myriocarpus*, when infected by *M. incognita* and *M. javanica* (Ramatsitsi, 2017). Moreover, giant cells observations in the current study are in contrast to the observation of no giant cell induced nor feeding site established in roots of resistant coffee cv. 'UFV 408-28', inoculated with *M. incognita* race 1, 2 and 3 (Albuquerque *et al*., 2010). The observations could reflect delayed hypersensitive response, which had been widely reported in numerous mechanisms of nematode resistant experiments on plants (Escobar and Fenoll, 2015; Gheysen and Fenoll, 2002; Johansson *et al*., 2015; Künstler *et al*., 2016; Zhang *et al*., 2003).

Giant cells are multinucleate structures that form in nematode-susceptible plant species when the feeding cell and adjacent cells undergo continuing mitosis without cytokinesis in response to sedentary feeding of *Meloidogyne* species. However, failure to develop root galls in nematode resistant crops does not imply that J2 would not develop to maturity (Dutta *et al*., 2023; Ravichandra and Ravichandra, 2014). According to Mashela *et al*. (2016), the transgenic plants generate a variety of plant genes referred to as antigene products, antiplant gene and RNAi in relation to nematode resistance, these plant genes are antagonistic to nematode-produced genes, often known as gene products. Therefore, the formation of giant cells is dependent on the interaction between gene products and plant genes as it dictates whether giant cells are successfully or unsuccessfully formed (Mashela *et al*., 2016). According to Liu *et al*. (2024), giant cells serve as a sole source of nutrients for the development and growth of nematodes. Thus, the secretion of gene products from the dorsal glands in the developing J2 is crucial for the establishment of nematode feeding sites, nematode development and to subsequent reproductive stages (Favery *et al*., 2016; Siddique *et al*., 2014). Failure of giant cells development in the current study as denoted by minute sizes and numbers, suggested that both *M. incognita* and *M. javanica* failed to establish feeding sites on the two test sweet potato lines '2015-2-1' and 'Khumo'. Chiuta (2021) suggested that plants with post-infectional nematode resistance have a common trait, which is failure of giant cell development.

4.4.3 Proliferation of rootlet interference

Periodic harvest intervals also had highly significant effects on rootlet interference for both sweet potato lines '2015-2-1' and 'Khumo' relations infected with *M. incognita* and *M. javanica*, with the exception of non-significant effects on rootlet interference for line 'Khumo'-*M. incognita* relations. Rootlet interferences were observed 4 days after inoculation for both lines '2015-2-1' and 'Khumo' inoculated with *M. incognita* and *M. javanica.* The optimum rootlet interference in lines '2015-2-1' and 'Khumo' were both attained at Days 28 post *M. incognita* infection. However, post *M. javanica* infection, optimum rootlet interference were both attained at Days 30 for the two sweet potato lines '2015-2-1' and 'Khumo'. In the current study, rootlet interference has never declined in all experiments until the last days of harvest, showing a strong resistance to the development of this variable. The observations of the current study supported those observed by Makhwedzhana (2018), where proliferation of rootlet interference were similarly reported from Days 4 and never declined in nematode-resistant sweet potato cv. 'Bosbok' and 'Mvuvhelo' exposed to *M. incognita* race 2. On the other hand, it has been reported that rootlet interference was observed at later stages in resistant *C. africanus-M. incognita* relations from 19 days post infection, *C. africanus-M. javanica* relations from 16 days post infection and *C. myriocarpus-M. javanica* at 24 days post infection (Ramatsitsi, 2017).

The formation of excessive rootlets is a common feature in nematode-resistant crops infested with *Meloidogyne* species, with the phenomenon referred to as lateral root formation, which is the initiation of new roots from parent roots (Jansen *et al*., 2013). Adult nematodes produce gene products via dorsal gland cells that mimic plant genes by producing plant growth regulators during sedentary phases, particularly the auxins and cytokinins, which are known to be vital for the initiation of lateral roots (Mashela *et al*., 2016; Noureddine, 2021). Additionally, plant growth regulator mechanism is viewed as an important process during the initiation and establishment of the feeding sites of sedentary plant-parasitic nematodes (Fukaki and Tasaka, 2009; Mashela *et al*., 2016). In this study, the observation of rootlets formation adjacent to the poorly developed giant cells confirmed findings from previous studies that observed proliferation of rootlet interference after nematode penetration in resistant crops with post-infectional nematode resistance (Das *et al*., 2008; Makhwedzhana, 2018; Ramatsitsi, 2017)

4.4.4 Nematode juveniles

The current study observed the absence of nematode juveniles inside the roots of the two resistant sweet potato lines '2015-2-1' and 'Khumo' infected with *M. incognita* and *M. javanica* even at 30 days after inoculation. The findings in the current study correspond with the findings observed and recorded with no detectable nematode juveniles in roots of resistant sweet potato cultivars ('Bosbok' and 'Mvuvhelo') and resistant *Cucumis* species (*C. africanus* and *C. Myriocarpus*) at 30 days after inoculation with *Meloidogyne* species (Makhwedzhana, 2018; Ramatsitsi, 2017).

In nematode-resistant plants, Hypersensitive response (HR) or localized tissue necrosis occurs at or near the site where feeding would normally be initiated after the nematode enters the plant root, this occurs within two days post-infection, resulting to no development of the feeding site and consequently cell death. Therefore, nematodes fail to establish feeding sites and either leave the roots or die due to starvation (Abd-

Elgawad, 2019). However, in some nematode-resistant plants, plants may secrete pre-formed root metabolites within the rhizosphere that reduce or repel mobility of plant-parasitic nematode and consequently inhibit nematode penetration (Chin *et al*., 2018). The latter states that, this could be the reason for the undetectable juveniles in roots of the two resistant sweet potato lines '2015-2-1' and 'Khumo' in the current study.

4.4.5 Root galls

According to Mashela *et al*. (2016), second-stage juveniles (J2) in nematode-susceptible hosts form root galls when they penetrate roots to create a feeding site, which is known as a giant cell that is usually inside the pericycle and vascular tissues. However, in the current study, the reduced number of giant cells and the absence of root galls on roots of the two sweet potato lines '2015-2-1' and 'Khumo' inoculated with *M. incognita* and *M. javanica* in the 30-days cycle indicate failure of feeding sites and therefore J2 development and reproduction which concludes that nematode infection did not succeed. Similar observations were recorded on roots of two sweet potato cultivars 'Bosbok' and 'Mvuvhelo' inoculated with *M. incognita* race 2 displaying no root galls (Makhwedzhana, 2018). In contrast, small undeveloped roots galls were noticeable on *Cucumis* species (*C. africanus* and *C. Myriocarpus*), on the other hand, developed root galls were also noticeable on *C. africanus* all inoculated with *M. incognita* and *M. javanica* (Ramatsitsi, 2017; Ramatsitsi and Ramachela, 2023). The differences of the latter may be due to different crops used as the current study used sweet potato lines and other studies used *Cucumis* species, so the degree of root galling also depends on the host plant species and cultivar as each crop responds differently to *Meloidogyne* species infection, different environmental conditions also play a role on root-knot nematode infection and therefore root galling, as the current study was conducted in Limpopo and the other study in Northwest (Anwar *et al*., 2007; Bautista-Garfias *et al*., 2019).

4.5 Conclusion

The responses in roots of the two resistant sweet potato lines '2015-2-1' and 'Khumo' to infection by *M. incognita* and *M. javanica* were similar as depicted by (1) presence of necrotic spots, (2) poorly developed giant cell, (3) proliferation of rootlet interference (4) absence of root galls and (5) non detectable juveniles in roots. Based on these observations, the two sweet potato lines were suggested to have the existence of post-infectional nematode resistance against the test *Meloidogyne* species. Therefore, the null hypothesis which suggested that resistant ARC sweet potato lines would not have post-infectional nematode resistance was therefore rejected.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

The two major indicators of nematode resistance without using traditional methods, namely, (a) reproductive potential (RP = Pf/Pi) and (b) relative susceptibility (rs_a $=RP_{unknown}$ /host−$_{RPsusceptible\ host}$) of *Meloidogyne incognita* and *M. javanica* were compared on 18 sweet potato (*Ipomoea batatas* L.) lines, with mechanism of nematode resistance in resistant sweet potato lines '2015-2-1' and 'Khumo'. Results demonstrated that the two indicators for *M. incognita* and *M. javanica* each singled out the two test cultivars as being resistant, with RP and rs_a values for each being greater than 1 on most test cultivars but being less than 1 on sweet potato lines '2015-2-1' and 'Khumo', suggesting that the lines were resistant to the test nematodes. Subsequently, the null hypothesis which suggested that RP and rs_a would illustrate that all test sweet potato lines were each not resistant to the test nematodes, since nematode resistance comprises either pre- or post-nematode resistance mechanism, the two lines were each subjected to test of mechanism of resistance, which involved inoculation with second-stage juveniles (J2), regular harvesting of roots, dyeing of roots and identification of physical structures under the microscope. In each test sweet potato line, the physical responses that depict mechanism of nematode resistance included (1) the presence of necrotic spots on roots, (2) failure of the development of giant cells (3) the proliferation of multiple rootlets (4) the absence of root galls and (5) the non-detectable of J2 in roots. Most of the observations exhibited linear relationship over time. Thus, the two sweet potato lines exhibited post-infectional nematode resistance each against the test *Meloidogyne* species.

5.2 Significance of findings

Findings in the current study demonstrated that reproductive potential (RP) and relative susceptibility (r_{sa}) were suitable for use in testing nematode resistance in sweet potato lines. The two resistant sweet potato lines '2015-2-1' and 'Khumo' would add to the existing nematode resistant sweet potato cultivars in South Africa (Chapter 3). Additionally, the identified post-infectional mechanism of resistance in the two test sweet potato lines (Chapter 4), suggested that the genetic pool for use in nematode molecular resistance breeding of sweet potatoes is being widened.

5.3 Recommendations

The inconsistencies of RP and rs_a values of most sweet potato lines to infection by the two test *Meloidogyne* species across seasons. Experiment 1 and Experiment 2, which were conducted during spring and autumn, respectively, suggested that plant genotype × environment interactions could have played determining role in some of the observed findings. The observation suggested that validation should be conducted during the same season (in time) or in space during the same season.

5.4 Conclusions

Out of 18 sweet potato lines tested for a host-status against *Meloidogyne incognita* and *M. javanica*, sweet potato lines '2015-2-1' and 'Khumo' had a consistent non host status to two test *M. incognita* and *M. javanica* except for *M. enterolobii*. Consequently, the current study demonstrated that the two sweet potato lines had attributes of being highly resistant to the two *Meloidogyne* species, and that therefore, they could be used in crop rotations intended to manage high population densities of such *Meloidogyne* species. Moreover, in sweet potato lines '2015-2-1' and 'Khumo', the mechanism of nematode resistance against the test *Meloidogyne* species was suggested to be post-infectional, which have desirable attributes in management of population densities of nematodes.

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APPENDICDES

Appendix 3.1 Analysis of variance for reproductive potential (RP) of sweet potato lines inoculated with *Meloidogyne incognita* under greenhouse conditions 56 days after inoculation (Experiment 1).

Source	DF	SS	MS	F	P
Replication	5	13219	2643.86		
Treatment	18	48607	2700.40	1.94	0.0217
Error	90	125093	1389.92		
Total	113	186920	6734.18		

Appendix 3.2 Analysis of variance for reproductive potential (RP) of sweet potato lines inoculated with *Meloidogyne incognita* under greenhouse conditions 56 days after inoculation (Experiment 2).

Source	DF	SS	MS	F	P
Replication	5	1730.3	346.05		
Treatment	18	63172.7	3509.60	5.56	0.0000
Error	89	56177.0	631.20		
Total	12	121080	4486.85		

Appendix 3.3 Analysis of variance for reproductive potential (RP) of sweet potato lines inoculated with *Meloidogyne javanica* under greenhouse conditions 56 days after inoculation (Experiment 1).

Source	DF	SS	MS	F	P
Replication	5	25351	5070.23		
Treatment	18	86651	4813.96	1.74	0.0475
Error	89	246926	2774.45		
Total	112	298928	12658.64		

Appendix 3.4 Analysis of variance for reproductive potential (RP) of sweet potato lines inoculated with *Meloidogyne javanica* under greenhouse conditions 56 days after inoculation (Experiment 2).

Source	DF	SS	MS	F	P
Replication	5	286005	57201		
Treatment	18	2068601	114919	2.06	0.0000
Error	90	5025037	55834		
Total	113	7379643	197957		

Appendix 3.5 Analysis of variance for reproductive potential (RP) of sweet potato lines inoculated with *Meloidogyne enterolobii* under greenhouse conditions 56 days after inoculation (Experiment 1).

Source	DF	SS	MS	F	P
Replication	5	7265	3464.0		
Treatment	18	1574037	55017.5	4.15	0.0000
Error	89	1896166	5328.7		
Total	112	3477468	63810.2		

Appendix 3.6 Analysis of variance for reproductive potential (RP) of sweet potato lines inoculated with *Meloidogyne enterolobii* under greenhouse conditions 56 days after inoculation (Experiment 2).

Source	DF	SS	MS	F	P
Replication	5	53210	118497		
Treatment	18	165645	198134	2.06	0.0184
Error	90	496801	121381		
Total	113	715656	438012		

Appendix 4.1 Analysis of variance for necrotic spots of sweet potato line '2015-2-1' inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	746.8	248.93		
Treatment	14	15130.3	1080.74	4.56	0.0001
Error	42	9962.2	237.20		
Total	59	25839.3	1566.87		

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Appendix 4.2 Analysis of variance for giant cells of sweet potato line '2015-2-1' inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	68.2	19.728		
Treatment	14	9724.4	694.602	3.10	0.0023
Error	42	9419.6	194.275		
Total	59	19212.2	941.602		

Appendix 4.3 Analysis of variance for rootlet interference of sweet potato line '2015-2-1' inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	4921.1	1640.36		
Treatment	14	41459.3	2961.38	2.34	0.0172
Error	42	53246.9	1267.78		
Total	59	99627.3	5869.52		

Appendix 4.4 Analysis of variance for necrotic spots of sweet potato line '2015-2-1' inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	258.8	86.283		
Treatment	14	7886.1	563.293	5.33	0.0000
Error	42	4435.9	105.617		
Total	59	12580.9	755.193		

Appendix 4.5 Analysis of variance for giant cells of sweet potato line '2015-2-1' inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	466.5	155.511		
Treatment	14	5414.2	386.731	3.09	0.0023
Error	42	5255.0	125.118		
Total	59	11135.7	667.36		

Appendix 4.6 Analysis of variance for rootlet interference of sweet potato line '2015-2-1' inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	5339.0	1779.67		
Treatment	14	42150.9	3010.78	2.41	0.0142
Error	42	52504.0	1250.10		
Total	59	99993.9	6040.55		

Appendix 4.7 Analysis of variance for necrotic spots of sweet potato line 'Khumo' inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	79.3	26.444		
Treatment	14	5857.7	418.410	2.29	0.0192
Error	42	7660.7	182.397		
Total	59	13597.7	627.251		

Appendix 4.8 Analysis of variance for giant cells of sweet potato line 'Khumo' inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	197.9	65.972		
Treatment	14	6527.4	466.243	2.3	0.0036
Error	42	6684.3	159.151		
Total	59	13409.7	691.666		

Appendix 4.9 Analysis of variance for rootlet interference of sweet potato line 'Khumo' inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	4643	1547.66		
Treatment	14	54265	3876.07	1.71	0.0908
Error	42	95364	1970.58		
Total	59	154277	7694.31		

Appendix 4.10 Analysis of variance for necrotic spots of sweet potato line 'Khumo' inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	431.4	143.794		
Treatment	14	9693.5	692.393	8.12	0.0000
Error	42	3582.4	85.294		
Total	59	13707.2	921.481		

Appendix 4.11 Analysis of variance for giant cells of sweet potato line 'Khumo' inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	7.33	2.444		
Treatment	14	6895.73	492.552	15.69	0.0000
Error	42	1318.67	31.397		
Total	59	8191.73	526.393		

Appendix 4.12 Analysis of variance for rootlet interference of sweet potato line 'Khumo' inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Replication	3	18996	6331.87		
Treatment	14	124742	8910.17	7.89	0.0000
Error	42	47412	1128.87		
Tota	59	191150	16370.91		