POTENTIAL USES OF INDIGENOUS *CUCUMIS AFRICANUS* AND *CUCUMIS MYRIOCARPUS* AS ROOT-KNOT NEMATODE-RESISTANT ROOTSTOCKS IN WATERMELON (*CITRULLUS LANATUS*) HUSBANDRY

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[redacted]

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DECLARATION

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

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DEDICATION

To my exquisite kids, Dineo, Thoriso and Dumisane

I say Halalaa!

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ABSTRACT

Global withdrawal of synthetic fumigant nematicides like methyl bromide due to their eco-unfriendliness resulted in serious consequences in production of crops which do not have genotypes that are resistant to plant-parasitic nematodes. Watermelon (*Citrullus lanatus*) is one such crop, where infection by highly aggressive root-knot nematodes (*Meloidogyne* species) invariably results into as high as 50% yield loss, with occasional total crop failures. Initial screening for nematode resistance in *Cucumis* species indigenous to South Africa suggested the possibility of the existence of nematode resistance, with the probability of these species being compatible with *Citrullus* species in inter-generic grafting technology. Uses of indigenous genera in Cucurbitaceae family as nematode-resistant seedling rootstocks in watermelon production could promote the South African watermelon industry as outlined in ISO 9001 certification guidelines to have competitive advantage in lucrative watermelon export markets. The objectives of this study were to determine the: (1) host-status and host-sensitivity of *C. africanus* and *C*. *myriocarpus* seedlings using a series of inoculation levels of *M. incognita* race 2 under various conditions, (2) host-status and host-sensitivity of *C. africanus* and *C*. *myriocarpus* seedlings using a series of inoculation levels of *M. incognita* race 4 and *M. javanica*, including the resistance form in these plant species, at least, under selected environmental conditions, (3) host-status and host-sensitivity of *C. africanus* and *C*. *myriocarpus* seedlings using a series of inoculation levels of *M. incognita* race 2 with multi-nematode

infestations in order to establish whether the observed nematode resistance was sustainable when the plant was attacked by various pests at the root system level, (4) compatibility of inter-generic grafting of *Citrullus* and *Cucumis* seedlings in order to establish the potential uses of *Cucumis* species in olericulture, and (5) influence of the greenhouse whitefly (*Trialeurode vaporariorum*) infection on resistance of *C. africanus* to *Meloidogyne* species in order to establish whether the observed nematode resistance was sustainable when the plant was attacked by pests on complimentary organs. Reliability of measured variables was ensured by using statistical levels of significance ($P \leq 0.05$) and coefficient of determination (R^2), with validity being ensured by conducting experiments at the same location over two seasons or conducting one experiment during one season at two different locations, *viz.* the University of Limpopo and the Agricultural Research Council – Institute for Industrial Crops, and/or by setting up factorial treatments. Results consistently demonstrated that *C. africanus* and *C. myriocarpus* were non-hosts to *M. incognita* races 2 and 4 and *M. javanica,* without the test nematodes inflicting any damage to plants, which in plant-parasitic nematodes is described as nematode resistance. Quadratic relationships between RF values and $\log_{10}(Pi + 1)$ transformations, in addition to confirming the density-dependent growth patterns of plant-parasitic nematodes, also suggested that chemical compounds responsible for suppression of nematodes in the two *Cucumis* species were different. The two *Cucumis* species were resistant to *M. incognita* races 2 and 4 and *M. javanica*, regardless of the environment under which the experiments were conducted. In field studies, the

two *Cucumis* species supported the ring nematodes (*Criconema mutabile*) and the spiral nematodes (*Helicotylenchus dihystera*), without these exo-parasitic nematodes inflicting any damage to plants, which in plant-parasitic nematodes is described as tolerance. Interactions among *Meloidogyne* species, *C. mutabile* and *H. dihystera* were either stimulatory or inhibitory, depending on whether *Meloidogyne* species were in the soil or inside the roots. Mechanisms of nematode resistance in the two *Cucumis* species were different, with *C. africanus* and *C. myriocarpus* depicting pre-infectional and post-infectional forms of resistance, respectively, without any sign of hypersensitivity in roots. When, seeds of *Citrullus* species were primed in water to hasten germination. Using the developed technology, survival of grafts improved from 36% to 100%, translating to relative improvement of 178%, with nematode-resistant rootstocks retaining their nematode resistant capabilities, while watermelon scions flowered earlier, with relatively higher fruit yield, without any deleterious effect on accumulation abilities of essential nutrient elements in leaves. Resistance of *C. africanus* to *M. javanica* was invariably broken by the greenhouse whitefly infection at high population levels, possibly through loss of non-structural carbohydrates, which are essential in synthetic pathways of secondary metabolites. *Cucumis africanus* and *C. myriocarpus* contain cucurbitacin B ($C_{32}H_{48}O_8$) and cucurbitacin A [cucumin ($C_{27}H_{40}O_9$), leptodermin ($C_{27}H_{38}O_8$)], respectively, which have high demand for carbon and energy. Consequently, the efficacy of indigenous *Cucumis* species as nematode-resistant rootstocks in suppression of *Meloidogyne* species would be dependent upon the management of the

greenhouse whitefly population densities. In conclusion, *C. africanus* and *C. myriocarpus* have the potential for use as nematode-resistant rootstocks in the production of watermelon cultivars ‘Congo’ and ‘Charleston Gray’ in South Africa, where nematode population densities of *M. incognita* races 2 and 4 and *M. javanica* are widely distributed and are highly injurious to watermelons. Although nematode resistance in the two *Cucumis* species had attributes of sustainability, populations of the greenhouse whitefly broke the resistance. Proposed future research areas included influence of cucurbitacins in fruit quality of watermelons and protocols for mass culturing the nematode-resistant *Cucumis* rootstocks using tissue culture technology.

PICTURES OF INDIGENOUS *CUCUMIS* SPECIES

Legend A: Plants and fruit of wild watermelon (*Cucumis africanus*).

Legend B: Plants and fruit of wild cucumber (*Cucumis myriocarpus*).

CHAPTER 1
RESEARCH PROBLEM

1.1 Introduction

Following the 2005 international withdrawal of methyl bromide (MB) technique in management of plant-parasitic nematodes due to its eco-unfriendliness (Speth, 2004), the root-knot nematodes (*Meloidogyne species*) became increasingly the most debilitating nematode genus in watermelon (*Citrullus lanatus* Thunb.) husbandry (Thies and Levi, 2007). The genus has more than 63 species (De Waele and Elsen, 2007) and paratisizes more than 3 000 host-plants (Rizvi and Rizvi, 1992), with cosmopolitan distribution (Taylor and Sasser, 1978). Also, due to the existence of numerous biological races within the genus, it is almost impossible to import nematode-resistant genotypes from one country to another (Robertson and Diez-Rojo, 2008). For instance, the predominant biological races in South America and the USA include *M. incognita* races 1 and 3 (Montalvo and Esnard, 1994), in Europe and Northern Africa, *M. incognita* race 4 (Sikora and Fernandez, 2005) and in South Africa, *M. incognita* races 2 and 4 (Kleynhans *et al*., 1996). In South Africa, *M. javanica* also has a wide distribution and is often ranked the most injurious root-knot nematode in various crops (Kleynhans *et al*., 1996).

Prior to the suspension of MB technique, the estimated global annual crop yield losses due to all plant-parasitic nematodes were US$125 billion (Chitwood, 2003). In particular, the southern root-knot nematode *(Meloidogyne incognita)*

causes substantial economic yield losses in watermelons – ranging annually to as high as 50%, at times to total crop failure (Lamberti, 1979). Worldwide, watermelon is one of the four major commercially-produced fruiting vegetables (tomato, melon, cucumber) – with estimated annual market value of R1.5 billion in South Africa (Anon., 2011a) and ca. US$435 million (ca. R4.35 billion) in the USA (Thies and Levi, 2007). Annually, South Africa produces ca. 141 409 tons of watermelons and other melons, with Northern Cape, Western Cape, Eastern Cape and Limpopo Provinces contributing 84%, 8%, 7% and 0.34% to the total tonnage, respectively (Anon., 2011a). Infection by *Meloidogyne* species induces formation of root galls, causing stunted growth, decreased water uptake, imbalances of essential nutrient elements, low evapo-transpiration and increased root exudation of amino acids, which invariably reduce soil pH (Bird, 1974; Maqbool *et al*., 1987; Mashela, 2002). The availability of most essential nutrient elements in soil to plants is sensitive to slight changes in soil pH (Bohn *et al*., 1985), which may partly provide some explanation on how nematode affect nutrient uptake, thus, limiting responses to fertiliser application (Fouche *et al*., 1977), all of which translate into reduced crop yield and profit.

1.2 Research statement

Phasing out of ozone-depleting and greenhouse-inducing chemical compounds after the adoption of the 1987 Montreal Protocol and the 1997 Kyoto Protocol, respectively (Speth, 2004), there is increased focus towards the investigation of host-plant resistance as a nematode control intervention strategy. Host-plant

resistance technique is the most eco-friendly, cost effective and integratable management intervention available for reducing crop losses due to infection by plant-parasitic nematodes (Boerma and Hussey, 1992; Roberts, 1992; Starr *et al*., 2002). Among the available alternative tactics to methyl bromide (MB) technique, plant resistance is the most investigated technique in plant-parasitic nematology (Sikora and Fernandez, 2005; Thurau *et al*., 2010). Mass screening within the genus *Citrullus* and exotic *Cucumis* species suggested that there was hardly any resistance in these genera to *Meloidogyne* species (Montalvo and Esnard, 1994; Thomason and McKinney, 1959; Winstead and Riggs, 1959). Lack of interest in developing watermelon genotypes with nematode-resistance might be ascribed to costly attempts that produced seedless fruits, which could not be propagated using conventional sexual propagation methods (Thies and Levi, 2007). Also, attempts to introgress nematode-resistance genes from landraces into high yielding watermelon cultivars using the Bt technique were avoided due to vehement and widespread consumer rejection of genetically modified food (Thies and Levi, 2007).

Nematode-resistance has a number of distinct non-commodity-specific advantages over the use of MB technique, including (1) complete prevention of nematode reproduction, (2) no requirement for special application techniques and equipment and (3) less production costs when compared to MB or organic matter application (Fourie, 1997; Fourie and Mc Donald, 2003; Stirling, 1991). Nematode-resistance technique is also highly rated for its compatibility with most

other nematode management intervention tactics (Cook and Evans, 1987; Dunn and Sydenham, 2003), including the ground leaching technique (Mashela, 2002). However, Thurau *et al*. (2010) identified several limitations for the use of natural nematode resistance genes in practice, which include sensitivity to certain abiotic and biotic factors (Dropkin, 1969; Mashela *et al*., 1992a). Due to climatic change, outbreaks of certain pests are becoming a serious concern. In Limpopo Province, the greenhouse whitefly (*Trialeurode vaporariorum*) is becoming a serious pest in cucurbits (Celix *et al*., 1996; Cohen *et al*., 1992; Duffus *et al*., 1996a,b; Tzanetakis, 2004; Winter *et al*., 1992; Wisler *et al*., 1998), but its effect on nematode resistance is not documented.

Preliminary mass screening of wild genera in the family Cucurbitaceae demonstrated that two species in the genus *Cucumis*, *viz*. wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) – both endemic to South Africa (Kristkova *et al*., 2003), have resistance to *M. incognita* race 2 (Land Bank Chair of Agriculture – University of Limpopo, 2002: unpublished report). Later, a micro-plot study demonstrated that *C. myriocarpus* seedlings were resistant to *M. incognita* race 2 at various levels of inoculation (Mofokeng, 2005).

1.3 Motivation

Crop breeding of new cultivars resistant to plant-parasitic nematodes is time-consuming and promoted the resurgence of virulent new races (Thurau *et al*., 2010). Also, intensive efforts to clone nematode-resistant genes associated with

time-linked genetic resistance have had limited success (Ballvora *et al*., 1995; Ganal *et al*., 1995; Ho *et al.,* 1992; Klein-Lankhorst *et al*., 1994; Thurau *et al*., 2010), with seedless watermelons resulting from such efforts (Thies *et al*., 2009). In contrast, grafting technique might eventually enable the sustainable suppression of nematodes using wild plants adapted to a particular region. Use of nematode-resistant wild *Cucumis* species as rootstocks in inter-generic grafting with watermelon cultivars might have three-fold benefits: (1) enable farmers to produce watermelons in areas with high densities of virulent root-knot nematodes, (2) suppress root-knot nematodes to allow farmers to produce watermelons without grafting in subsequent seasons, and (3) suppress root-knot nematodes to allow farmers to produce other highly nematode-susceptible cash crops in a resistant-susceptible crop-rotation system.

1.4 Aim and objectives

1.4.1 Aim

The aim of the study was to assess host-status and host-sensitivity of wild *Cucumis* species to *Meloidogyne* species with the overall intention of developing nematode-resistant seedling rootstocks for inter-generic grafting with nematode-susceptible watermelon cultivars for the South African watermelon industry.

1.4.2 Objectives

The study comprised five objectives, outlined as follows:

1. To determine whether *C. africanus* and/or *C*. *myriocarpus* seedlings would be resistant to *M. incognita* race 2 under greenhouse and micro-plot conditions.

2. To determine whether *C. africanus* and *C*. *myriocarpus* seedlings would be resistant to *M. incognita* race 4 and *M. javanica* under greenhouse conditions and whether the resistance was introgressible.

3. To determine whether resistance of *C. africanus* and *C. myriocarpus* seedlings to *M. incognita* race 2 would be retained under field conditions with multi-nematode infestations.

4. To determine whether inter-generic grafting of *Citrullus* cultivars onto *Cucumis* seedlings would be compatible under greenhouse and field conditions.

5. To determine whether *T. vaporariorum* infection would compromise resistance of *C. africanus* to *M. javanica* under greenhouse conditions.

1.5 Hypotheses

The study consisted of five hypotheses, summarised as follows:

1. *Cucumis africanus* and *C*. *myriocarpus* seedlings would not be resistant to *M. incognita* race 2 under greenhouse and micro-plot conditions.

2. *Cucumis africanus* and *C*. *myriocarpus* seedlings would not be resistant to *M. incognita* race 4 and *M. javanica* under greenhouse conditions and the resistance was introgressible.

3. Resistance of *C. africanus* and *C. myriocarpus* seedlings to *M. incognita* race 2 would not be retained under field conditions with multi-nematode infestations.

4. Inter-generic grafting of *Citrullus* cultivars onto *Cucumis* seedlings would not be compatible under greenhouse and field conditions.

5. *Trialeurodes vaporariorum* infection would not compromise resistance of *C. africanus* to *M. javanica* under greenhouse conditions.

1.6 Reliability, validity and objectivity

Reliability is described previously as the extent to which a measuring instrument yields consistent results when the variable being measured repeatedly had not changed (Leedy and Ormrod, 2005). Statistical analyses provide various reliability checks on the data (Berenson and Levine, 1996). In this study, reliability in various experiments was ensured by using appropriate levels of statistical significance for mean separation and when evaluating the variance explained by models as measured by coefficients of determination (R^2). Validity is described as an extent to which the instrument measures what was actually

intended to be measured (Leedy and Ormrod, 2005). In empirical research, experiments are either replicated in time or space in order to increase the range of validity of conclusions drawn from it (Little and Hills, 1981). A factorial set of treatments is another way for increasing the range of validity. Validity is ensured by conducting the experiment at the same location over two seasons, or during one season at different locations or by setting up factorial treatments (Little and Hills, 1981). Objectivity is described as striving, as far as possible or practicable, to reduce or eliminate biases, prejudices or subjective evaluations by relying on verifiable data (Leedy and Ormrod, 2005). Objectivity is 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).

1.7 Bias

Bias is described as any influence, conditions or set of conditions that singly or altogether distort the data (Leedy and Ormrod, 2005). In this study, bias was minimised by ensuring that the experimental error in each experiment was reduced through increased replications and randomisation (Little and Hills, 1981).

1.8 Significance of the study

The study was intended to produce *Meloidogyne*-resistant seedlings from indigenous wild *Cucumis* species to serve as rootstocks for highly nematode-susceptible watermelon cultivars. The envisaged inter-generic grafting technique

would probably serve as a sustainable alternative to the eco-unfriendly MB technique for the South African watermelon industry.

1.9 Format of thesis

Following the description and detailed outlining of research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the five subsequent chapters (Chapter 3-7) addressed each of the objectives in sequence. In the final chapter (Chapter 8), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion which tied the entire study together.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Watermelon (*Citrullus lanatus*) cultivars do not have resistant genotypes to the root-knot nematodes (*Meloidogyne* species). Breeding new watermelon cultivars that are resistant to *Meloidogyne* species have had setbacks that led to less research interest in the area (Ballvora *et al*., 1995; Ganal *et al*., 1995; Ho *et al.,* 1992; Klein-Lankhorst *et al*., 1994; Thies *et al.,* 2009). Overall, the aim of this study was to investigate the host-status and host-sensitivity of wild *Cucumis* species to *Meloidogyne* species with the intention of developing nematode-resistant seedling rootstocks for inter-generic grafting with highly nematode-susceptible *Citrullus* cultivars for the South African watermelon industry. Literature review was restricted to work done and not yet done in the research problem, as described previously (Chapter 1). In this review, genera in the Cucurbitaceae family were repeatedly written out in full in order to eliminate confusion.

2.2 Work done on research problem

Work done on the research problem included nematode-plant interactions, mechanisms of nematode resistance, Cucurbitaceae family and grafting technique in fruit-bearing vegetables.

2.2.1 Nematode-plant interactions

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

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

competition due to nematode infection (Duncan and McSorley, 1987; Ferris, 1981). The concept of minimum yield suggests that nematodes do not kill their hosts, but rather expose them to opportunistic infection, which may result in killing the host plant.

Host-sensitivity measures the responses of a plant to nematode infection and is a function of (i) nematode type, (ii) inoculum level, (iii) plant type, (iv) age of plant and (v) biotic and abiotic factors (Seinhorst, 1965). Generally, certain nematode species, for example, the root-knot nematodes, the burrowing nematodes (*Radopholus* spp.), the sting nematode (*Belonolaimus longicaudatus* Rau, 1958) and the root-lesion nematode (*Pratylenchus* spp.), are more aggressive than others, for example, the citrus nematode (*Tylenchulus semipenetrans* Cobb, 1913), and result in high yield losses (Duncan, 2009; Mashela, 1992). When interacting with abiotic factors like salinity, plant-parasitic nematodes like *T. semipenetrans*, the sting nematode and *M. incognita,* induce maximum damage in crops (Duncan, 2009; Duncan *et al*., 1995; Hixson *et al*., 2005; Mashela and Nthangeni, 2002; Mashela *et al*., 1992a, b). Review of host-status and host-sensitivity of plants to nematodes was, in this study, limited to the description of three concepts: (1) susceptible plants, (2) tolerant plants and (3) resistant plants (Seinhorst, 1967; Trudgill, 1985; 1992). Since the concepts are extensively used in this study, they are, along with mechanisms of resistance, reviewed in some detail.

(a) Susceptible plants

Susceptible hosts are plants that have the ability to build up nematode populations and suffer subsequent damage in terms of yield reduction (Trudgill, 1992). Generally, host types respond to attack by root-knot nematodes by forming galls on roots, referred to as root galls (Agrios, 2005). Feeding cells induced by root-knot nematodes, termed giant cells, are formed from host root cells during parasitism to sustain the growth, development and reproduction of the nematode (Hussey and Grundler, 1998).

(b) Tolerant plants

Seinhorst (1967) defined tolerance to nematodes as the capacity of the plant to withstand nematode damage. Most nematodes can reproduce in tolerant hosts without causing any significant reduction in growth and yield (Kleynhans *et al*., 1996; Trudgill, 1985). However, tolerant hosts are not suitable for use in crop rotation systems since they invariably result in the build-up of nematode population densities, which may eventually produce virulent biological races.

(c) Resistant plants

Resistant plants neither allow nematode reproduction nor suffer nematode damage (Taylor and Sasser, 1978; Seinhorst, 1967). Resistance to nematodes is usually associated with the inability of the nematode to induce normal feeding, develop or reproduce inside the host (Miller and Guyla, 1987). Introduction of well-adapted and high-yielding nematode-resistant cultivars is currently the focus in nematode management. Genotypes with superior levels of resistance to a

particular plant-parasitic nematode are continuously being selected for planting and/or breeding efforts to minimise nematode population increases and crop damage (Miller and Guyla, 1987). Traditionally, nematode resistance genes were introduced into susceptible hosts through a process called introgression (Campbell, 1990; Kaplan and Davis, 1987).

Plant breeders introgress natural nematode resistance genes from resistant landraces into nematode-susceptible crops to improve their resistance (Lambrides and Miller, 1998). For instance, successful introgression of Mi resistance genes in tomato (*Solanum* l*ycopersicum* L.) cultivars resulted in intensive use of the Mi genes in agriculture (Thurau *et al*., 2010). However, due to the pathogenic variability of the root-knot nematodes with multiple biological races, introgression of resistance genes were raising concerns with respect to the durability of the engineered resistance genes (Faghihi *et al*., 1995, Castagnone-Sereno, 2002). Although the Mi genes blocked nematode development at an early stage, due to the occurrence of biological races and variation in *Meloidogyne* species, successful development of infective stages and their subsequent reproduction on Mi-resistant tomato genotypes were documented (Roberts and Thomason, 1989). Also, virulent nematode biotypes against the Mi gene were described from various tomato-growing regions (Eddaoudi *et al*., 1997; Kaloshian *et al*., 1996; Ornat *et al*., 2001). Generally, introgressed plant resistance lost its practical utility when, under certain conditions, resistant cultivars were challenged by new virulent nematode races

(Eddaoudi *et al*., 1997; Ornat *et al*., 2001), which might be exacerbated by environmental factors such as temperature (Dropkin, 1969) and salinity (Duncan, 2009; Mashela, 1992).

2.2.2 Mechanisms of nematode resistance

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

(a) Pre-infectional resistance

Pre-infectional resistance is mainly due to pre-formed chemicals, which are fully expressed in root tissues before infection and do not rise to higher levels in response to attacks by invading nematodes (Ferraz and Brown, 2002). Marigold species (*Tagetes* spp.) suppress populations of the lesion and the root-knot nematodes through pre-formed chemical compounds (Motsinger *et al*., 1977; Richard and DuPree, 1978), which were identified as alpha-terthienyl and bi-thienyl (Veech, 1981). Among 175 plant species from different families surveyed for resistance to *P. penetrans*, resistance in 70 species was closely correlated with pre-infectional resistance (Gommers and Voor In't Holt, 1976). In the same study, populations of *P. penetrans* were reduced by 99%, 55% and 63% in *Tagetes patula* L., *T. erecta* L. and *T. minuta* L., respectively. Asparagus (*Asparagus officinalis* L.) contains glycosides, which have nematicidal properties responsible for pre-infectional resistance (Rhode, 1972). Similarly, Griffin and Waite (1971) noted that certain varieties of alfalfa (*Medicago sativa* L.) released substances that were repellent to the tulip root nematode (*Ditylenchus dipsaci* Kuhn, 1857).

(b) Post-infectional resistance

Post-infectional resistance is the ability of a plant to defend itself against nematode parasitism by releasing chemicals present in low levels to higher levels in the host tissues after penetration of nematodes (Kaplan and Davis, 1987). Induced chemicals are triggered to higher levels by the invading nematodes,

where the antimicrobial chemicals either inhibit feeding, development or kill the invading nematode. Induced chemicals, called phytoalexins, are believed to confer resistance to most plant-parasitic nematodes (Harborne, 1999). Generally, post-infectional nematode resistance is introgressible (Kaplan and Davis, 1987), with three distinct variations, *viz.* (i) phytoalexins, (ii) time-unlinked genetic resistance (instant hypersensitivity) and (iii) time-linked genetic resistance (gradual hypersensitivity):

(i) Phytoalexins

Phytoalexins are defensive phytochemicals present in cells in inactive forms and are instantly activated by the penetration of pathogens (Veech, 1981). Generally, phytoalexins represent effective plant resistance mechanisms to nematodes, particularly the sedentary types like the root-knot nematodes (Huang, 1985; Veech, 1981). Giebel (1974) and Roy (1981) each considered the role of nematodes and plant enzymes in resistant plant reactions and suggested that enzymes might influence changes in plant growth regulators, free bound phenols and composition of amino acids in plants, with changes inducing lignifications in order to limit nematode development.

Studies on expression of the reporter gene *GUS,* fused to the promoter of TobRB7 (a tobacco gene), strongly suggested that gene expression occurred in root tips (Oppermann *et al*., 1994a), where TobRB7 gene encoded a membrane protein believed to function as water channel, where the antisense construct of the resultant gene suppressed nematode reproduction (Oppermann *et al*.,

1994b). Changes in gene expression in potato leaves that occurred after root infection by the potato cyst nematode (*Globodera rostochiensis* Wollenweber, 1923*)* included the induction of pathogenesis-related proteins (Hammond-Kosack *et al*., 1989). In tomato, roots infected with the root-knot nematode, genes with homological responses similar to several known plant defence enzymes such as peroxidase, chitinase, lipoxygenase and proteinase inhibitors were observed at the infection site within 12 h of inoculation (Lambert *et al*., 1990). Crammer *et al*. (1993) reported that the tomato gene *hmg*2, which encoded HMG-CoA reductase induced by pathogens other than nematodes, was unregulated after infection by root-knot nematodes. Genetic resistance associated with phytoalexins is post-infectional (Kaplan and Davis, 1987).

(ii) Time-unlinked genetic resistance

Time-unlinked genetic resistance is usually referred to as hypersensitivity since there is, upon penetration of roots by a pathogen, a rapid dying of host cells that produce localised necrosis around the pathogen, thus, limiting its spread and growth (Wallace, 1971). Hypersensitivity can be in response to facultative fungi, bacteria, viruses and nematodes (Klement *et al*., 1964; Muller, 1959).

In certain sources of resistance, inheritance is polygenic, whereas in other cases it is recessive (Faghihi *et al*., 1995; Kreike *et al*., 1993; Trudgill, 1992; Wang and Goldman, 1996). In some cases, polygenic resistance were divided into two classes: dominant and recessive genes, which modulate the responses to

attacks (Kreike *et al*., 1994; Webb *et al.,* 1995). With most of these resistance genes, localised necrosis or hypersensitive responses resembled those described for other resistance genes (Dangl *et al*., 1996; Hammond-Kosack and Jones, 1996).

One of the most studied hypersensitivity resistance genes is Mi, which confers resistance to several root-knot nematode species in tomato (Thurau *et al*., 2010). The Mi-mediated resistance is characterised by a localised necrosis of host cells near the invading nematode (Dropkin, 1969). Also, Dropkin (1969) demonstrated that resistance responses were not always entirely necessary for expression of plant resistance towards nematodes. The earliest noticeable hypersensitive response occurred at 2-4 h after inoculation (Paulson and Webster, 1972). Hypersensitivity may also be invoked at different times following the initiation of a feeding site by a sedentary nematode. For instance, when *Meloidogyne* species were infecting susceptible tomato plants, hypersensitivity was invoked relatively earlier, before appreciable giant cell formation occurred (Paulson and Webster, 1972), whereas for other sedentary nematodes and plant species, hypersensitivity was gradual (Cotten and Hayes, 1969; Endo, 1965). This distinction, as reviewed below, is important to note because the absence of root galls in short-term experiments of *Meloidogyne* species may not necessarily mean that infection was not inducing root galls. Time-unlinked genetic resistance, which is post-infectional (Kaplan and Davis, 1987), should be taken into consideration when assessing nematode-plant interactions.

(iii) Time-linked genetic resistance

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

Generally, in time-linked genetic resistance, molecular changes occurred rapidly after nematode infection. For instance, activity levels of the enzymes phenylalanine, ammonia-lyase and anionic peroxidase increased in resistant plants (Brueske, 1980; Zacheo *et al*., 1993). Cucumbers were observed to have a repulsion mechanism towards nematodes that was associated with time-linked genetic resistance (Thurau *et al*., 2010). Haynes and Jones (1976) found that cucumber plants carrying a dominant allele at the bitter (Bi) locus attracted fewer *M. incognita* juveniles to their roots than did the near-isogenic non-bitter (*bibi*) genotypes. The Bi locus permits plants to accumulate cucurbitacins, which are highly toxic triterpenoids that are important in conferring resistance against various pathogens, particularly in the family Cucurbitaceae (Da Costa and Jones, 1971).

Intensive efforts to clone nematode-resistance genes associated with time-linked genetic resistance had generally not been successful (Ballvora *et al*., 1995; Ganal *et al*., 1995; Ho *et al.,* 1992; Klein-Lankhorst *et al*., 1994). Time-linked genetic resistance can either be pre-infectional or post-infectional, with post-infectional nematode resistance being an active process, where energy is required (Thurau *et al*., 2010). The synthesis of secondary metabolites, as shown by elaborate pathways, used in both pre- and post-infectional nematode resistance, is an active process. Therefore, any abiotic or biotic factor which may interfere with energy-inducing systems in plants, may compromise active nematode resistance. The mechanisms involved in breaking nematode resistance were previously described in citrus-nematode inter-relations (Mashela and Nthangeni, 2002).

2.2.3 Cucurbitaceae family

The family Cucurbitaceae has 115 genera, with *Citrullus*, *Cucumis*, *Cucurbita* and *Lagenaria* being of great economic importance in agriculture (Pitrat *et al.*, 1999). South Africa is considered as the centre of diversity for wild *Cucumis* species (Kristkova *et al*., 2003), mainly the wild watermelon (*Cucumis africanus* L.F.) and the wild cucumber (*Cucumis myriocarpus* Naude.).

(a) Plant-nematode resistance

The family Cucurbitaceae contains genera which are highly susceptible to the root-knot nematodes, with emerging evidence suggesting the presence of

resistance in certain wild genera (Mofokeng, 2005). Thomason and McKinney (1959) in the USA demonstrated that there was no resistance in 34 varieties of cantaloupe (*Cucumis melo*) and watermelon cv. 'Striped *Klondira'* to *M. incognita acrita*, *M. javanica* (Treub, 1885) and *M. hapla* (Chitwood, 1949). Seventy-eight watermelon cultivars and five breeding lines evaluated for resistance to the root-knot nematodes in the USA were all susceptible (Winstead and Riggs, 1959). Also, 10 watermelon cultivars in Puerto Rico were susceptible to *M. incognita* race 3 (Montalvo and Esnard, 1994). However, in these studies (Montalvo and Esnard, 1994; Thomason and McKinney, 1959; Winstead and Riggs, 1959), the methodologies, as explained later, allowed for screening and not for resistance.

Thies and Levi (2006) in the USA found some moderate non-host status to *M. arenaria* race 1 in certain landraces of watermelons. Also, in the USA, Fassuliotis (1970) demonstrated that some non-host status to *M. incognita acrita* occurred in "fig-leafed" gourd (*Cucumis ficifolia* Bouche.) and African horned cucumber (*Cucumis metuliferus* E Mey.), with the results being briefly outlined below. Although most juveniles penetrated roots of *Cucumis ficifolia* and *Cucumis metuliferus* as in the susceptible *Cucumis melo* L., few developed to the adult female stage, suggesting the existence of post-infectional non-host status. In *Cucumis ficifolia* and *Cucumis metuliferus* non-host status was associated with hindrance of juvenile development beyond the second-stage juvenile, delayed development of juveniles to adults and increased stimulation toward maleness. Hypersensitivity was not associated with juvenile penetration. However,

comparison of cells in histopathology studies 26 days after infection of *Cucumis ficifolia* and *Cucumis metuliferus* roots showed noticeable giant cells that developed in regions of roots associated with adult females. However, in *Cucumis metuliferus*, immature female nematodes were associated with formation of small giant cells which were limited to a few cells near the head of the nematode. Later, moderate resistance to the root-knot nematodes using a series of inoculum levels was observed in *Cucumis metuliferus* (Fassuliotis, 1977). The observation was important in the sense that it showed for the first time, the importance of screening (at one level) from resistance (at several levels) in nematode-plant relations. However, attempts to genetically introgress resistance from *Cucumis metuliferus* into *Cucumis melo* were unsuccessful (Chen and Adelberg, 2000; Norton and Granberry, 1980; Soria *et al*., 1990).

(b) Uses of indigenous *Cucumis* species

Mofokeng (2005) demonstrated that *Cucumis myriocarpus* has some resistance to *M. incognita* race 2. Mashela (2002) demonstrated that crude extracts of *Cucumis myriocarpus* fruit have the potential for suppressing *M. incognita* race 2, when applied at quantities ranging from 0.2 to 0.7 mt/ha. The material increased tomato productivity and improved soil electrical conductivity, without affecting soil pH. The efficacy of crude extracts of *Cucumis myriocarpus* fruit on nematode suppression and improving tomato productivity was comparable to that of synthetic nematicides, *viz.* aldicarb and phenamiphos (Mashela *et al*., 2008). In bioactivity tests, Muedi *et al.* (2005) demonstrated conclusively the nematicidal

properties of crude extracts of *Cucumis myriocarpus* fruit to *M. incognita* and *T. semipenetrans in vitro* cultures. Crude extracts of *Cucumis myriocarpus* fruit did not interact with *Bacillus* species under greenhouse, micro-plot and field conditions (Mphosi, 2004) nor did the material had any suppressive effect on *Bradyrhizobium japonicum* species (Shakwane *et al*., 2005).

Local people use leaves of *Cucumis myriocarpus* as greens. Another indigenous plant, the wild watermelon (*Cucumis africanus*), has more or less similar features to those of *Cucumis myriocarpus,* but its leaves are bitter and not suitable for use as leafy vegetable. Both plants have short stems, ca. 5-cm high, with multiple vines at the terminal end (Grubben and Denton, 2004). The two species are perennial, with vines and leaves dying-back in winter, whereas the stems and roots remain intact. Consequently, the stems are hardy and suitable for use in grafting technique.

2.2.4 Grafting technique in vegetables

Grafting is a technique where two plant parts are combined, with the lower part serving as the rootstock and the upper part as scion for producing the desired fruit (McMahon *et al*., 2005). Generally, in fruit-bearing vegetables, scions are grafted onto rootstocks by using one of the three methods, *viz*. (i) cleft grafting, (ii) tube grafting and (iii) tongue grafting or any of their modifications (Kurata, 1994; Siguenza *et al*., 2005). Tongue grafting is mainly used for cucumbers (Kurata, 1994), whereas cleft or tongue grafting is suitable for melons (Siguenza

et al., 2005). In Japan, a large number of commercially available seedling rootstocks are being bred and released for use by growers (Lee, 2003). Since grafting gives increased disease tolerance to rootstocks and vigour to scions, it is a useful technique in alternatives to methyl bromide in the management of plant-parasitic nematodes.

(a) Using grafting to suppress soil pathogens

Cultivation of melons (*Cucumis sativus* L.) grafted on squash (*Cucurbita maxima* Duchesne.) began in Korea and Japan in late 1920s (Yamakawa, 1983). Development and use of resistant rootstocks had since been viewed as one of the most promising technique in management of plant-parasitic nematodes. Generally, in *Cucumis* species a large and vigorous seedling rootstock has increased tolerance to diseases caused by fungi such as *Verticilium* and *Fusarium,* although the tolerance may vary depending on the genotype of the rootstock used (Oda *et al.*, 1997).

Grafted cucumbers are widely used in management of soil-borne pathogens, primarily *Fusarium oxysporium* [Schltdl, 1824] (Oda *et al.*, 1997). Di Vito *et al*. (1983) and Ferris (1985) each demonstrated that the susceptible melon cv. ‘Durango’, when grafted onto *Cucumis metuliferus*, performed well under high nematode pressures, with dry shoot mass being significantly higher than that of intact plants. Also, *Cucumis metuliferus* reduced root galling and nematode reproduction (Di Vito *et al*., 1983; Ferris, 1985). Siguenza *et al*. (2005) studied

responses of *Meloidogyne*-susceptible melons grafted on *Cucumis metuliferus* and *Cucurbita moschata* [Duchesne ex Poir.] seedlings in management of *M. incognita*. In the study, Siguenza *et al*. (2005) observed that susceptible melons grafted on *Cucurbita moschata* had lower root-gall ratings at high nematode densities and higher shoot mass than in control plants. However, final nematode levels were not lower on grafted than on control plants. Siguenza *et al*. (2005) demonstrated that grafting melons onto *Cucumis metuliferus* rootstocks accorded tolerance, that is, nematode numbers increased, without reducing plant yield. In contrast, grafting melons onto *Cucurbita moschata* resulted in resistance to *M. incognita* (Siguenza *et al.,* 2005), as shown by reduced nematode numbers and no nematode effect in plant yield. Plant tolerance to nematodes in crop rotation systems is not desirable since it increases initial nematode population densities for subsequent crops.

In Japan, by the early 1980s, 100% of cucumbers grown under greenhouse conditions were grafts, with 70% grafted on gourd (Tsambanakis, 1984). In the southern parts of Greece, the ratio of the production area using grafted plants to the total production area amounted to almost 90-100% for cucumbers and 40-50% for melons in the late 1990s (Traka-Mavrona *et al*., 2000). Consequently, the technique is gaining widespread use, particularly in countries with stringent environmental laws towards eco-unfriendly synthetic pesticides. Due to the stringent ISO 9001 certification requirements, eco-unfriendly synthetic materials are also being pushed out of production systems by consumers.

(b) Using grafting to ameliorate other stresses

Grafting is also used to ameliorate stresses induced by abiotic and biotic factors (Traka-Mavrona *et al*., 2000). In addition to suppressing pathogens, grafting technology also serves to boost growth and development, which strengthen tolerance to extremes in temperatures, ameliorate salinity stress and also increases uptake of essential nutrient elements to shoots (Lee, 1994). Generally, grafted plants have strong vigour and are able to develop well under toxicity ions such as B, Cu, Cd and Mn (Arao *et al*., 2008; Edelstein *et al*., 2005; Matsuo *et al.,* 1985; Savvas *et al*., 2009; Rouphael *et al*., 2008).

(c) Challenges in grafting technique

The major challenge in inter-generic grafting technique is incompatibility of rootstock and scion, which is mainly due to differences in stem diameters of plant parts. Tiedermann (1989) demonstrated that when two stem diameters of the two parts were not comparable in size, there was the likelihood of high graft mortalities. Scion-rootstock compatibility is also influenced by the technique used, age of the plant and the pre- and post-grafting management practices (Traka-Mavrona *et al*., 2000). Also, in most inter-generic grafting, the rootstock has some effect on the scion (Traka-Mavrona *et al*., 2000). For instance, the trifoliate orange (*Poncirus trifoliata* [L.] Raf.) rootstock has a dwarfing effect on *Citrus* trees (Mashela, 1992). Bales *et al.* (1989) proposed that the root system was responsible for the synthesis of substances that enable grafted plants to be resistant to foliar pathogens and that the compounds were transported to shoots.

Apparently, also the chemical compounds which are required for nematode resistance are produced in roots, where leaves serve as an energy source for activating the pathways through which the compounds are anabolised (Thurau *et al*., 2010). A classical example of success in inter-generic grafting occurs in the family Rutaceae where Citrus, *Poncirus, Fortunella* (Kumquat) and various hybrids are widely used in management of plant-parasitic nematodes in citrus production (Duncan, 2009).

2.3 Work not yet done

Screening of 21 plant species in 10 genera of the family Cucurbitaceae in Limpopo Province, South Africa, showed that certain endemic *Cucumis* species had no root galls when each was infected with 600 second stage juveniles of *M. incognita* race 2 (Land Bank Chair of Agriculture – University of Limpopo, 2002, unpublished data). Earlier in this literature review, a distinction was made between screening for nematode resistance and investigating nematode resistance in plant-parasitic nematology. Basically, in screening, one inoculum level of nematode is used, whereas in investigating nematode resistance a series of nematode levels are used. Consequently, five areas were identified as the work not yet done with respect to the research problem, which included:

- Host-status and host-sensitivity of *Cucumis africanus* and *Cucumis myriocarpus* seedlings using a series of inoculation levels of *M. incognita* race 2 under various conditions.

- Host-status and host-sensitivity of *Cucumis africanus* and *Cucumis myriocarpus* seedlings using a series of inoculation levels of *M. incognita* race 4 and *M. javanica* including the resistance form in these plant species, at least, under selected environmental conditions.
- Host-status and host-sensitivity of *Cucumis africanus* and *Cucumis myriocarpus* seedlings using a series of inoculation levels of *M. incognita* race 2 with multi-nematode infestations in order to establish whether the observed nematode resistance was sustainable when the plant was attacked by various nematodes at the root system level.
- Compatibility of inter-generic grafting of *Citrullus* and *Cucumis* seedlings in order to determine the potential uses of *Cucumis* species in olericulture.
- Influence of the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) infection on resistance of *Cucumis* species to *Meloidogyne* species in order to establish whether the observed nematode resistance was sustainable when the plant was attacked by both pests on complimentary organs.

In short, host-status and host-sensitivity of *Cucumis africanus* and *Cucumis myriocarpus* would be investigated at various levels of inoculation for all *Meloidogyne* species that are dominant in South Africa, particularly in watermelon-producing regions. Inter-generic grafting of indigenous wild *Cucumis* species as seedling rootstocks to the highly nematode-susceptible *Citrullus* cultivars would be explored. Assurance was also taken throughout the study to

ascertain that factors which might later on compromise the observed nematode resistance were identified. For instance, influence of attack by *T. vaporariorum* on nematode resistance in *Cucumis* species was suspected.

CHAPTER 3
HOST-STATUS AND HOST-SENSITIVITY OF *CUCUMIS* SPECIES TO *MELOIDOGYNE INCOGNITA* RACE 2

3.1 Introduction

Watermelon (*Citrullus lanatus*) cultivars are highly sensitive to the root-knot nematodes (*Meloidogyne* species), with yield losses ranging up to 50%, with complete crop losses being common under certain conditions (Lamberti, 1979). Suspension of methyl bromide and related fumigant nematicides due to their eco-unfriendliness shifted the focus of plant-parasitic nematodes from control to management options in various cropping systems (Fourie and Mc Donald, 2003; Mashela, 2002; Stirling, 1991). One such management option was the use of nematode resistance, which is a function of host-status and host-sensitivity (Seinhorst, 1965). Despite, watermelon being one of the four major fruit-bearing vegetables (tomato, melon, cucumber, watermelon), in all commercial cultivars there are currently no genotypes that are resistant to *Meloidogyne* species.

Worldwide, the total production of watermelon was 101 billion metric tons in 2010/2011 growing season, whereas South Africa produced ca. 74 000 metric tons (Anon., 2011b). Over 50% of the overall watermelon production in the world occurs in China (Anon., 2011b). South Africa has had access to the international watermelon markets from 1996, with the 2010/2011 export sales being less than US$1 million (Anon., 2011b). Northern Cape, Western Cape, Eastern Cape and Limpopo Provinces contribute 84%, 8%, 7% and 0.34% to the total production tonnage of South African watermelon, respectively (Mashela and Morudu, 2009).

Focus on nematode management options in other fruit-bearing vegetables was on the use of inter-generic grafting, where nematode-resistant rootstocks from different genera within the same family are being used (Thies *et al*., 2010). The *Citrullus* genus, in the family Cucurbitaceae, is endemic to the Kalahari Desert in the Republic of Namibia (Jarret *et al*., 1996). Wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*), both within the family Cucurbitaceae, are indigenous to South Africa (Kristkova *et al*., 2003). Evidence suggests that the family Cucurbitaceae is endemic to Africa (Grubben and Denton, 2004), supported by identified centres of biodiversity of wild *Cucumis* species and *Citrullus* species.

Preliminary screening trials suggested that the *Cucumis* species have some compatibility with *Citrullus* cultivars when used in inter-generic (genus-to-genus) grafting technique, although the survival ratios were low, at 35% (Land Bank Chair of Agriculture – University of Limpopo, 2002, unpublished report). Using inter-generic grafting technique in watermelon production for nematode management requires detailed investigations of nematode resistance on *Cucumis* seedlings. An earlier study under the auspices of the Land Bank Chair of Agriculture – University of Limpopo, suggested that *C. myriocarpus* seedlings were resistant to the southern root-knot nematode (*M. incognita*) under micro-plot conditions (Mofokeng, 2005). The observed nematode resistance had not been validated under greenhouse conditions. The objective of this study was to determine the host-status and host-sensitivity of *C. africanus* and *C. myriocarpus*

to *M. incognita* race 2 at various levels of inoculation under greenhouse conditions and to confirm previous results under micro-plot conditions.

3.2 Materials and methods

Both greenhouse and micro-plot trials were conducted at the Plant Protection Skills Centre, University of Limpopo, in Limpopo Province of South Africa (23°53'10"S, 29°44'15"E).

3.2.1 Greenhouse trials

Greenhouse trials were conducted in summer (November-January) during the 2008/2009 growing season and repeated in autumn (February-April) 2009. When required, *M. incognita* race 2 inoculum was prepared by extracting eggs and second-stage juveniles (J2s) from roots of greenhouse-grown nematode-susceptible tomato (*Solanum lycorpersicum*) cv. 'Floradade' in 1% water solution of sodium hypochlorite (Hussey and Barker, 1973).

Preparation of experimental units and material

Fruits of *C. africanus* and *C. myriocarpus* were collected from the local field, cut into pieces, seeds removed and shade-dried for 14 days. Dry seeds of *C. africanus* and *C*. *myriocarpus* were separately wrapped in hand-sewn cotton handkerchief bags and submerged in running tapwater for 8 h to leach out the germination-inhibiting chemicals prior to planting in seedling trays containing

Hygromix (Hygrotech, Pretoria North, South Africa) growing medium (Mafeo and Mashela, 2009).

Thirty-cm-diameter plastic pots, filled with 10 L steam-pasteurised sand (300^{o}C for 45 min.) and Hygromix at 3:1 ratio (v/v), were placed on greenhouse benches at 0.5 m inter-row and 0.6 m intra-row spacing. Each plant was fertilised using 5 g 2:3:2 NPK (22) per pot which provided 310 mg N, 210 mg P and 260 mg K and 3 g 2:1:2 NPK (43) which provided a total of 0.70 mg N, 0.64 mg K and 0.64 mg P per ml water. Also, the 2:1:2 (43) mixture provided 1.8 mg Mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo/ml tapwater. Uniform three-week-old, nematode-free *Cucumis* seedlings were transplanted to pots for each separate *Cucumis* species one day after irrigating the growing medium to field capacity. Ambient day/night temperatures averaged 28/21^{o}C, with maximum temperatures controlled using thermostatically-activated fans. Other greenhouse variables such as relative humidity, photosynthetically active radiation and solar radiation were not measured.

A day after transplanting, pots were each infested by dispensing 0, 500, 750, 1 000, 1 250, 1 500, 1 750 and 2 000 approximate numbers of *M. incognita* eggs and second stage juveniles (J2s) using a 20-ml plastic syringe by placing into 2½-cm-deep holes on cardinal points of the stems per replication. Untreated control plants (0 nematodes) received filtrate (25-µm-mesh sieve) of nematode

suspension to establish any microbes associated with *M. incognita* race 2 in their rhizosphere.

Experimental design and cultural practices

Eight treatments, *viz.* 0, 500, 750, 1 000, 1 250, 1 500, 1 750 and 2 000 eggs and J2s, were arranged in a randomised complete block design (RCBD), with 5 replicates. Four sets of Hadeco Moisture Meter (Hadeco, Magic®, New Delhi, India) were inserted to 20-cm depths in randomly selected pots to monitor soil moisture tension. Plants were irrigated with 1 000 ml tapwater as soon as 50% of the moisture meters had readings just below 2 units. Plants were scouted for greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood), and sprayed with 1.33 ml Lebaycid (a.i. fenthion 50% ml) per litre water when populations increased to above 10 whiteflies per plant.

Data collection

Fifty six days after inoculation with nematodes, plant length was measured from the crown end of the short stem to the tip of the vines, which were combined to constitute vine length/plant. Shoots were cut at the soil level and stem diameters measured in the middle of the stem using a digital vernier caliper. Shoots were oven-dried for 72 h at 70°C for recording of dry matter. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and fresh mass measured to facilitate the calculation of nematode density per total root system per plant. Fully-developed root galls were assessed using the North

Carolina Differential Scale of 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% water solution of NaOCl (Hussey and Barker, 1973) and passed through top-down nested 150-µm, 45-µm and 25-µm mesh sieves. Contents of the 25-µm mesh sieve were poured into 100-ml plastic containers for counting under a stereomicroscope. The remaining roots were oven-dried for dry matter determination with allowance for convention of the 5 g roots collected for nematode extraction being taken into consideration.

Soil per pot was thoroughly mixed and a 250-ml soil sample was collected. Nematodes were extracted from soil samples using the modified sugar-floatation and centrifugation method (Jenkins, 1964). Briefly, the soil sample was washed through a 45-µm-aperture sieve into a bucket, which was then filled with water and mixed in a swill. After the swill had stopped, the aliquot was poured through a 25-µm sieve, with the contents being washed into 100-ml plastic centrifuge tubes. A teaspoon (2½ g) of kaolin was then added in each tube and contents centrifuged at 1 750 RPM for five minutes. The kaolin solution was then decanted with nematodes having settled at the bottom of the tubes with soil particles. A 469 g sugar/L tapwater was poured into the centrifuge tubes and stirred once prior to centrifuging for one minute at 1 750 RPM. The aliquot was then decanted onto 25-µm sieve with sugar being rinsed off the nematodes, which were then collected from the sieve into 100-ml plastic containers for counting under a

stereomicroscope. During counting, which was completed in less than 10 days, samples were stored at 5^{o}C. Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 10 L soil per pot to estimate the final nematode population density (Pf). Reproductive factor (RF = Pf/Pi), which is a proportion of Pf and initial nematode population density (Pi), was computed for each inoculum level.

Data analysis

The RF and plant data were subjected to analysis of variance (ANOVA) procedure using SAS software (SAS Institute, Inc., Cary, NC., U.S.A., 2008). When treatments were significant at the probability level of 5%, the degrees of freedom and their associated sum of squares were partitioned (Appendices 3.1 – 3.8) to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the treatment means (Steyn *et al*., 2003). Mean separation was achieved using Waller–Duncan multiple-range test. The RF (y-axis) and the $\log_{10}$(Pi + 1) values (x-axis) were assessed using lines of the best fit. Responses of RF values to increasing $\log_{10}$(Pi + 1) levels were modelled by the regression curve estimations, resulting to a quadratic equation: $Y = b_2x + b_1x + a$, where Y = RF values, $x = – b_1/2b_2$, where x = the optimum nematode population level, which is a Pi value where nematodes had maximum RF value (Keller and Warrack, 2003). Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

3.2.2 Micro-plot trials

Trials were conducted in autumn (March-May) 2009 and repeated in spring (September-November) 2009. The site was fallowed prior to the various experiments. Artificial micro-plots were established by inserting 30-cm-diameter plastic pots into 20-cm-deep holes at 1.0 m intra-row and 1.0 m inter-row spacing. Each pot was filled with 10 L steam-pasteurised sand and Hygromix at 3:1 (v/v). *Cucumis africanus* and *C. myriocarpus* experiments were separate but conducted concurrently.

Experimental design and cultural practices

Cucumis seedlings were produced from seeds under greenhouse conditions as described in the greenhouse trials. Uniform 3-week-old nematode-free seedlings were transplanted into pots one day after irrigating to field capacity. Each pot received one seedling. *Meloidogyne incognita* inoculum was raised and prepared as in the greenhouse trials. However, adjustments were made in inoculation levels to accommodate the equilibrium points (E) of *M. incognita* for the two *Cucumis* species. Eight treatments, *viz*. 0, 25, 50, 125, 250, 625, 1 250 and 3 125 eggs and J2s of *M. incognita* race 2, were arranged in a RCBD, with five replicates. Nematode placement was as in the greenhouse trial, with control plants also receiving equivalent filtrate from aliquots of eggs and J2s to establish associated microbes. Fertilisation and irrigation were as described previously. Weeds among the pots were removed using hand-held hoes prior to surface

covering by the vines. Total rainfall in autumn and spring was 9 mm and 15 mm, respectively.

Data collection and analysis

At harvest, 56 days after inoculation, nematode and plant variables similar to those in the greenhouse trials were collected and analysed.

3.3 Results

Under both greenhouse and micro-plot conditions, interactions between the growing seasons for variables in each *Cucumis* species were not significantly different ($P \leq 0.05$) and data were pooled ($n = 10$) by season and subjected to statistical analysis. RF values of *M. incognita* race 2 under both environments were less than one on *C. africanus* and *C. myriocarpus* (Table 3.1). RF values at different levels of inoculation differed, allowing for fitting of the best lines, which resulted in quadratic relationships (Figure 3.1). Under greenhouse conditions, the log-transformed Pi contributed 86% and 99% to the TTV in RF values of *M. incognita* race 2 on *C. africanus* and *C. myriocarpus*, respectively. Similarly, under micro-plot conditions, Pi contributed 88% and 81% to TTV on RF values of the test nematode on the two respective test plant species.

Using the relationship – $b_1/2b_2$, derived from the quadratic relationships, in *C. africanus* under greenhouse conditions, $\log_{10}(Pi + 1)$ optimised RF values at $x = 0.40$ units for the optimum RF value of 0.39, while under micro-plot conditions, x

= 2.08 units for the optimum RF value occurred at x = 0.35 (Table 3.2). In contrast, in *C. myriocarpus*, the minimum RF values occurred at x = 7.88 units for RF value of 0.17 under the greenhouse conditions, while under the micro-plot conditions, RF values were optimised at x = 2.40 units for the RF value of 0.68. Under both plant species and growing conditions, the nematode treatment had no effect on dry shoot mass, dry root mass, root/shoot ratio, stem diameter (Table 3.3) and root galls (data not shown). Also, the root/shoot ratios of either plant species were below 0.10 under both growing conditions.

Table 3.1 Initial population density (Pi), final population density (Pf) and reproductive factor (RF = Pf/Pi) values of *Meloidogyne incognita* race 2 on *Cucumis africanus* and *Cucumis myriocarpus* under greenhouse and micro-plot conditions at 56 days after inoculation (n = 10).

Greenhouse					Micro-plot				
	C. africanus		*C. myriocarpus*			*C. africanus*		*C. myriocarpus*	
Pi	Pf	RF[z]	Pf	RF[z]	Pi	Pf	RF[z]	Pf	RF[z]
500	205	0.41a	231	0.46a	25	7	0.28a	10	0.40bc
750	250	0.33a	287	0.38a	50	17	0.34a	28	0.56b
1 000	293	0.29b	343	0.34a	125	49	0.39a	66	0.53b
1 250	395	0.32ab	319	0.26b	250	85	0.34a	168	0.67ab
1 500	497	0.33ab	294	0.20b	625	169	0.27ab	444	0.74a
1 750	374	0.21bc	323	0.18b	1 250	313	0.25ab	613	0.49b
2 000	251	0.13c	351	0.18b	3 125	563	0.18b	1187	0.23c

[z]Column means followed by the same letter were not different according to Waller–Duncan multiple-range test at the probability level of 5%.

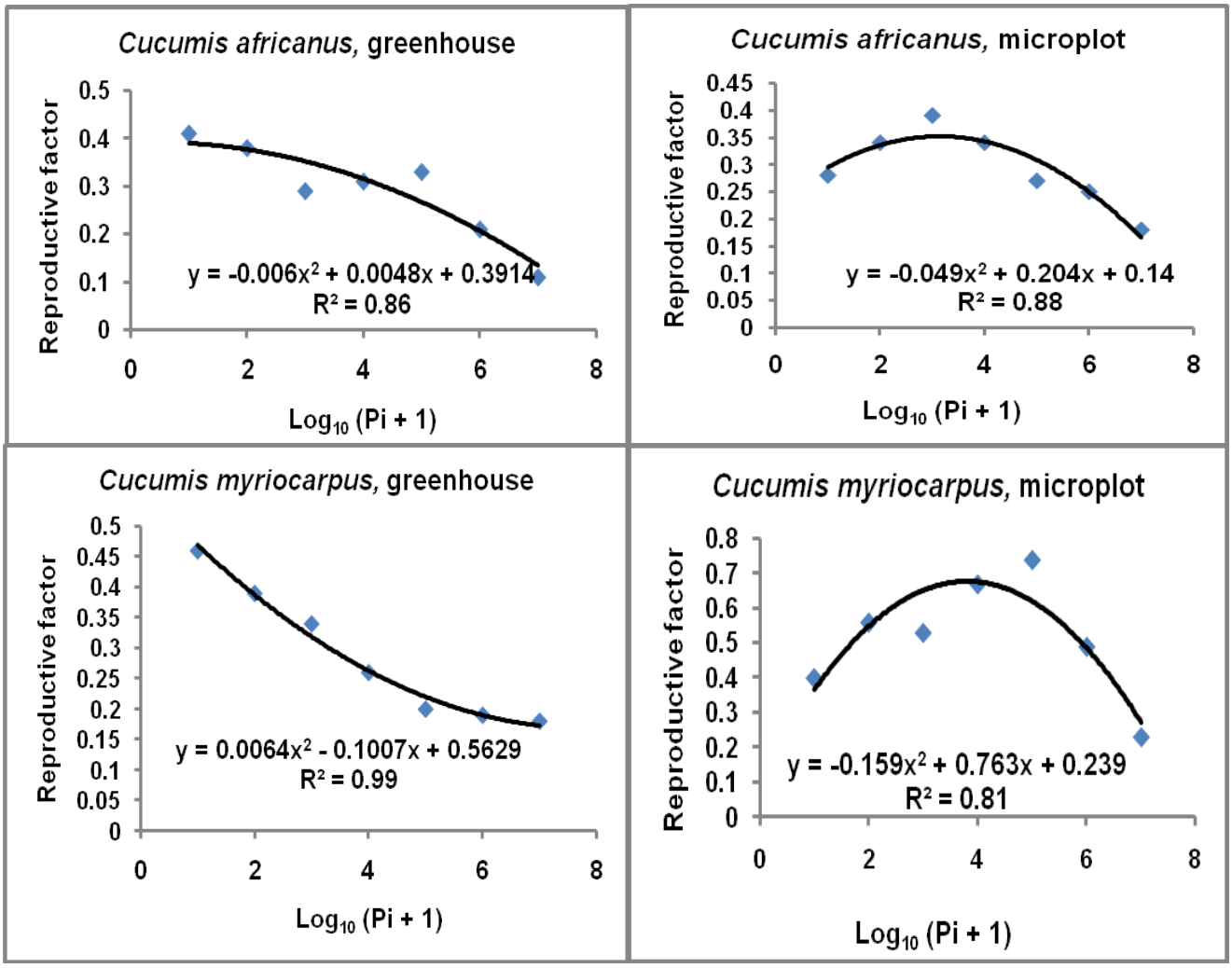

Figure 3.1 Relationship between reproductive factor (RF) values and $\log_{10}$(Pi + 1) of *Meloidogyne incognita* race 2 on *Cucumis africanus* and *Cucumis myriocarpus* under greenhouse and micro-plot conditions at 56 days after inoculation (n = 10).

Table 3.2 Estimates of the independent Pi-values (x) which optimise the reproductive factor (RF) values of *Meloidogyne incognita* race 2 on *Cucumis africanus* and *Cucumis myriocarpus* under greenhouse and micro-plot conditions at 56 days after inoculation (n = 10).

Greenhouse study				
Plant	Model	R^2	X	a
C. africanus	$y = -0.006x^2 + 0.0048x + 0.3914$	0.86	0.4	0.39 max
C. myriocarpus	$y = 0.0064x^2 - 0.1007x + 0.5629$	0.99	7.88	0.17 min
Micro-plot study				
C. africanus	$y = -0.049x^2 + 0.204x + 0.14$	0.88	2.08	0.35 max
C. myriocarpus	$y = -0.159x^2 - 0.763x + 0.239$	0.81	2.40	0.68 max

$x = -(b_1/2b_2)$, where in *C. africanus* $b_1 = 0.0048$ and $b_2 = 0.006$.

a = multiplication rate, determined by substituting the value of x in the model.

Table 3.3 Infestation with *Meloidogyne incognita* race 2 on *Cucumis africanus* and *Cucumis myriocarpus* had no significant ($P \leq 0.05$) effect on dry shoot mass (DSM), dry root mass (DRM), root/shoot ratio and stem diameter (SD) at 56 days after inoculation (n = 10).

Greenhouse								
Pi	*Cucumis africanus*				*Cucumis myriocarpus*			
	DSM (g)	DRM (g)	Root/shoot ratio	SD (mm)	DSM (g)	DRM (g)	Root/shoot ratio	SD (mm)
0	189.32	4.83	0.03	4.57	61.60	3.55	0.06	8.87
500	189.95	4.74	0.02	4.66	53.23	3.26	0.06	8.35
750	187.60	4.40	0.02	4.86	51.44	3.35	0.07	8.22
1 000	185.24	4.06	0.02	5.06	49.64	3.44	0.07	8.09
1 250	192.49	4.15	0.02	5.02	50.52	3.54	0.07	8.14
1 500	199.74	4.24	0.02	4.98	51.39	3.63	0.07	8.18
1 750	188.46	4.24	0.02	4.97	53.51	3.42	0.06	8.48
2 000	177.18	4.24	0.02	4.95	55.63	3.21	0.06	8.77
$LSD_{0.05}$	32.55	0.88	0.04	1.49	13.96	1.42	0.05	1.87
Micro-plot								
0	200.86	6.89	0.03	11.23	103.49	2.94	0.03	12.51
25	230.44	7.15	0.03	10.94	98.93	2.90	0.03	8.53
50	190.54	7.07	0.04	10.68	112.06	2.93	0.03	9.21
125	150.63	7.00	0.05	10.41	125.18	2.96	0.02	9.88
250	165.73	7.11	0.04	10.39	112.56	2.62	0.02	10.04
625	180.83	7.22	0.04	10.36	99.93	2.27	0.02	10.19
1 250	183.77	7.15	0.04	10.45	94.86	1.98	0.02	10.26
3 125	186.71	7.08	0.04	10.53	89.78	1.68	0.02	10.33
$LSD_{0.05}$	83.18	2.41	0.05	0.51	30.25	1.53	0.03	7.98

3.4 Discussion

In greenhouse and micro-plot trials, all RF values of *M. incognita* race 2 on *C. africanus* and *C. myriocarpus* were below unity. Host-status is described using RF, which is a measure of the reproductive potential of a nematode on a given host (Seinhorst, 1967; Windham and Williams, 1988). All RF values below unity suggested that the nematode failed to reproduce on a given host, whereas those above one indicated that the nematode established the feeding site and reproduced. Interpretation of RF values requires the appreciation of the impact of the equilibrium point (E) on reproductive rates of nematodes. Beyond E all RF values are invariably below unity since competition for infection sites is intense, resulting in reduced reproductive rates (Seinhorst, 1967).

In support of the view of using infection levels beyond E, in their study of the citrus nematode (*Tylenchulus semipenetrans*) race in South Africa, Kwaye *et al*. (2008) used Pi = 40 000 nematodes, which was far above E on various differential hosts that were hosts and non-hosts, with the result that all RF values, including those of susceptible hosts, were below unity. Consequently, RF values should be viewed with caution, more especially when the screening data are considered.

The Pi used in determining host-status should invariably be a series that includes levels below and above E. Duncan and McSorley (1987) argued that for most plants where RF values are greater than unity at Pi values below E, plants are

hosts regardless of whether or not the nematode populations decrease in a density-dependent growth pattern. This argument suggested that even at inoculum levels of Pi lower than E, with increasing infection time, nematode populations may reach E, resulting into a situation where RF values are below unity, with subsequent unconscious confounding inference that the plant was a non-host, whereas in actual fact it was a host. Literature is replete with results that confound nematode competition for non-host status (Kwaye *et al*., 2008; Pofu, 2008).

Equilibrium points of *M. incognita* race 2 in both *C. africanus* and *C. myriocarpus* had not been established. Consequently, a series of Pi levels were adjusted for micro-plot trials after the greenhouse trials had shown that the quadratic curves of RF values versus $\log_{10}(Pi + 1)$ had different shapes – concave or convex. Because RF values were below unity at all inoculum levels, one can safely infer that this was due to the incompatibility between the test nematode race and the test plant species. Also, to guard against the impact of time-related final nematode population densities, which may lead to the misinterpretation of RF values due to the cyclic nature of Pf values with time (Ferris, 1985), experiments were terminated 56 days after inoculation. This duration allowed for approximately three generations for this nematode species (Sikora and Fernandez, 2005).

Graphic representation of RF values demonstrated that RF variables had a density-dependent growth pattern (Ferris and Noling, 1987), which supported the view of the existence of reproductive cycles in plant-parasitic nematology as observed in the citrus nematode (*Tylenchulus semipenetrans*) on citrus and in *M. javanica* on hemp (Mashela *et al*., 1992a; Pofu, 2008). Generally, growth of nematode population densities has upswings and downswings, which are important in analysing the observed nematode resistance with respect to the level of inoculation. In plant-parasitic nematology, should there be a positive linear relationship between RF and $\log_{10}(Pi + 1)$, although the RF is less than unity, this may suggest improper selection of inoculum ranges (Ferris, 1985). The major difference between the current relationships and those of others (Ferris and Noling, 1987; McSorley and Gallaher, 1993) was that in both greenhouse and micro-plot studies quadratic relationships occurred, which are characteristic of most biological systems (Liu *et al*., 2003; Mafeo *et al*., 2011; Mamphiswana *et al.,* 2010; Salisbury and Ross, 1992). Generally, where linear relationships were depicted (Ferris and Noling, 1987; McSorley and Gallaher, 1993), Pi ranges might have been below E (for positive relationship) or above E for negative relationship. Alternatively, where there was no RF-Pi relationships (McSorley and Gallaher, 1993), sampling for determination of Pf might have been collected when the nematode population densities were at E point (Seinhorst, 1967).

In the greenhouse trials for both *Cucumis* species, it appeared that starting Pi at 500 nematodes was already beyond or approaching E for *M. incognita* race 2, as

depicted by negative quadratic relationships between RF and $\log_{10}(Pi + 1)$. In the micro-plot, the inoculum range of less and above 500 nematodes resulted in a situation where the quadratic curves were convex-shaped, with maximum multiplication rates. The convex-shaped quadratic relationship with maximum rates of multiplication being less than unity, suggested that the resistance in *Cucumis* species was sustainable. In both the greenhouse and the micro-plot experiments the major factor to consider was that RF values were less than unity, which implied non-host status of *Cucumis* species to *M. incognita* race 2. Consequently, in subsequent studies on *Meloidogyne* – *Cucumis* relationships, inoculum levels should be below and above 500 nematode levels.

The less than unity in RF values of *M. incognita* race 2 on the two *Cucumis* species also suggested that this nematode did not have the potential to feed on either plant species, since feeding is a pre-requisite for nematode development and reproduction (Ferraz and Brown, 2002). RF values in this study confirmed those of *M. incognita* race 2 on *C. myriocarpus* under micro-plot studies, where inoculum levels included those below and above 500 nematodes (Mofokeng, 2005). Generally, RF values between 0 and 1 indicate a poor host or non-host to nematodes (Ferris *et al.,* 1993), as demonstrated for *M. chitwood* race 1 in three onion and three carrot cultivars (Motjahedi *et al*., 1998; Sasser *et al*., 1984).

Failure of *M. incognita* race 2 to feed and reproduce on both *Cucumis* species under both growing conditions could be explained in terms of the cucurbitacins

which are highly toxic chemical compounds in the two plant species (Chen *et al.,* 2005). Plants in the family Cucurbitaceae contain a total of 12 cucurbitacins; with cucurbitacin A in *C. myriocarpus* fruit and roots being the only water-soluble cucurbitacin (Chen *et al*., 2005). Plants in the family Cucurbitaceae contain a dominant allele at the bitter (Bi) locus, which is responsible for accumulating cucurbitacins (Haynes and Jones, 1976). Cucurbitacin A comprises two phyto-chemical compounds, *viz.* cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffery, 1978; Rimington, 1938), which are quite different from those of aldicarb ($C_7H_{14}N_2O_2S$) and fenamiphos ($C_{13}H_{22}NO_3PS$) [Mashela, 2007]. *Cucumis africanus* has cucurbitacin B ($C_{32}H_{48}O_8$), which is insoluble in water. Crude extracts from ground fruits of *C*. *myriocarpus* suppressed numbers of *M. incognita* race 2 on nematode-susceptible tomato cultivars (Mashela, 2002), with the related efficacy being comparable to that of aldicarb and fenamiphos (Mashela *et al*., 2008). Results of the current experiments suggested that the cucurbitacins are also active against nematodes *in vivo,* since both plant species have cucurbitacins in roots (Jeffery, 1978).

All yield components measured in both *Cucumis* species in greenhouse and micro-plot experiments were not affected by *M. incognita* race 2 infections. The low root/shoot ratios in the two *Cucumis* species supported the view that these two plant species were resistant to *M. incognita* race 2, since successful infection with root gall formation invariably increases the root/shoot ratios (Taylor and Sasser, 1978). The observed ratios, in the range 0.02–0.03, normally

characterise plant species that are viewed as being drought tolerant (Griffiths and Parry, 2002; Hsiao and Xu, 2000; Kirnak *et al*., 2001; Wu and Cosgrove, 2000). Incidentally, the two *Cucumis* species were previously classified as being drought tolerant (Grubben and Denton, 2004), with current root/shoot ratios confirming those observed previously in *C. myriocarpus* (Mafeo, 2006). Drought tolerance features would also be an added admirable characteristic of these test plants, should they have sustainable resistance to *Meloidogyne* species and serve as seedling rootstocks for watermelons. Lack of empirical evidence on gall formation in roots of *Cucumis* species supported the work of Fassuliotis (1970), who observed that galls were not observed in certain exotic *Cucumis* species with moderate nematode-resistance.

Seinhorst (1967) model defines nematode-plant relationships using three concepts: (1) susceptible host as allowing nematode reproduction and incurring damage, (2) tolerant host as allowing nematode reproduction without incurring damage and (3) resistant host as inhibiting nematode reproduction without incurring damage. Consequently, under both growing environments, the two *Cucumis* species were resistant to *M. incognita* race 2.

3.5 Conclusion

Cucumis africanus and *C. myriocarpus* are each resistant to *M. incognita* race 2, with the resistance having the characteristics of being sustainable. In addition to *M. incognita* race 2, *M. incognita* race 4 and *M. javanica* are also widely

distributed in South Africa, and cause high yield losses in various crops (Kleynhans *et al.,* 1996). Consequently, for the *Cucumis* species to be useful as inter-generic rootstocks for watermelon cultivars, their host-status and host-sensitivity to *M. incognita* race 4 and *M. javanica* have to be established.

CHAPTER 4
RESISTANCE OF *CUCUMIS* SPECIES TO *MELOIDOGYNE INCOGNITA* RACE 4 AND *MELOIDOGYNE JAVANICA*

4.1 Introduction

Root-knot nematodes (*Meloidogyne* species), with more than 63 species (De Waele and Elsen, 2007), are highly injurious to more than 3 000 plant species (Rizvi and Rizvi, 1992). Existence and widespread distribution of species and biological races in this genus (Hartman and Sasser, 1985) make it difficult to use nematode resistance to manage population densities of this nematode unless the species and biological races were properly identified. Misidentification of biological races in plant-parasitic nematology had on a number of occasions resulted in dire consequences for various crop industries. A classical example is the South African citrus industry and the biological race of the citrus nematode (*Tylenchulus semipenetrans*), which was misidentified as the mediterranean race (Cohn, 1976), resulting in widespread use of trifoliate orange (*Poncirus trifoliata*) rootstock, while the actual race was poncirus, which aggressively and virulently infects the trifoliate orange rootstock (Kwaye *et al*., 2008). Over the years, the use of *P. trifoliata* in South Africa declined from 21% of the total rootstock used to less than 5% due to its being highly susceptible to *T. semipenetrans* (Rabe and Von Broembsen, 1991), particularly in areas with salinity (Kwaye *et al*., 2008).

The southern root-knot nematode (*M. incognita*) has, traditionally, four biological races, *viz. M. incognita* races 1, 2, 3 and 4 (Hartman and Sasser, 1985), with races 5 and 6 recently reported using molecular markers (Devran and Sogut,

2011; Robertson and Diez-Rojo, 2008). Widespread existence of a particular race in a given country dictates the direction of plant breeding programmes for resistance against plant-parasitic nematodes. In a country where *M. incognita* race 2 is predominant, for instance, the focus of plant breeding programmes against the root-knot nematodes would be on this race. Existence of different biological races in developing countries where expertise in breeding for nematode resistance is scant makes it difficult to import nematode-resistant genotypes from developed countries with robust plant breeding programmes.

A recent study (Chapter 3) demonstrated that wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) were each highly resistant to *M. incognita* race 2. The two plant species are indigenous to South Africa (Kristkova *et al*., 2003). In South Africa, due to high labour costs, coupled with high population densities of the cotton nematode (*M. incognita* race 4), most cotton farmers are turning to the production of alternative crops, such as watermelon (*Citrullus lanatus*) [Mashela and Morudu, 2009]. Also, in addition to *M. incognita* races 2 and 4 population densities, *M. javanica* population densities are widely distributed in most crop-producing regions of South Africa (Kleynhans *et al*., 1996; Ngobeni *et al*., 2011). The host-status and host-sensitivity of the two indigenous *Cucumis* species are not documented. The objective of this study was, therefore, to determine the host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *M. incognita* race 4 and *M. javanica,* including the resistance form and mechanisms of resistance.

4.2 Materials and methods

Meloidogyne incognita race 4 and *M. javanica* experiments were conducted in summer (November–January) separately in the greenhouse at two locations, *viz.* the Plant Protection Skills Centre, University of Limpopo (UL), South Africa (23°53'10"S, 29°44'15"E) and at the Agricultural Research Council – Institute for Industrial Crops (ARC – IIC), Rustenburg, North West Province (25°43'40"S, 27°17'30"E). Ambient day/night temperatures in both locations averaged 29/22^{o}C, with maximum temperatures controlled using thermostatically-activated fans. Relative humidity, photosynthetically active radiation (PAR) and solar radiation (SR) were not measured.

Experimental design and cultural practices

Fruits of *C. africanus* and *C. myriocarpus* were collected, seeds extracted, prepared, sown and seedlings raised as described previously (Chapter 3). Uniform 3-week-old, nematode-free *Cucumis* seedlings were transplanted to pots for separate studies one day after irrigating the growing medium to field capacity. Twenty-cm-diameter plastic pots, filled with 2 700 ml steam-pasteurised sand and Hygromix at 3:1 (v/v), were placed on greenhouse benches at 0.5 m inter-row and 0.6 m intra-row spacing. One week before transplanting, 3 g 2:3:2 (22) fertiliser mixture was applied to provide a total of 186 mg N, 126 mg K and 156 mg P per ml water along with 2 g 2:1:2 (43), which provided a total of 0.35 mg N, 0.32 mg K and 0.32 mg P per ml water. Also, the 2:1:2 (43) fertiliser mixture provided 0.9 mg Mg, 0.75 mg Fe, 0.075 mg Cu, 0.35 mg Zn, 1.0 mg B, 3.0 mg

Mn and 0.07 mg Mo per ml water. The greenhouse whitefly (*Trialeurodes vaporariorum*) was sprayed with 1.33 ml Lebaycid (a.i. fenthion 50% ml) per L water on biweekly basis.

Seven treatments, *viz*. 0, 200, 600, 1 000, 1 400, 1 800 and 2 200 eggs and second-stage juveniles (J2s) of *M. incognita* race 4 and *M. javanica*, were arranged in a randomised complete block design, with six replicates. Inoculum was prepared by extracting eggs and J2s of any of the two test nematode species from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) plants in 1% water solution of NaOCl (Hussey and Barker, 1973). A day after transplanting, pots were each infested by dispensing approximate number of eggs and second stage juveniles (J2s) using a 20-ml plastic syringe and placing into 2½-cm-deep holes on the cardinal points of the stem per replication. Untreated control plants received 20 ml filtrate of nematode suspension to establish microbes associated with *M. incognita* race 4 or *M. javanica* in their rhizosphere. Four sets of Hadeco Moisture Meters (Hadeco Magic®, New Delhi, India) were inserted to 20-cm depths in randomly selected pots to monitor soil moisture tension. Plants were irrigated with 500 ml tapwater as soon as at least 50% of the moisture meters have average readings below 2 units.

Data collection

At harvest, 56 days after inoculation, vine length, stem diameter, dry shoot mass, dry root mass and root galls were determined as described previously (Chapter 3). Roots were assessed for necrotic spots under a stereomicroscope at 60 × magnification. Nematodes from both locations (UL & ARC) were extracted from total roots per plant by maceration in a blender for 30 s in 1% NaOCl (Hussey and Barker, 1973) and passed through top-down nested 150-µm and 38-µm mesh sieves at the ARC Nematology Laboratory. Contents of the 38-µm mesh sieve were poured into 100-ml plastic containers for counting under a stereomicroscope. Soil per pot from each location was thoroughly mixed and a 250-ml sample collected and nematodes extracted using the sugar-floatation and centrifugation method (Jenkins, 1964). Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 2 700 ml soil per pot and added to provide the final nematode population density (Pf). Reproductive factor (RF) values were computed as a proportion of the Pf to the initial nematode population density (Pi).

Data analysis

Prior to analysis of variance (ANOVA), nematode data were transformed through $\log_{10}(x + 1)$ to normalise the data (Gomez and Gomez, 1984), but untransformed data were recorded. Data were subjected to ANOVA through the SAS software (SAS Institute, Inc., Cary, N.C., U.S.A., 2008) to determine the effects of Pi on RF values and the yield components. Mean separation for significant ($P \leq 0.05$)

treatments was achieved through Waller–Duncan multiple-range test. RF values (y-axis) and the $\log_{10}$-transformed Pi values (x-axis) were subjected to the lines of the best fit. Responses of RF values to increasing Pi levels were modelled by the regression curve estimations resulting to a quadratic equation: $Y = b_2x^2 + b_1x + a$ as described previously (Chapter 3). Total nematodes from root and soil at all levels of inoculation were integrated and compared using the two sample t-test to determine relative penetration indices. When the treatments were significant at the probability level of 5%, the degrees of freedom and their associated sum of squares were partitioned (Appendices 4.1 – 4.6) to determine the percentage contribution of sources of variation to the total treatment variation (TTV). Also, males and females at all levels were each integrated and compared to determine the sex ratios. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

4.3 Results

Interactions between locations for variables from each *Cucumis* species and nematode species were not significantly different ($P \leq 0.05$) and therefore, data were pooled ($n = 12$) and subjected to statistical analysis. Root galls were in all cases invisible, without any evidence of necrotic spots.

In both *Cucumis* species, RF values of the test nematodes were less than unity, but differed in different inoculation levels (Table 4.1). The RF values versus $\log_{10}(Pi + 1)$ had quadratic relationships on both *Cucumis* species, which

accounted for 84% and 82% of the total treatment variation (TTV) in RF values of *M. incognita* race 4 on *C. africanus* and *C. myriocarpus*, respectively, but accounted for 77% and 83% of TTV in RF values of *M. javanica* in the two test plants (Figure 4.1).

In the two *Cucumis* species, for both test nematodes, RF values initially increased, reached saturation levels and started to decline as $\log_{10}(Pi + 1)$ increased, resulting into quadratic relationships (Figure 4.1). In *C. africanus*, $\log_{10}(Pi + 1)$ optimised RF values at x = 3.5 units for the optimum RF value of 0.44, whereas in *C. myriocarpus* optimisation occurred at x = 3.2 for RF value of 0.43, with units for *M. javanica* being optimised at RF values of 0.26 and 0.15 in *C. africanus* and *C. myriocarpus,* respectively (Table 4.2).

Aggregated relative penetration indices (RPIs) for *M. incognita* race 4 were 2.20 and 3.57 on *C. africanus* and *C. myriocarpus*, respectively, whereas those of *M. javanica* were 0.79 and 4.41, respectively (Table 4.3). Aggregated relative maleness indices (RMIs) of *M. incognita* race 4 were 3.80 and 7.00 on *C. africanus* and *C. myriocarpus*, respectively, whereas those of *M. javanica* were 4.86 and 2.00 on the respective test plants. In both plant species, the two nematode species, at all levels of inoculation, had no effect on dry shoot mass, dry root mass, vine length and stem diameter (Table 4.4). Root/shoot ratios in all experiments were less than 0.1, but were not different (data not shown).

Table 4.1 Initial population density (Pi), final population density (Pf) and reproductive factor (RF = Pf/Pi) of *Meloidogyne incognita* race 4 and *Meloidogyne javanica* on *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Pi	*Meloidogyne incognita* race 4				*Meloidogyne javanica*			
	C. africanus		*C. myriocarpus*		*C. africanus*		*C. myriocarpus*	
	Pf	RF	Pf	RF	Pf	RF	Pf	RF
200	17c	0.09c	20c	0.10b	29	0.15	27	0.14a
600	201b	0.34a	256b	0.43a	103	0.23	24	0.15a
1000	511a	0.51a	501a	0.50a	246	0.25	53	0.13a
1400	507a	0.36a	447a	0.32a	312	0.24	186	0.14a
1800	405a	0.23ab	252b	0.14b	417	0.23	241	0.13a
2200	296b	0.14bc	159b	0.07c	480	0.15	155	0.07b

Table 4.2 Predictive models of population densities (x) at which *Meloidogyne incognita* race 4 and *Meloidogyne javanica* had optimum reproductive factor values in *Cucumis africanus* and *Cucumis myriocarpus* (n = 12).

Cucumis species	Predictive model	R^2	X	RF	P <
	Meloidogyne incognita race 4				
C. africanus	$y = -0.052x^2 + 0.362x – 0.193$	0.84	3.5	0.44	0.05
C. myriocarpus	$y = -0.053x^2 + 0.341x –0.121$	0.82	3.2	0.43	0.05
	Meloidogyne javanica				
C. africanus	$y = -0.016x^2 + 0.110x + 0.068$	0.77	3.4	0.26	0.05
C. myriocarpus	$y = -0.005x^2 + 0.027x + 0.115$	0.83	2.7	0.15	0.05

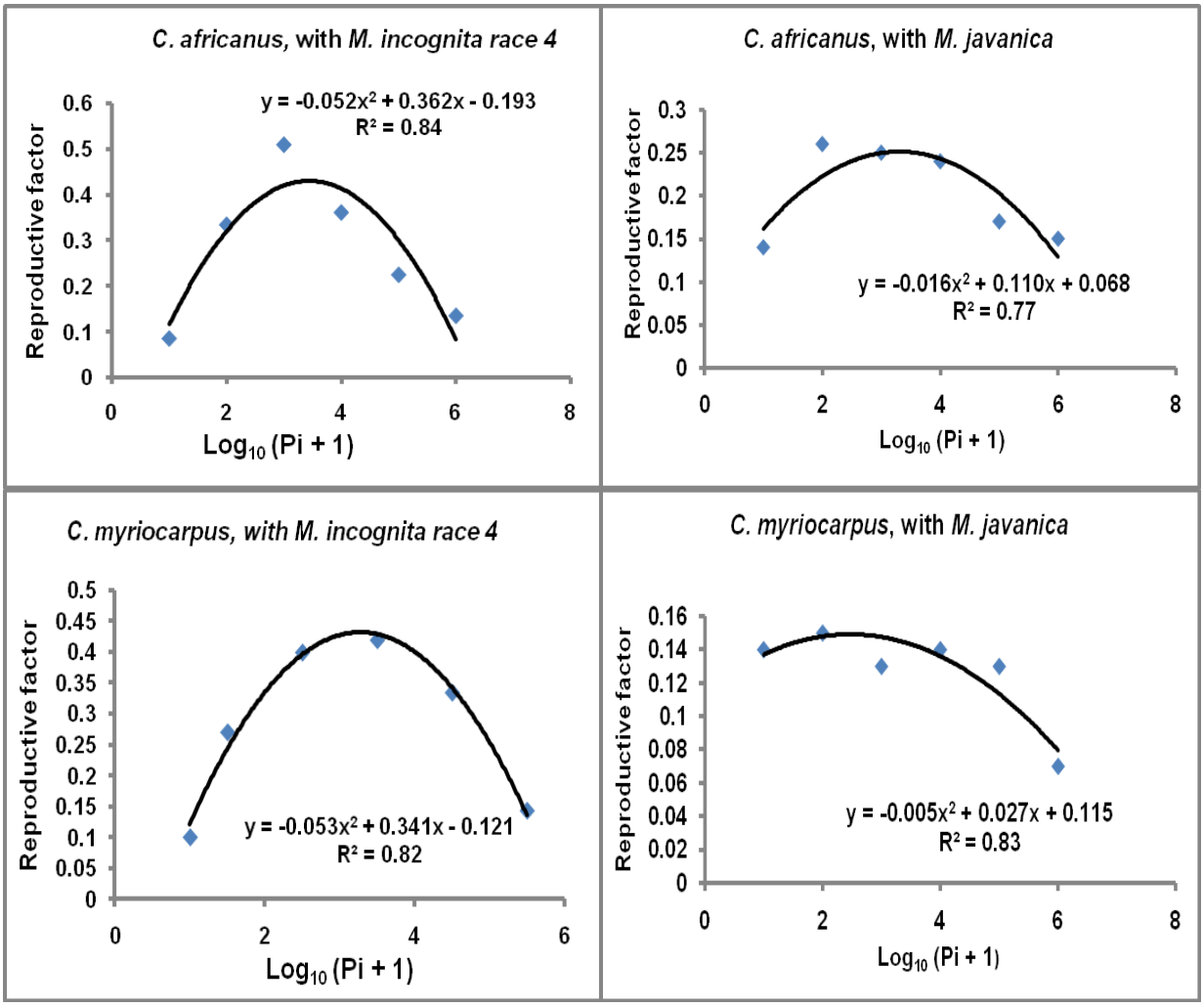

Figure 4.1 Relationship between reproductive factor (RF) values and $\log_{10}$(Pi + 1) of *Meloidogyne incognita* race 4 and *Meloidogyne javanica* on *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Table 4.3 Aggregated relative penetration index (RPI) and aggregated relative maleness index (RMI) of second stage juveniles (J2s) of *Meloidogyne incognita* race 4 (Mi-r4) and *Meloidogyne javanica* (Mj) in roots of *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Cucumis species	Relative penetration index			Maleness index		
		Mi-r4	Mj		Mi-r4	Mj
	Source	Pf	Pf	Gender	Pf	Pf
C. africanus	$Soil_{total}$	40	95	Female	5	7
	$Root_{total}$	128	170	Male	24	41
	RPI	2.20**	0.79**	RMI	3.80**	4.86**
C. myriocarpus	$Soil_{total}$	21	17	Female	1	1
	$Root_{total}$	96	92	Male	8	3
	RPI	3.57**	4.41**	RMI	7.00**	2ns

** = Different at P ≤ 0.05, ns = not different at P ≤ 0.10.

Table 4.4 Infestation with *Meloidogyne incognita* race 4 and *Meloidogyne javanica* had no significant (P ≤ 0.05) effect on dry shoot mass (DSM), dry root mass (DRM), vine length (VL) and stem diameter (SD) at 56 days after inoculation (n = 12).

Pi	*Meloidogyne incognita* race 4								*Meloidogyne javanica*							
	Cucumis africanus				*Cucumis myriocarpus*				*Cucumis africanus*				*Cucumis myriocarpus*			
	DSM (g)	DRM (g)	VL (cm)	SD (mm)	DSM (g)	DRM (g)	VL (cm)	SD (mm)	DSM (g)	DRM (g)	VL (cm)	SD (mm)	DSM (g)	DRM (g)	VL (cm)	SD (mm)
0	71.8	6.8	129.9	5.1	133.4	4.3	1342.7	6.2	68.2	6.7	134.3	5.0	118.8	2.8	1433.5	6.3
200	73.8	9.2	145.9	5.1	124.7	3.3	1368.0	6.4	72.9	7.0	143.2	5.0	108.5	2.3	1556.0	5.1
600	80.9	8.5	146.2	5.0	120.1	4.0	1219.8	6.4	71.5	6.1	146.0	5.0	125.8	2.9	1054.5	6.4
1000	77.3	8.6	158.6	5.1	154.2	5.5	1276.7	6.0	66.6	7.7	139.5	5.0	131.5	2.8	1598.7	6.0
1 400	76.2	9.7	159.6	5.0	112.6	4.2	1167.8	6.9	70.9	6.2	126.0	5.0	99.6	3.2	1216.7	6.3
1800	80.0	12.4	158.6	5.0	121.2	4.4	1233.2	6.2	65.4	7.8	130.9	5.0	125.0	4.1	1717.7	6.5
2200	90.9	9.4	152.1	5.1	126.4	4.6	1007.8	6.9	64.1	7.0	126.3	5.1	123.1	2.7	1596.7	6.2
$LSD_{0.05}$	17.4	4.3	26.2	0.2	38.4	1.5	422.56	1.8	15.4	1.9	26.7	0.2	32.0	1.2	679.7	1.3

4.4 Discussion

The less than one RF values of *M. incognita* race 4 and *M. javanica,* supported by the failure of root galls to develop, suggested that the two test nematodes did not feed and reproduce on any of the two *Cucumis* species. In both *Cucumis* species, for both test nematodes, few root galls, when present, hardly developed. Results confirmed those of *M. incognita* race 2, where the two plant species were non-host under both greenhouse and micro-plot conditions (Chapter 3; Mofokeng, 2005). Knowledge of density-dependent-growth patterns and quadratic equation curves as described previously (Chapter 3), are important in investigations of host-status and host-sensitivity in plant-parasitic nematology. Consequently, although screening for resistance to plant-parasitic nematodes could be done at one level of inoculation, nematode resistance cannot be conclusively established at one level of inoculum.

Using the regression relationships of RF values and Pi, Ferris (1985) and others (Chapter 3; Duncan and McSorley, 1987; Elston *et al.*, 1991) demonstrated that RF and Pi levels had density-dependent relationships. Generally, biological entities respond to extrinsic or intrinsic factors through quadratic relationships (Chen *et al*., 2005; Mafeo and Mashela, 2011; Mamphiswana *et al*., 2010; Salisbury and Ross, 1992). Good fits of RF values versus $\log_{10}$ (Pi + 1) were observed in the study with relationships depicting convex-shaped quadratic relationships, which confirmed some of the relationships observed previously (Chapter 3). Also, it should be indicated that for *M. incognita* the relationships were, in this study, similar for *C. africanus* and *C. myriocarpus,* which was

probably due to the reduction of inoculation levels further below 500 eggs and J2s as recommended previously (Chapter 3).

The convex quadratic curves as observed in this and the other study (Chapter 3) were important in several ways. First, the maximum reproductive multiplication rates in all trials were less than one, which is indicative of non-host status. Secondly, the density-dependent growth pattern of plant-parasitic nematodes also occurred in the two non-host *Cucumis* species. In short, all the principles of density-dependent growth patterns (Salisbury and Ross, 1992) were observed in nematode-resistant *Cucumis* species.

In most plant species where resistance involves endo-parasitic nematodes, resistance forms were described as either being pre- or post-infectional (Acedo *et al*., 1984; Huang 1986; Ibraham *et al*., 1980; Raja and Dasgupta, 1986; Steele and Savitsky, 1981; Weischer, 1982). Aggregated RPIs, which were all greater than one for both nematode tests in both plant species except for *M. javanica* in *C. africanus*, suggested that the resistance form in the two plant species was post-infectional, which confirmed results of *M. incognita* race 2 infection in *C. myriocarpus* trials (Pofu and Mashela, 2011). Mechanisms of resistance in post-infectional resistance involve the release of phytoalexins for hypersensitivity inside plant tissues (Harborne, 1999), which is either time-linked (Thurau *et al*., 2010) or time-unlinked (Wallace, 1971). In hypersensitivity resistance, nematodes are allowed to enter roots, resulting in a situation where cells around the nematode wither and prevent further movements of the nematode inside the

root system (Kaplan and Keen, 1980). In this study, necrotic spots were not observed, suggesting that hypersensitivity did not occur.

Pre-infectional resistance observed in *C. myriocarpus* against *M. javanica* confirmed that of *M. incognita* race 2 in *C. myriocarpus* (Pofu and Mashela, 2011). Also, *Rotylenchulus reniformis* in *Tagetes patula* and *Meloidogyne* juveniles in *T. erecta* (Caswell and Robert, 1991; Ploeg and Marris, 1999), *Pratylenchus* species in *Tagetes species* (Siddiqui and Alam, 1988; Veech, 1981), *M. incognita acrita* in some landraces of "fig-leafed" gourd (*Cucurbita ficifolius)* and African horned cucumber (*Cucumis metuliferus)* plants (Fassuliotis, 1970), all had post-infectional resistance, with no evidence of necrotic spots. Generally, in pre-infectional nematode-resistance, resistance is expressed prior to penetration into roots (Kaplan and Keen, 1980), through exudation of potent chemical compounds into the rhizosphere, whereas in post-infectional resistance, induced-resistance is expressed after penetration.

Sorghum (*Sorghum bicolour* L.), cowpea (*Vigna unguiculata* L.), marigold (*Tagetes species*), castor bean (*Ricinus communis* L.), velvet bean (*Mucuna pruriens* L.) and sunn hemp (*Crotalaria juncea* L.) also released potent chemical compounds into the rhizosphere that inhibited infection by nematode juveniles (McSorley and Gallaher, 1991; Roberts, 1993). In contrast, rootstocks such as *P. trifoliata* and Swingle citrumelo (*Citrus paradisi* × *P. trifoliata)*, allow nematode juveniles of *T. semipenetrans* to penetrate root systems and prevent damage by having cells around the nematode undergoing hypersensitivity (Duncan, 2005). Unlike pre-infectional resistance, post-

infectional resistance as observed in *C. myriocarpus* is introgressible (Kaplan and Keen, 1980) and could be used in plant breeding programmes to develop nematode-resistant genotypes.

In both *Cucumis* species, the aggregated RMIs of *M. incognita* race 4 and *M. javanica* were each greater than unity. Apparently, failure to feed on cells of the two plant species, resulted in J2s being converted to males. In *Meloidogyne* species, maleness is common when root cells are not suitable for juvenile feeding (Ferraz and Brown, 2002). Similar responses occurred when *C. myriocarpus* was infested with *M. incognita* race 2 (Pofu and Mashela, 2011). In *Cucurbita ficifolia* and *Cucumis metuliferus*, within the family Cucurbitaceae, infection by *Meloidogyne* species also increased the tendency towards maleness (Fassuliotis, 1970). Increase in maleness which is an irreversible process whenever feeding is not possible, and is another mechanism through which *Meloidogyne* population densities are reduced, since the process inherently reduces the number of females.

Host-sensitivity describes the responses of host plants to nematode infection (Seinhorst, 1967). Infection by *M. incognita* race 4 and *M. javanica* had no effect on all yield components of the two *Cucumis* species, which confirmed observations of *M. incognita* race 2 on both plant species (Chapter 3). In plant-parasitic nematology, when RF values are less than unity and there is no yield loss, test plants are said to be resistant hosts to test nematodes (Seinhorst, 1967). Using the Seinhorst (1967) model,

both *C. africanus* and *C. myriocarpus* were resistant to *M. incognita* race 4 and *M. javanica*.

4.5 Conclusion

Cucumis africanus and *C. myriocarpus* are resistant to *M. incognita* race 2, *M. incognita* race 4 and *M. javanica*. Since the widely distributed *Meloidogyne* species in South Africa are *M. incognita* races 2 and 4 and *M. javanica* (Kleynhans *et al*., 1996), it can be inferred that the two indigenous *Cucumis* species are resistant to all *Meloidogyne* species in most crop-producing regions of South Africa. Consequently, the two *Cucumis* species have the potential for use in crop rotation systems for the management of *Meloidogyne* species, as well as serving as rootstocks in watermelon production. However, *Meloidogyne*-resistant capabilities of the two *Cucumis* species have not been tested under multi-nematode infestations in the field to assess if this nematode resistance is sustainable.

CHAPTER 5
RESISTANCE OF *CUCUMIS* SPECIES TO *MELOIDOGYNE* SPECIES UNDER MULTI-NEMATODE INFECTIONS

5.1 Introduction

Host-status and host-sensitivity of plants to plant-parasitic nematodes differ in monospecific and multi-nematode population densities (Kraus-Schmidt and Lewis, 1981). In some cases, monospecific resistance is lost under multi-nematode population densities (Kraus-Schmidt and Lewis, 1981). Wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) seedlings were shown to be highly resistant to the southern root-knot nematode (*Meloidogyne incognita*) at various levels of inoculation under monospecific nematode population densities in the greenhouse and the micro-plot (Chapter 3,4; Mofokeng, 2005; Pofu and Mashela, 2011).

Challenges of validating endo-parasitic nematode resistance under field conditions are that total root systems per plant and soil volumes occupied by root systems can hardly be estimated with accuracy. Thus, under field conditions, one cannot estimate the final nematode population density (Pf) - an indispensable estimate for computing reproductive factor (RF) values, which are used as indicators for host-status in plant-nematode relations (Seinhorst, 1967). In contrast, for ecto-parasitic nematodes, the initial nematode population density (Pi) and Pf are each estimated from a unit subsample soil (Duncan and McSorley, 1987). Using a similar unit to estimate Pi and Pf for endo-parasitic nematodes may not be justifiable because soil Pf may be lower just because most of the second-stage juveniles (J2s) had earlier penetrated into the root system and could not be retrieved from the unit soil subsample at harvest.

Another challenge, which might result in confounding the interpretation of nematode-resistance capabilities under field conditions, is the existence of multi-nematode infestations, which may altogether have negative effects on the test plant (Eisenback and Griffin, 1987), resulting into potential loss of nematode resistance. Also, under field conditions weeds may confer to the invading nematode juveniles a wide selection in terms of hosts of different age groups, with diverse nematode-attraction cues.

A stepwise regression model, using the backward elimination approach, may serve as a useful tool for testing variables as observed in nematode resistance under field conditions, where numerous potential interacting factors are at play (Draper and Smith, 1981), including plants of different ages, variation in soil, multi-nematodes, etc. Khan *et al*. (2000) demonstrated that infection of *M. incognita* in tomato seedlings decreased with the age of the transplants. The influence of multi-nematode infection and the age of *Cucumis* seedlings on resistance to *Meloidogyne* species are not documented. Therefore, the objective of this study was to validate the host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* seedlings at three different ages to *M. incognita* race 2 under field conditions with multi-nematode infestations.

5.2 Materials and methods

Field experiments were carried out at the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E), with a Hutton sandy loam (65% sand, 30% clay, 5% silt) containing 1.6% organic C, with EC = 0.148 dS m^{-1} and pH(H_2O) = 6.5. The site is characterised by having hot dry summers (November–January), with

maximum day temperature (MDT) range of 28-38°C and cool dry winters (May–July), with DMT range of 5-18°C. Two experimental plots were established at 20 m away from each other and 15 m away from a 10-year old citrus trial.

The experimental sites were initially cropped with a nematode-susceptible tomato (*Solanum lycorpersicum*) cv. 'Floradade' in order to ensure even distribution of *Meloidogyne* species. A day after transplanting, each tomato plant was inoculated by dispensing 860 *M. incognita* race 2 eggs and second-stage juveniles (J2s), which were prepared by extracting from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) plants in 1% NaOCl (Hussey and Barker, 1973). A 20-ml plastic syringe was used to place eggs and J2s into 2½-cm-deep holes on cardinal points of the stems.

Tomato plants were uprooted 56 days after inoculation and primary land preparation achieved through levelling using hand-held forks and rakes. Each plot of 5 m^2 comprised five *Cucumis* seedlings at 1 m × 1 m, with the first and the last seedlings of each *Cucumis* species in a row being a border plant, while the middle three plants with different ages within a row were used for data collection. The study was conducted in autumn (February–April) 2009, without being repeated. *Cucumis* seedlings were raised in the greenhouse as described previously (Chapter 3).

Experimental design and cultural practices

Treatments, *viz*. 3-, 4½- and 6-week-old seedlings of *C. africanus* and *C. myriocarpus* were transplanted in their respective trial sites. In each plant species, treatments were arranged in a randomised complete block design, with 12 replicates. After planting, the site was irrigated to field capacity using overhead sprinkler irrigation system and three days later, sampled for initial nematode population density (Pi). Each soil sample consisted of five composited cores (2.5 cm d × 20 cm deep) per plot, which were collected within plant rows in a systematic pattern. Total soil cores were mixed and nematodes extracted from a 250 ml soil subsample using the sugar-floatation and centrifugation method (Jenkins, 1964). In the *C. africanus* trial, the mean Pi comprised 103 *Meloidogyne* species and 23 ring nematodes (*Criconema mutabile* [Taylor, 1936] Raski and Luc, 1985), whereas in *C. myriocarpus* trial, the mean Pi consisted of 158 *Meloidogyne* species, 27 *C. mutabile* and 38 spiral nematodes (*Helicotylenchus dihystera* Cobb, 1893).

Two days after transplanting, 5 g 2:3:2 (22) and 2 g 2:1:2 (43) fertilizer mixtures were applied around each seedling to provide essential nutrients elements described previously (Chapter 3). Seedlings were irrigated initially for 28 days with 1 L tapwater per plant every other day, which was then increased to 2 L until harvest. Weeds were removed using hand-held hoes when necessary. Total rainfall during the trial period was 6 mm. Pests and diseases were not observed.

Data collection

At harvest, 56 days after inoculation, vine length was measured, stem cut off at soil level and stem diameter measured at 3 cm above the severed end using a digital vernier caliper. Shoots were oven-dried for at 70°C for 72 h and recorded. Root samples per plant were collected, immersed in water to remove soil particles and blotted dry. Nematodes were extracted from 10 g roots per plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey and Barker, 1973) and passed through nested 150-µm, 45-µm and 25-µm-pore sieves. One L soil sample was collected around the roots of each plant, with nematodes being extracted from a 250-ml soil subsample (Jenkins, 1964). Juveniles were counted from a 10-ml aliquot using a stereomicroscope.

Data analysis

Data for reproductive factor (RF) values for the three nematode species were subjected to analysis of variance using SAS software (SAS Institute, Inc., Cary, NC., U.S.A., 2008). Using stepwise regression model, plant age was eliminated in all variable candidates of nematode and therefore, the data were pooled across the three age groups (n = 36). Nematode variables were subjected to stepwise regression analysis in order to determine the relation among different nematode species on one another (Draper and Smith, 1981). Also, yield variables were subjected to stepwise regression analysis in order to determine the effect of different nematode species on yield components. Unless otherwise stated, only predictive models which were significant at the probability level of 5% were discussed.

5.3 Results

Partitioning the degrees of freedom and their associated sum of squares suggested that the nematode treatment contributed 35% ($P \leq 0.05$) to TTV on RF values of *Meloidogyne* species on *C. africanus* and 18% ($P \geq 0.10$) on *C. myriocarpus* (data not shown). Using 250 ml soil subsample as a unit of nematode population density, the soil RF values for *C. mutabile* on *C. africanus* and *C. myriocarpus* were approximately 26 and 3, respectively, whereas those for *Meloidogyne* species were 0.86 and 0.46, respectively (Table 5.1). Soil RF value for *H. dihystera* on *C. myriocarpus* was approximately one.

Table 5.1 Initial and final population densities in the soil (250 ml) with reproductive factors of *Criconema mutabile, Helicotylenchus dihystera* and *Meloidogyne* species under *Cucumis africanus* and *Cucumis myriocarpus* production at 56 days after transplanting (n = 36).

Nematode	*Cucumis africanus*			*Cucumis myriocarpus*		
Species	Pi	Pf	RF	Pi	Pf	RF
C. mutabile	23	590	25.65	27	87	3.22
H. dihystera	-	-	-	38	41	1.08
Meloidogyne spp.	103	89	0.86	158	73	0.46

RF = Pf/Pi, where Pf = final population density of nematodes in 250 ml subsample soil and Pi = initial population density of nematodes in 250 ml soil subsample.

Predictive models for nematode densities

In the *C. africanus* trial, nematode variables did not predict the variability of other nematode species (data not shown), whereas in the *C. myriocarpus* trial, Pf variables predicted the variability of other nematode species (Table 5.2). In the ensuing results,

only predictive models for nematodes on *C. myriocarpus* trial were described. Also, since the root-knot nematode was not identified to the species level, it was referred to as *Meloidogyne* species.

Predictive model for *Meloidogyne* in soil

In the predictive model for *Meloidogyne* in soil, the nematode variables, *viz. Meloidogyne* in root, *C. mutabile* and *H. dihystera*, contributed 98% to the TTV in *Meloidogyne* population density in soil (Table 5.2). The predictive model, generated from the associated coefficients (Table 5.2), was summarised as follows: $Meloidogyne_{soil} = 0.53 + 1.64\ Meloidogyne_{root} + 0.27\ C.\ mutabile - 0.58\ H.\ dihystera.$

Briefly, the model suggested that when *C. mutabile* and *H. dihystera* were held constant, for each additional unit of *Meloidogyne* in root*, Meloidogyne* in soil increased on average by 1.64 units. Alternatively, when *Meloidogyne* in root and *H. dihystera* were held constant, for each additional unit of *C. mutabile, Meloidogyne* in soil increased on average by 0.27 units. However, when *Meloidogyne* in root and *C. mutabile* were held constant for each additional unit of *H. dihystera, Meloidogyne* in soil decreased on average by 0.58 units.

Predictive model for *Meloidogyne* in root

In the predictive model for *Meloidogyne* in root, *C. mutabile, H. dihystera* and *Meloidogyne* in soil variables contributed 99% to the TTV in *Meloidogyne* population density in root (Table 5.2), with the predictive model being: $Meloidogyne_{root} = 0.359 -$

0.18 *C. mutabile* + 0.40 *H*. *dihystera* + 0.58 *Meloidogyne* soil (Mashela and Nthangeni, 2002).

In this model, when *C. mutabile* and *H. dihystera* were held constant, for each additional unit of *Meloidogyne* in soil, *Meloidogyne* in root increased on average by 0.58 units. Also, when *C. mutabile* and *Meloidogyne* in soil were held constant, for each additional unit of *H. dihystera, Meloidogyne* in root increased on average by 0.40 units. Similarly, when both *Meloidogyne* in root and soil were held constant, for each additional unit of *C. mutabile, H. dihystera* increased on average by 0.44 units and when *Meloidogyne* in soil and *C. mutabile* were held constant, for each additional unit of *Meloidogyne* in root, *H. dihystera* increased on average by 2.01 units. In contrast, when *Meloidogyne* in root and *H. dihystera* were held constant, for each additional unit of *C. mutabile, Meloidogyne* in root decreased on average by 0.18 units. Similarly, when *H. dihystera* and *C. mutabile* were held constant, for each additional unit of *Meloidogyne* in soil, *H. dihystera* decreased on average by 1.03 units.

Predictive model for *Helicotylenchus dihystera*

In the predictive model for *H. dihystera*, *Meloidogyne* in root, *Meloidogyne* in soil and *C. mutabile*, contributed 98% to the TTV in *H. dihystera* population densities in soil (Table 5.2), with the model being: *Helicotylenchus dihystera* = –2.87 + 2.01 $Meloidogyne_{root}$ – 1.03 $Meloidogyne_{soil}$ + 0.44 *C. mutabile.*

When *Meloidogyne* in root and in soil were held constant, for each additional unit of *H. dihystera, C. mutabile* increased on average by 0.44 units. Similarly, when *C. mutabile* and *Meloidogyne* species in soil were held constant, for each additional unit of *H. dihystera*, *Meloidogyne* in root increased by 2.01 units. In contrast, when *Meloidogyne* in root and *C. mutabile* were held constant, for each additional unit of *H. dihystera*, *Meloidogyne* in soil decreased on average by 1.03 units.

Predictive model for *Criconema mutabile*

In this model, *Meloidogyne* in root, *Meloidogyne* in soil and *H. dihystera*, contributed 98% to the TTV in *C. mutabile* population densities in soil (Table 5.2), with the model being: *Criconema mutabile* = 7.19 – 4.55 $Meloidogyne_{root}$ + 2.39 $Meloidogyne_{soil}$ + 2.21 *H*. *dihystera.*

Table 5.2 Predictive stepwise regression models for *Meloidogyne* in soil, *Meloidogyne* in root, *Helicotylenchus dihystera* and *Criconema mutabile* on multi-nematode infestations in *Cucumis myriocarpus seedlings* at 56 days after transplanting (n = 36).

Nematode	Variable	Coefficient	t-value	P ≤	R^2
Meloidogyne$_{soil}$	Constant	0.53	0.16	-	0.98
	Meloidogyne$_{root}$	1.64	9.78	0.01	
	C. mutabile	0.27	3.31	0.02	
	H. dihystera	–0.58	–3.01	0.02	
Meloidogyne$_{root}$	Constant	0.36	0.19	-	0.99
	C. mutabile	–0.18	–5.26	0.01	
	H. dihystera	0.40	4.84	0.01	
	Meloidogyne$_{soil}$	0.58	9.78	0.01	
H. dihystera	Constant	–2.87	–0.69	-	0.96
	Meloidogyne$_{root}$	2.01	4.84	0.01	
	Meloidogyne$_{soil}$	–1.03	–3.01	0.02	
	C. mutabile	0.44	16.20	0.01	
C. mutabile	Constant	7.19	0.78	-	0.98
	Meloidogyne$_{root}$	–4.55	–5.26	0.01	
	Meloidogyne$_{soil}$	2.39	3.31	0.02	
	H. dihystera	2.21	16.20	0.01	

The model demonstrated that, when *Meloidogyne in* root and soil were held constant, for each additional unit of *H. dihystera, C. mutabile* increased on average by 2.21 units (Table 5.2). Similarly, when *Meloidogyne* in root and *H. dihystera in soil* were held constant, for each additional unit of *Meloidogyne* in soil, *C. mutabile* in soil increased on average by 2.39 units. In contrast, when the two ecto-parasitic nematodes were held

constant, for each additional unit of *Meloidogyne* in root, *C. mutabile* decreased on average by 4.55 units.

Predictive models for yield components

In the *C. africanus* trial*, C. mutabile* was the only predictor of dry shoot mass and fresh fruit mass, where the nematode species increased the two yield components (Table 5.3). However, in the *C. myriocarpus* trial, all three nematode species were not predictors of any yield component (Table 5.4).

Table 5.3 Predictive stepwise regression models of yield components in *Cucumis africanus* infested with *Criconema mutabile, Helicotylenchus dihystera* and *Meloidogyne* species under multi-nematode infestations at 56 days after transplanting (n = 36).

Component	Variable	Coefficient	t-value	P ≤	R^2
Dry shoot mass (g)	Constant	177.98	8.26	-	0.50
	C. mutabile	0.09	3.13	0.01	
Fresh fruit yield (g)	Constant	712.99	5.60	-	0.32
	C. mutabile	0.37	2.18	0.05	
Vine length (m)	Constant	9.73	8.44	-	0.24
	C. mutabile	0.01	1.80	0.10	
Vine-diameter (mm)	Constant	10.66	24.43	-	0.22
	C. mutabile	9.80	1.68	0.12	

Table 5.4 Predictive stepwise regression models for *Cucumis myriocarpus* dry shoot mass, fruit yield, plant length and stem diameter in soil infested with *Criconema mutabile, Helicotylenchus dihystera* and *Meloidogyne* species at 56 days after transplanting (n = 36).

Component	Variable	Coefficient	t-value	P ≤	R^2
Dry shoot mass	Constant	189.80	3.50	-	0.22
(g)	*C. mutabile*	0.89	1.10	0.31	
	H. dihystera	–0.88	–0.55	0.60	
Fresh fruit yield	Constant	317.87	3.13	-	0.06
(g)	*Meloidogyne*$_{root}$	–1.43	–0.45	0.66	
	H. dihystera	1.11	0.63	0.55	
Vine length (m)	Constant	313.83	6.77	-	0.37
	Meloidogyne$_{root}$	8.50	1.91	0.97	
	Meloidogyne$_{soil}$	–7.29	–2.01	0.08	
Vine diameter	Constant	8.05	15.22	-	0.31
(mm)	*C. mutabile*	0.01	1.67	0.14	
	Meloidogyne$_{root}$	0.01	0.30	0.77	

5.4 Discussion

The non-host status of both *Cucumis* species to *Meloidogyne* species confirmed results in greenhouse and micro-plot studies (Chapter 3,4; Mofokeng, 2005; Pofu and Mashela, 2011). In contrast, RF values of two ecto-parasitic nematodes suggested that the two *Cucumis* species were hosts. In South Africa, *C. mutabile* and *H. dihystera* were associated with various field and horticultural crops (Kleynhans *et al*., 1996). However, this is the first record of the association of the two ecto-parasitic nematodes with *C.*

africanus and *C. myriocarpus*. The source of the two ecto-parasitic nematodes was apparently the adjacent citrus trial, since citrus is an established host for both *C. mutabile* and *H. dihystera* (Kleynhans *et al.*, 1996).

In the *C. myriocarpus* trial, the predictive models demonstrated that the population densities of *Meloidogyne* species, *C. mutabile* and *H. dihystera* either had stimulatory or antagonistic effects, depending on whether *Meloidogyne* numbers were in soil or roots. *Meloidogyne* in soil stimulated *C. mutabile* population numbers, but inhibited those of *H. dihystera*. In contrast, *Meloidogyne* in roots simulated populations of *H. dihystera* and inhibited those of *C. mutabile*. Generally, host-status responses of a plant to a nematode species may be altered by the presence of another nematode species (Eisenback and Griffin, 1987), with either stimulatory or suppressive effects. Kraus-Schmidt and Lewis (1981) observed that the spiral nematode (*Scutellonema brachyurum*) on cotton at 60 days after inoculation increased population densities of *M. incognita*, whereas the cotton nematode (*Hoplolaimus columbus*) suppressed population densities of the same nematode species. Similarly, in interaction of *M. hapla* and *C. xenoplax* on grape seedlings, the reproductive potential of *M. hapla* was inhibited, whereas that of *C*. *xenoplax* was stimulated (Santo and Bolander, 1977). Populations of *C. mutabile* stimulated those of *H. dihystera, vice versa*. Reasons for inhibition and stimulation are not clear, although it appears to be a general phenomenon on pests relying on the same organ for feeding and development.

Generally, upon egress in root tissues, J2s of *Meloidogyne* species migrate into the soil, where they locate new roots for infection (Ferraz and Brown, 2002). Migration may explain some of the observed interactions between soil and root stages of *Meloidogyne* species with *C. mutabile* and *H. dihystera*.

Reproduction of an endo-parasitic nematode could also be suppressed by an ecto-parasitic nematode through direct or indirect competition for feeding sites (Eisenback and Griffin, 1987). The number and quality of feeding sites in roots for an endo-parasitic nematode can be reduced if the nematode is attracted to and penetrated a feeding site which was previously used by the ecto-parasitic nematode. Generally, ecto-parasitic nematodes reduce the number and quality of feeding sites available for endo-parasitic nematodes by physically damaging the root systems. For instance, *Paratrichodorus minor* (Colbran, 1964) and *M. naasi* feed near root tips and compete for feeding sites on the basis of first come first served (Franklin, 1965). Also, *Tylenchorynchus arri* (internet) and *P. minor* on creeping bentgrass (*Agrostis stolonifera* L.) reduced infection of *M. naasi* since they compete for the same feeding sites (Sikora *et al.,* 1979), with all three test nematodes in this study preferring root tips (Eisenback and Griffin, 1987).

Meloidogyne species had no effect on all yield components measured, confirming results under greenhouse and micro-plot conditions (Chapter 3,4; Mofokeng, 2005; Pofu and Mashela, 2011). Except for *C. mutabile* which increased dry shoot mass and fresh fruit mass of *C. africanus*, the two ecto-parasitic nematodes had no effect on yield components of the two *Cucumis* species. In plant-parasitic nematology, two or more

nematodes may act dependently or independently of each other on yield components of the host plant (Johnson and Nusbaum, 1970). Actually, before the nematode damage threshold population density is attained, most plant-parasitic nematodes have stimulatory effect on yield variables (Mashela, 2002; Mashela and Nthangeni, 2002; Wallace, 1971). Apparently, *C. mutabile,* which stimulated *C. africanus* fruit yield and dry shoot mass, was still below the damage threshold density for this plant species.

Using the Seinhorst (1967) model as described previously (Chapter 3), *Meloidogyne*-*Cucumis* inter-relations in this study fit the description of the resistance concept, which implies that *C. africanus* and *C. myriocarpus* were resistant to the test *Meloidogyne* species. Also, the observation confirmed those under greenhouse and micro-plot conditions, where both *Cucumis* species were resistant to *M. incognita* races 2 and 4, along with *M. javanica* (Chapter 3,4; Mofokeng, 2005; Pofu and Mashela, 2011). In contrast, the two *Cucumis* species were tolerant to *C. mutabile* and *H. dihystera*.

5.5 Conclusion

Under multi-specific nematode infestations, *C. africanus* and *C. myriocarpus* retained their resistance capabilities to *Meloidogyne* species, but were tolerant to *C. mutabile* and *H. dihystera*. Consequently, the two *Cucumis* species could successfully serve as seedling rootstocks for watermelons in fields with multi-nematode infestations. However, caution should be taken to ensure that the successor crops are not susceptible to *C. mutabile* and *H. dihystera*. In this and the previous studies (Chapter 3,4), it became apparent that *C. africanus* and *C. myriocarpus* are highly resistant to *Meloidogyne*

species in South Africa under diverse environments. Consequently, detailed studies on inter-generic grafting with watermelon cultivars should ensue in order to establish whether the observed nematode resistance could be retained in inter-generic grafting of *Citrullus* cultivars on *Cucumis* species.

CHAPTER 6
INTER-GENERIC GRAFTING OF *CITRULLUS* CULTIVARS ON *CUCUMIS* SPECIES

6.1 Introduction

Under greenhouse, micro-plot and field conditions, wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) seedlings were highly resistant to *Meloidogyne incognita* races 2 and 4 and *M. javanica* (Chapter 3,4,5; Mofokeng, 2005; Pofu and Mashela, 2011), which are predominant in watermelon-producing regions of South Africa (Kleynhans *et al*., 1996). *Cucumis* species and *Citrullus* species are both within the family Cucurbitaceae (Pitrat *et al*., 1999), with *Cucumis* species being indigenous to South Africa (Kristkova *et al*., 2003), whereas the genus *Citrullus* is endemic to the Kalahari Desert, in south-western Africa (Jarret *et al*., 1996).

Inter-specific (species-to-species) grafting technique was widely used in plant production for managing soil-borne diseases (Lee, 1994). However, in some instances, species within the same genus do not have genotypes that are resistant to pathogens and/or nematodes. For instance, all species within the genus *Citrullus* do not have genotypes that are resistant to *Verticilium oxysporum* and *Meloidogyne* species, which are both highly injurious to *C. lanatus* cultivars (Davis, 2005, Oda *et al.,* 1997). Genera within the same family have since conferred some limited promises in inter-generic (genus-to-genus) grafting technique for suppression of soil-borne pathogens due to incompatibility limitations.

Inter-generic grafting is replete with contradictions on influences of seedling rootstocks on survival, flowering, fruit production, bio-mass, fruit quality and accumulation abilities of essential nutrient elements in scions (Colla *et al.,* 2006a,b; Davis *et al*., 2008; Flores *et al*., 2010; Rouphael *et al*., 2010; Traka-Mavrona *et al*., 2000). The latter, for instance, may result in deficiency and/or toxicity of ions for certain essential nutrient elements in scions. *Citrullus-Cucumis* grafting technique would be more appealing to watermelon-producing farmers in South Africa, because since 1996, South Africa has penetrated the watermelon export market (Anon., 2011b), which might in the long-term be lucrative, but demanding in terms of the ISO 9001 specifications (Corbett *et al*., 2005). Due to the withdrawal of eco-unfriendly synthetic nematicides, alternatives in the management of plant-parasitic nematodes are highly desirable.

In addition to suppressing *Meloidogyne* species, *Citrullus-Cucumis* grafts would be more attractive to watermelon growers if they were to improve fruit yield and quality, without having deleterious effects on accumulation abilities of essential nutrient elements in the scion component. The objective of this study, therefore, was three-fold: (i) to investigate the compatibility of *Citrullus-Cucumis* grafts in a series of greenhouse trials and to determine the influence of *Citrullus-Cucumis* grafting on (ii) nematode resistance and (iii) productivity of *Citrullus* cultivars.

6.2 Materials and methods

Three separate studies were conducted to achieve the above sub-objectives. Materials and methods, including results for each sub-objective were described separately in

order to enhance clarity. However, the discussion integrated the findings of the three sub-objectives.

6.2.1 *Citrullus-Cucumis* grafting and compatibility

Location and preparation of materials

The experiment was conducted in the greenhouse at the Agricultural Research Council – Institute for Industrial Crops (ARC – IIC), Rustenburg, North West Province, South Africa (25°43'40"S, 27°17'30"E) in late spring (September–October 2010). Only the technique which was successful is being described. Fruits of *C. africanus* and *C. myriocarpus* were collected and seeds extracted and prepared for sowing as described previously (Chapter 3). Seeds of *Cucumis* species were sown in a 160-hole seedling tray containing Hygromix (Hygrotech, Pretoria North, South Africa) and irrigated daily to field capacity. Seven days after sowing *Cucumis* seeds, primed seeds of watermelon cultivars 'Congo' and 'Charleston Gray' were sown at one seed/cone in separate 200-hole seedling trays containing Hygromix and irrigated daily to field capacity. Ungrafted *Citrullus* (A & B) and *Cucumis* (C & D) seedlings are illustrated to demonstrate reduced and increased stem diameters, respectively (Figure 6.1).

Grafting was performed 14 days after emergence of watermelon seedlings using the modified approach method (Cushman, 2006). Briefly, rootstock and scion seedlings were removed from their respective seedling trays, with rootstock being cut at 45° upward underneath the axis and scions cut at 45° downward just above the same height. The two companions were snagged into each other; joined using the grafting

(foil) peg and set in two adjacent cones of the 160-hole seedling trays, with Hygromix added to firm the seedlings (Figure 6.2). Five days after setting, parts of watermelon and those of *Cucumis* species below and above the graft union, respectively, were severed.

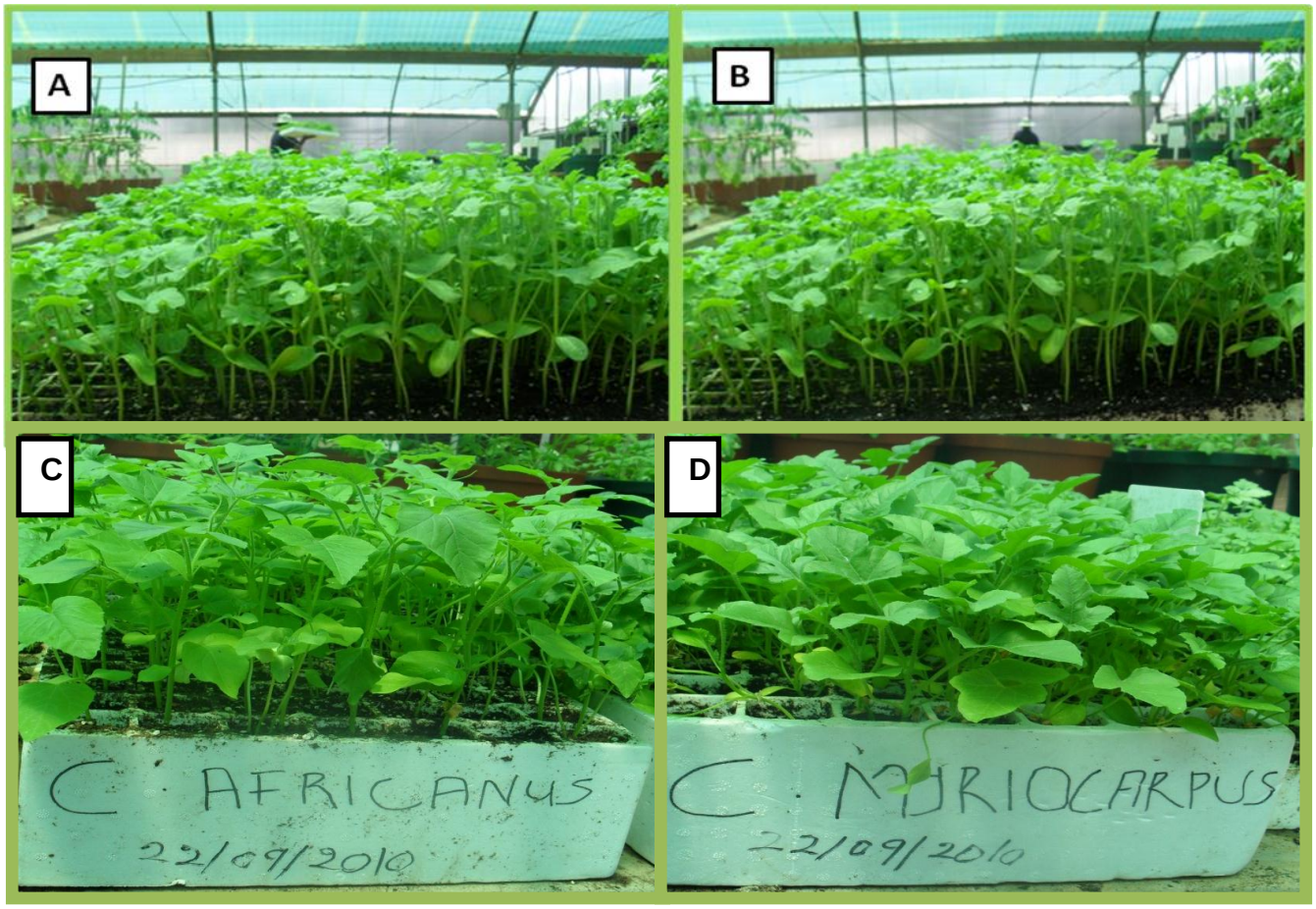

Figure 6.1 Seedling scions of *Citrullus* (A & B) and rootstocks of *Cucumis* (C & D) sown in 200- and 160-holes seedling trays, respectively, for equating stem diameters of two genera.

Figure 6.2 Two watermelon cultivars grafted onto *Cucumis africanus* and *C. myriocarpus*, joined using grafting pegs (above) and grafting foils (below).

Data collection

At harvest, 25 days after grafting, the graft union was marked with a grafting clip for measuring stem diameter from the clip to 5 cm above (D_2) and 5 cm below (D_1) using a digital vernier caliper. Survival of the grafted seedlings was also recorded.

Data analysis

Data were subjected to analysis of variance with SAS software (SAS Institute, Inc., Cary, N.C., U.S.A.). Mean separation was achieved through the Waller–Duncan multiple-range test.

6.2.2 *Citrullus-Cucumis* grafting and nematode resistance

Location and preparation of materials

The experiments were carried out during summer (November–January) 2010/2011 growing season under greenhouse conditions at the Plant Protection Skills Centre, University of Limpopo (UL), South Africa (23°53'10"S, 29°44'15"E) and at the ARC – IIC. Seedlings were prepared as described in the compatibility trials. Five days after severing the components of unwanted scions and rootstocks, grafts were transplanted into 20-cm-diameter plastic pots containing 2 700 ml growing mixture of steam-pasteurised sand and Hygromix at 3:1 (v/v). From planting of seedling rootstocks to transplanting, a total of 24 days were required. Cultivars ‘Congo’ and ‘Charleston Gray’ experiments were conducted concurrently.

Experimental design and cultural practices

Three treatments in the cv. ‘Congo’ experiment, *viz*. control (cv. ‘Congo’ alone), cv. *‘*Congo’ grafted onto *C. africanus* and cv. ‘Congo’ grafted onto *C. myriocarpus*, were arranged in a randomised complete block design, with 10 replicates. The cv. ‘Charleston Gray’ experiment, with similar experimental design, ran alongside the cv. ‘Congo’ experiment. A week after transplanting, seedlings were fertilised using 3 g 2:3:2

(22) and 2 g 2:1:2 (43) per pot which provided a total of essential nutrient elements described previously (Chapter 4). Approximately 300 ml tapwater was applied when the three Hadeco Moisture Meters (Hadeco Magic®, New Delhi, India) readings averaged below 2 units. The greenhouse whitefly (*Trialeurodes vaporariorum*) was sprayed with 1.33 ml Lebaycid (a.i. fenthion 50% ml) per litre water on biweekly basis.

Nematode inoculum was prepared by extracting eggs and second-stage juveniles (J2s) from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus*) plants in 1% NaOCl (Hussey and Barker, 1973). On transplanting day, each treatment was inoculated by dispensing ca. 1 000 *M. incognita* race 2 eggs and J2s using a 20-ml plastic syringe by placing into 2½-cm-deep holes on cardinal points of the base of the vines and then covering with the growing mixture.

Data collection

Survival of the grafts was recorded from grafting through harvest, at 56 days after inoculation. At transplanting and harvest, diameter of scions at 5 cm above the grafting union (D_2) and rootstocks at 5 cm below the grafting union (D_1), were measured using a digital vernier caliper. Stem diameter data were used to compute the quotient (D_2/D_1) of the graft union (Traka-Mavrona *et al*., 2000). Vine length was measured from the crown end of the stem to the tip of the vines, with all measurements per plant added together to constitute vine length. Shoots were oven-dried at 70°C for 72 h for dry matter determination. Root and soil nematodes were sampled and extracted using the maceration and blending method (Hussey and Barker, 1973) and the sugar-

centrifugation method (Jenkins, 1964), respectively. The reproductive factor (RF = Pf/Pi) values were computed, where Pf = final nematode population density and Pi = initial nematode population density, which was 1 000 eggs and J2s.

Data analysis

RF, root gall, data within a particular cultivar were subjected to analysis of variance with SAS software (SAS Institute, Inc., Cary, NC., U.S.A., 2008) and mean separation achieved using Fisher's least significant difference test. Where the interaction between locations for variables measured in each *Citrullus-Cucumis* combination was not significantly ($P \leq 0.05$) different, the data were pooled (n = 20) and subjected to statistical analysis. Unless otherwise stated, treatment means were different at the probability level of 5%.

6.2.3 *Citrullus-Cucumis* grafting and productivity

Location

Field experiments were conducted during summer (November–January) in 2010/2011 at two locations, *viz*. the Plant Protection Skills Centre, UL and the ARC – IIC. The UL location had Hutton sandy loam (65% sand, 30% clay, 5% silt) containing 1.6% organic C, with EC = 0.148 dS m^{-1}, pH(H_2O) = 6.5. The UL location has hot dry summers, with maximum day temperature (DMT) ranging from 28 to 38°C. Soil at the ARC – IIC location comprised 81% sand, 13% clay and 6% silt, containing 1.1% organic C, with EC = 0.152 dS m^{-1}, pH(H_2O) = 6.7, with hot dry summers and DMT ranging from 26 to

35°C. Average monthly rainfall during the growing period at the UL and the ARC – IIC locations averaged 50 mm and 80 mm, respectively.

Preparation of materials

Seedlings were raised and grafting was performed as described in compatibility trials. Each location was initially cropped with nematode-susceptible tomato (*Solanum lycorpersicum* L.) cv. 'Floradade', which was inoculated after transplanting by dispensing approximately 1 000 *M. incognita* race 2 eggs and J2s per plant. Eggs and J2s were prepared and inoculated as explained previously (6.2.2). Tomato plants were uprooted at 56 days after inoculation and primary land preparation done. Each plot of 22.5 m^2 comprised 10 seedlings at inter- and intra-row spacing of 1.5 m and 1.5 m, respectively. The first and the last seedlings in a row were used as border plants, with the middle eight plants within a row being used for harvesting and measurements.

Experimental design and cultural practices

Six treatments, *viz*. cv. 'Congo' alone, cv. 'Charleston Gray' alone, cv. 'Congo' onto *C. africanus,* cv. 'Congo' onto *C. myriocarpus*, cv. 'Charleston Gray' onto *C. africanus* and cv. 'Charleston Gray' onto *C. myriocarpus*, were arranged in a randomised complete block design, with 10 replicates. Irrigation was scheduled using four Hadeco Moisture Meters (Hadeco Magic®, New Delhi, India), with plants being irrigated when the average reading was below 2 units using drip irrigation system for 2 hours.

Two days after transplanting, soil samples were collected for the determination of Pi. Each soil sample was collected in a systematic pattern between the plants within a row and comprised five composited cores of 2.5-cm diameter at 20-cm deep per plot. Nematodes were extracted from a 250 ml soil subsample (Jenkins, 1964). In the UL and the ARC–IIC trials the mean Pi comprised 230 and 375 *Meloidogyne* species, respectively. Fertilisation and pest management were executed as prescribed for commercial watermelon production (Hygrotech Planting Chart, 1998, unpublished). Weeds were removed using hand-held hoes until full coverage (Figure 6.3).

Figure 6.3 *Citrullus-Cucumis* grafts under field conditions at 40 days after transplanting.

Data collection

Flowering and fruit set under field conditions were scored at 4, 6 and 8 weeks after transplanting. At harvest, 66 days after transplanting, soil samples for the determination of the final population nematode densities (Pf) were collected per plant as described under greenhouse conditions (6.2.2). Vine length was measured as described earlier

(6.2.2) and the graft union was marked with a grafting clip for measuring stem diameter from the clip to 5 cm above (D_2) and 5 cm below (D_1) the graft union using a digital vernier caliper. Shoots were cut from the crown, oven-dried for 72 h at 70°C, weighed and leaves ground in a Wiley mill to pass through a 1-cm-opening sieve. Root galls were assessed using the North Carolina Differential Scale (Taylor and Sasser, 1978). Nematodes were extracted from 10 g roots/plant by maceration in a blender for 30 s in 1% NaOCl solution and then passed through a nested series of 150-, 63- and 45-µm-pore sieves onto a 25-µm-pore sieve (Hussey and Barker, 1973). Eggs and juveniles from the 25-µm-pore sieve were collected for counting under the stereomicroscope. Essential nutrient elements in mature leaves were quantified (Jones, 1997).

Data analysis

Prior to analysis, data for numbers of nematodes, flower, fruits and micronutrients were transformed with $\log_{10}(x + 1)$ in order to normalise the data (Gomez and Gomez, 1984), but untransformed data were reported. All data were subjected to analysis of variance with SAS software (SAS Institute, Inc., Cary, NC., U.S.A., 2008), followed by mean treatment separation using Waller–Duncan multiple-range test or Fisher's least significant different test. The interaction between locations for each *Cucumis* species was not significantly different ($P \leq 0.05$) and data were pooled ($n = 20$) and subjected to statistical analysis. Only data where the F-test was significant ($P \leq 0.05$) are discussed, unless otherwise indicated.

6.3 Results

6.3.1 *Citrullus-Cucumis* grafting and survival

Survival of grafts after using the new technique from grafting to transplanting was 100%. Stem diameters above (D_2) and below (D_1) the graft union, as shown by the D2/D1 quotient, were comparable in size at termination of this trial (Table 6.1).

Table 6.1 Quotients of watermelon cultivars 'Congo' and 'Charleston Gray' scions raised in 200-hole seedling tray and *Cucumis africanus* and *Cucumis myriocarpus* rootstocks raised in 160-hole seedling trays 25 days after grafting (n = 20).

Scion-rootstock combination	stem diameter (mm)		Quotient (D_2/D_1)
	Rootstock (D_1)	Scion (D_2)	
cv. 'Congo' alone	5.33	5.33	1.0
Onto *C. africanus*	5.37	5.33	1.0
Onto *C. myriocarpus*	5.31	5.39	1.0
LSD $_{0.05}$	0.18	0.23	-
cv. 'Charleston Gray' alone	5.34	5.34	1.0
Onto *C. africanus*	5.38	5.33	1.0
Onto *C. myriocarpus*	5.34	5.36	1.0
LSD $_{0.05}$	0.11	0.19	-

6.3.2 *Citrullus-Cucumis* grafting and nematode resistance

Similarly, survival of grafts after using the new technique from grafting to harvest at 56 days after transplanting was 100% (data not shown). The interaction between seasons for the two trials was not significant and data were pooled (n = 20) and subjected to

statistical analysis. In the cv. ‘Congo’ experiment, the partitioning of the degrees of freedom and their associated sum of squares demonstrated that the nematode treatment contributed 70% to the total treatment variation (TTV) on RF values of *M. incognita* race 2, whereas in the cv. ‘Charleston Gray’ experiment the treatment contributed 78% to TTV on RF values of the test nematode (Appendix 6.2).

In the cv. ‘Congo’ experiment, relative to the control, the *C. africanus* and *C. myriocarpus* rootstocks reduced RF values by 92% and 93%, respectively (Table 6.2). In the cv. ‘Charleston Gray’ experiment, the rootstocks reduced the RF values by 94% and 96%, respectively. In the cv. ‘Congo’ experiment, relative to the control, the *C. africanus* and *C. myriocarpus* rootstocks reduced root galls by 96% and 94%, respectively (Table 6.3). Similarly, in the cv. ‘Charleston Gray’ experiment, the rootstocks reduced root galls by 98% and 90%, respectively. All yield components measured on grafts and controls were not different (Table 6.4). Also, with the exception of cv. ‘Charleston Gray’ grafted on *C. myriocarpus*, the stem diameters above the graft unions at transplanting were not different from those below the graft union, with the result that the stem diameter quotients were approximately equal to one. However, at harvest, stem diameters above the graft union were significantly ($P \leq 0.01$) larger than those below the graft union (data not shown).

Table 6.2 Rootstock effect on reproductive factor (RF) values of *Meloidogyne incognita* race *2* on ungrafted watermelon cultivars and grafted onto *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under greenhouse conditions at 56 days after inoculation with 1 000 nematodes (n = 20).

Seedling	Cultivar 'Congo'		Cultivar 'Charleston Gray'	
Rootstock	RF	Relative effect (%)	RF	Relative effect (%)
Ungrafted control	7.58a	–	7.07a	–
Cucumis africanus	0.62b	–92	0.45b	–94
Cucumis myriocarpus	0.54b	–93	0.29b	–96

Relative effect = [(Rootstock/Control) – 1) × 100]

Table 6.3 Rootstock effect on number of root galls induced by *Meloidogyne incognita* race *2* on ungrafted watermelon cultivars and grafted onto *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under greenhouse conditions at 56 days after inoculation with 1 000 nematodes (n = 20).

Seedling	Cultivar 'Congo'		Cultivar 'Charleston Gray'	
Rootstock	Root gall	Relative effect (%)	Root gall	Relative effect (%)
Ungrafted control	4.8a	–	4.6a	–
C. africanus	0.2b	–96	0.16b	–98
C. myriocarpus	0.3b	–94	0.4b	–90

Relative effect (%) = [(Rootstock/Control) – 1) × 100]

Table 6.4 Yield components of watermelon cultivars 'Congo' and 'Charleston Gray' grafted on *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under greenhouse conditions at 56 days after inoculation with 1 000 eggs and juveniles of *Meloidogyne incognita* race 2 (n = 20).

Plant growth variable	Treatment			
	Control	*C. africanus*	*C. myriocarpus*	$LD_{0.05}$
Watermelon cv. 'Congo'				
Dry vine mass (g)	14.65	16.05	15.36	6.40
Vine length (cm)	337.33	369.83	381.33	52.00
Stem diameter-above (mm): D_2	5.45	4.20	4.18	1.17
Stem diameter-below (mm): D_1	5.45	4.57	5.03	0.88
Stem diameter quotient (D_2/D_1)	1.00	0.92	0.83	0.97
Watermelon cv. 'Charleston Gray'				
Dry vine mass (g)	14.68	14.52	16.62	8.10
Vine length (cm)	381.33	403.33	353.67	54.66
Vine diameter-above (mm): D_2	5.50	3.58	3.52	2.48
Vine diameter-below (mm): D_1	5.50	4.98	5.38	0.82
Stem diameter quotient (D_2/D_1)	1.00	0.72	0.65	0.95

6.3.3 *Citrullus-Cucumis* grafting and productivity

From grafting to harvest, all grafts survived (data not shown). Using a 250-ml soil subsample as a unit of nematode population density, the soil RF values for *Meloidogyne* species at both locations were above one for intact watermelon plants and below one for the grafted plants. Relative to ungrafted cv. 'Congo', *C. africanus* and *C. myriocarpus* rootstocks grafted with cv. 'Congo' reduced RF values by 98% and 96%, respectively, whereas in the cv. 'Charleston Gray' the two rootstocks reduced the RF values by 97% and 95%, respectively (Table 6.5).

Table 6.5 Rootstock effect on reproductive factor (RF) values of *Meloidogyne incognita* race *2* on ungrafted watermelon cultivars and grafted onto *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under field conditions at 56 days after inoculation with 1 000 nematodes (n = 20).

Seedling	Cultivar 'Congo'		Cultivar 'Charleston Gray'	
Rootstock	RF	Relative effect (%)	RF	Relative effect (%)
Ungrafted control	11.05a	–	12.41a	–
Cucumis africanus	0.21b	–98	0.32b	–97
Cucumis myriocarpus	0.47b	–96	0.68b	–95

Relative effect = [(Rootstock/Control) – 1) × 100]

Four weeks after transplanting, relative to cv. 'Congo' alone, *C. africanus* and *C. myriocarpus* rootstocks increased flowering by 70% and 81%, respectively (Table 6.6). In cv. 'Charleston Gray' the rootstocks increased the variable by 96% and 77%, respectively.

Sixty-six days after transplanting, relative to cv. 'Congo' alone, *C. africanus* and *C. myriocarpus* rootstocks increased fresh fruit yield of cv. 'Congo' by 46% and 60%, respectively, whereas the two rootstocks each increased fresh fruit yield of cv. 'Charleston Gray' by 48% and 115%, respectively (Table 6.7). Relative to ungrafted watermelon cultivars, the two rootstocks increased dry shoot mass in cv. 'Congo' by 50% and 66%, respectively, whereas in cv. 'Charleston Gray' dry shoot mass was increased by 30% and 104%, respectively.

Rootstocks had no effect on vine length and stem diameter (Table 6.8). In all treatments, the stem diameter quotients were approximately equal to unity. The treatment had no effect on accumulation of essential nutrient elements in leaves, except for Mn and Zn (Table 6.9). *Cucumis africanus* rootstock increased leaf Mn in cv. 'Congo', but leaf Mn was not different in cv. 'Congo' alone and cv. 'Congo' grafted onto *C. myriocarpus*. However, leaf Mn in cv. 'Congo' grafted onto *C. myriocarpus* was higher than that in cv. 'Charleston Gray' alone and all its scion-rootstock combinations. In contrast, *C. myriocarpus* rootstock increased the accumulative capability of leaf Zn in cv. 'Charleston Gray', which was different from that of cv. 'Congo' grafted onto *C. myriocarpus* and cv. 'Charleston Gray' alone.

Table 6.6 Flower induction and fruit set in watermelon cultivars ‘Congo’ and ‘Charleston Gray’ grafted on nematode-resistant *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under field conditions at four, six and eight weeks after transplanting (n = 20).

Treatment	Number of flowers			Number of fruits	
	Week 4	Week 6	Week 8	Week 6	Week 8
‘Congo’ alone	2.00 (0.67b)	10.50 (2.01a)	3.50 (1.28a)	0.80 (0.41a)	1.30 (0.70a)
Onto *C. africanus*	2.40 (1.14a)	11.60 (2.49a)	5.30 (1.77a)	1.20 (0.69a)	1.80 (0.94a)
Onto *C. myriocarpus*	3.30 (1.21a)	11.10 (2.35a)	7.10 (1.75a)	0.60 (0.36a)	1.30 (0.70a)
‘Charleston Gray’ alone	2.20 (0.77b)	13.80 (2.64a)	8.00 (1.76a)	1.20 (0.69a)	1.80 (0.94a)
Onto *C. africanus*	4.10 (1.51a)	10.30 (2.03a)	3.10 (1.14a)	0.70 (0.40a)	1.00 (0.60a)
Onto *C. myriocarpus*	3.70 (1.36a)	11.10 (2.11a)	3.80 (1.30a)	1.50 (0.77a)	0.90 (0.57a)

Column means in brackets with the same letter were not different ($P \leq 0.05$) according to Waller–Duncan multiple-range test.

Table 6.7 Fresh fruit mass and dry shoot mass of two watermelon cultivars grafted on two nematode-resistant *Cucumis* seedling rootstocks under field conditions at 66 days after transplanting (n = 20).

Scion-rootstock Combination	Fresh fruit mass (g)	Impact (%)[z]	Dry shoot mass (g)	Impact (%)
'Congo' alone	239.02b[y]	-	62.66b	-
Onto *C. africanus*	347.81a	46	93.73a	50
Onto *C. myriocarpus*	383.41a	60	103.73a	66
'Charleston Gray' alone	240.86c	-	74.90b	-
Onto *C. africanus*	356.13b	48	97.58b	30
Onto *C. myriocarpus*	517.12a	115	153.13a	104

[y]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's least significant different test.

[z]Relative impact = [(Grafted/ungrafted) – 1] × 100

Table 6.8 Vine length, vine diameter and diameter quotient of two watermelon cultivars grafted on two nematode-resistant *Cucumis* seedling rootstocks under field conditions at 66 days after transplanting (n = 20).

Scion-rootstock Combination	Vine length (cm)	stem diameter (mm)		Quotient (D_2/D_1)
		Rootstock (D_1)	Scion (D_2)	
'Congo' alone	166.41	9.85	10.18	1.03
Onto *C. africanus*	198.01	9.46	10.25	1.08
Onto *C. myriocarpus*	237.14	9.36	9.75	1.04
$LSD_{0.05}$	76.73	0.94	0.85	0.53
'Charleston Gray' alone	291.20	10.90	10.19	0.93
Onto *C. africanus*	192.34	10.25	9.53	0.93
Onto *C. myriocarpus*	222.09	9.08	9.05	1.00
$LSD_{0.05}$	36.89	2.28	1.41	0.39

Table 6.9 Selected macro- and micro-nutrient elements in leaves of watermelon cultivars 'Congo' and 'Charleston Gray' grafted on nematode-resistant *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under field conditions at 66 days after transplanting (n = 20).

Scion-rootstock	Macro-nutrient elements (%)					Micro-nutrient elements (ppm)				
Combination	N	P	K	Ca	Mg	B	Cu	Fe	Mn[z]	Zn[z]
'Congo' alone	2.58	0.40	2.41	2.24	0.53	16.61	7.44	108.16	51.94a	45.44b
Onto *C. africanus*	2.48	0.40	2.27	2.40	0.54	17.67	7.17	131.79	55.89a	41.72b
Onto *C. myriocarpus*	2.63	0.39	2.36	1.91	0.51	15.72	6.78	107.42	44.56b	34.44c
'Charleston Gray' alone	2.66	0.39	2.57	2.10	0.47	17.00	7.83	106.74	37.33b	35.44b
Onto *C. africanus*	2.59	0.41	2.40	1.91	0.45	16.28	7.44	107.00	38.24ab	45.28b
Onto *C. myriocarpus*	2.54	0.40	2.49	1.77	0.43	15.72	7.39	107.78	44.44ab	52.50a
$LSD_{0.05}$	2.75	0.42	2.72	2.66	0.61	18.65	8.36	210.21	-	-

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

6.4 Discussion

Under all three studies (*viz*. compatibility of *Citrullus*-*Cucumis* grafts, influence of *Citrullus*-*Cucumis* grafting on nematode resistance and influence of *Citrullus*-*Cucumis* grafting on yield), the 100% survival rates in inter-generic grafts of *C. lanatus* cultivars 'Congo' and 'Charleston Gray' onto *C. africanus and C. myriocarpus*, did not support the view that inter-generic grafting was invariably incompatible, with high mortalities (Tiedermann, 1989). Prior to using the technique described under compatibility of *Citrullus*-*Cucumis* grafts, survival of grafts in this study was less than 36%, translating to 64% mortalities, with the new procedure resulting into a relative survival of 178% [(100/36 − 1) × 100].

In contrast, in inter-generic grafting of melons (*Cucumis melo*) on various lines of squash (*Cucurbita* species), within the family Cucurbitaceae, the scion-rootstock incompatibilities were relatively high, with cultivars 'Lefko Amynteou', 'Thraki', 'Kokkini Banana' and 'Peplo', each grafted onto the landrace 'Kalkabaki' (*C. moschata*), resulting in survival ratios of 52%, 0%, 14% and 8%, respectively (Traka-Mavrona *et al.,* 2000). Similar high percentage mortalities in inter-generic grafts were reported elsewhere (Thies *et al.,* 2010; Tiedermann, 1989).

Tiedermann (1989) identified the major cause of mortalities in inter-generic grafts as being the unequal sizes of stem diameters of scions and rootstocks at the graft union, where the phloem vessels were not able to combine to form a unified vascular bundle. The exceptional survival rates of inter-generic grafts in this

study could be ascribed to the new method of raising seedlings of the two genera for inter-generic grafting as shown by the stem diameter quotients, which were approximately equal to one. The similarity among the stem diameter quotients of grafts at transplanting suggested that the technique did not have physiological effects on the grafts at the early stages of grafting, however at harvesting the scion grew bigger suggesting that it was a time-linked physiological response.

Ungrafted watermelon cultivars under both greenhouse and field conditions had high RF values and root gall numbers, which confirmed the host-status of watermelon cultivars to *Meloidogyne* species (Davis, 2005; Ploeg and Phillips, 2001, Oda *et al*., 1997; Sikora and Fernandez, 2005). RF values lower than one on *Cucumis* rootstocks and the absence of noticeable root galls confirmed results in ungrafted *Cucumis* species where *C. africanus* and *C. myriocarpus* were non-hosts to *M. incognita* races 2 and 4 and *M. javanica* under greenhouse or micro-plot or field trials (Chapter 3,4,5). Root gall numbers and RF values measure the ability of a nematode to develop and to reproduce, respectively (Ferris and Wilson, 1987), all of which are dependent on the ability of the nematode to feed on root tissues. Generally, RF values less than one indicate that the test plant is a non-host to the test nematode (Sasser *et al*., 1984; Seinhorst, 1967). In this study, therefore, grafted *Cucumis* rootstocks retained their non-host status to *M. incognita* race 2.

Host-sensitivity measures yield loss due to nematode infection (Seinhorst, 1967). Fifty-six days after inoculation under greenhouse conditions, all yield components measured in grafted and intact watermelon cultivars were not different. In plant-parasitic nematology, resistance is measured in terms of host-status and host-sensitivity (Seinhorst, 1967). Since the *Cucumis* species did not support nematode reproduction and also, their respective scions did not suffer yield loss due to nematode infection, grafting nematode-susceptible watermelon cultivars 'Congo' and 'Charleston Gray' had, therefore, no influence on nematode resistance capabilities of the two *Cucumis* species against *M. incognita* race 2.

Under field conditions, the two *Cucumis* rootstocks induced early flowering in watermelon cultivars 'Congo' and 'Charleston Gray'. Incidentally, the rootstock-scion combination might have altered the concentration of plant-hormones and, therefore, influenced sex expression and flowering order in inter-generic grafts as observed by Satoh (1996) in cucumber grafted on squash (*Cucurbita moschata* L.) rootstocks. Also, early flowering of inter-generic grafts in this study confirmed observations where watermelon cultivars grafted onto bottle gourd (*Lagenaria siceraria)* flowered earlier than in intact watermelon cultivars (Kurata, 1976; Sakata *et al*., 2007). In contrast, Yamasaki *et al*. (1994) observed that when pumpkin (*Cucurbita maxima*), bottle gourd, wax gourd (*Benin-casa hispida*) and watermelon were each serving as seedling rootstocks to watermelon, all delayed flowering. Grafting of watermelon cultivars 'Congo' and 'Charleston Gray' onto *C. africanus* and *C. myriocarpus* in this study increased fresh fruit yield and dry

shoot mass of watermelon cultivars. However, this observation does not confirm that of Thies *et al*. (2009), where among the evaluated five lines of wild watermelon seedling rootstocks, only one line (RKVL 318) increased fruit numbers of seedless watermelon cv. 'Tri-X 313'. Satoh (1996) proposed that since roots of grafts are different from the original roots of scions, inter-generic grafting might alter the physiology of each of the components, affording different capabilities, which are non-existent in intact plants. The mechanisms involved are currently not clear.

In both watermelon cultivars, inter-generic grafting increased dry shoot mass of scions. Almost always, in other inter-generic grafting studies shoot mass was reduced (Tiedermann, 1989; Traka-Mavrona *et al*., 2000). Generally, watermelons are determinate plants, where flowering curtails shoot growth. Thus, in the field study, experiments were terminated at 66 days after transplanting in order to minimise confounding effects of flowering and treatments on shoot growth. Tiedermann (1989) proposed that the major cause of high mortalities in inter-generic grafts was the unequal stem diameter sizes D_2 and D_1, which resulted in symplastic phloem contact of two companions failing to form a unified vascular bundle. Exceptional survival ratios in this study could be ascribed to the differential procedure of raising seedlings of the two genera, where the larger stem diameter of watermelon was systematically decreased by relatively smaller-sized seedling trays, whereas the smaller stem diameter of *Cucumis* species was increased by larger-sized seedling trays.

The similarity between D_2/D_1 of intact and grafted watermelon at transplanting suggested that the technique did not have physiological effects on grafts during the early stages. However, as shown by D_2/D_1 ratios, which were different between intact and grafted watermelons at harvest, D_2 above the graft union appeared to have grown faster than D_1 below the graft union. The same phenomenon was observed in all grafts regardless of the rootstock used, but did not occur in intact plants, suggesting that it was a time-linked physiological response to grafting, with scant information as to the mechanism involved.

In the field study, grafting affected the accumulation abilities for Mn and Zn in leaves of watermelon scions. Generally, cv. 'Charleston Gray' alone was a poor accumulator of both Mn and Zn. Generally, inter-generic grafting affects the accumulation of mineral ions in various plants (Colla *et al*., 2006a,b). Rouphael *et al*. (2008) demonstrated that relative to intact cucumber cv. 'Akito', the accumulation of Cu in leaf and fruit tissues of cv. 'Akito' grafted onto 'Shintoza'-type rootstock (*C. maxima x C. moschata*) was 15 567% and 31 233%, respectively. Edelstein and Ben-Hur (2006) demonstrated that, when melon-pumpkin grafts were irrigated with marginal water under field conditions, the concentration of B, Zn, Mn and Cu in fruit of melons grafted onto pumpkin were lower in grafts than in control plants, suggesting incompatibility of scions and rootstocks in relation to the union of the vascular bundle (Tiedermann, 1989). Mashela (2002) demonstrated that fruit of *C. myriocarpus* from different regions of Limpopo Province, had excessively high concentrations of Fe, but the high

accumulative ability for Fe in *Cucumis* species was not observed in leaves of the grafts. Probably, leaves and fruits in these plant species have different accumulative abilities for Fe. Root systems of different plant species may have different or similar absorptive capabilities for absorbing essential nutrient elements (Mamphiswana *et al*., 2010). Essentially, failure to detect differences in all macro-nutrient and certain micro-nutrient elements could imply that the root systems of both *Cucumis* and *Citrullus* genera within the family Cucurbitaceae have similar absorptive capabilities.

Generally, cucurbitacin A occurs in roots and seeds of *C. myriocarpus*, whereas cucurbitacin B occurs in all organs of *C. africanus* (Chen *et al.,* 2005). The two phyto-chemicals were implicated in defense mechanisms against plant-parasitic nematodes (Mashela *et al*., 2008). Bales *et al*. (1989) proposed that the root system was responsible for the synthesis of phyto-chemicals that enabled the grafted plants to be resistant to foliar pathogens and that the compounds were transported from roots to shoots. Because the two *Cucumis* seedling rootstocks retained their nematode-resistance capabilities, it is probable that the two potent phyto-chemicals were synthesised or activated in roots of the highly nematode-resistant *Cucumis* species.

6.5 Conclusion

When *Citrullus* seeds were sown in 200-hole seedling trays seven days after sowing *Cucumis* seeds in 160-hole seedling trays, there was 100% survival of

the inter-generic grafts as a result of successfully equating the stem diameters. Under greenhouse conditions, the two *Cucumis* species when serving as seedling rootstocks in inter-generic grafting with highly nematode-susceptible watermelon cultivars ‘Congo’ and ‘Charleston Gray,’ retained their nematode resistance capabilities. Under field conditions, *Cucumis* seedling rootstocks also retained their resistance capabilities to *Meloidogyne* species, with 100% survival, induced earlier flowering and increased productivity in terms of fruit yield and dry shoot mass. Generally, the rootstocks increased the accumulative abilities for Mn and Zn in leaves. The observed attributes confer potential economic benefits on inter-generic grafting technique of *Citrullus* cultivars onto the *Cucumis* seedling rootstocks in areas heavily infested with *Meloidogyne* species. The rootstock not only provided resistance to the *Citrullus* against nematode damage but also enhanced its productivity in terms of earliness to flower, fruit yield and accumulation of some micro-nutrients, in the leaves, especially Zn and Mn.

In all the greenhouse trials, throughout this study (Chapter 3,4,6) the major pest in the production of the two indigenous *Cucumis* species was the greenhouse whitefly. Consequently, it was imperative that the influence of this pest on nematode resistance of *Cucumis* species to *Meloidogyne* species be investigated.

CHAPTER 7
GREENHOUSE WHITEFLY (*TRIALEURODES VAPORARIORUM*) INFECTION BREAKS RESISTANCE TO *MELOIDOGYNE JAVANICA* IN *CUCUMIS AFRICANUS*

7.1 Introduction

Worldwide, the rising temperature and rainfall levels are promoting the resurgent of the greenhouse whitefly (*Trialeurodes vaporariorum*) populations into a serious economic pest of cucurbit, lettuce, pepper, potato, strawberry, sweet potato and tomato under both greenhouse and field conditions (Celix *et al*., 1996; Cohen *et al*., 1992; Duffus *et al*., 1996a,b; Tzanetakis, 2004; Winter *et al*., 1992; Wisler *et al*., 1998). The greenhouse whitefly (GHWF), a viral vector of the genus *Crinivirus*, reduces the longevity and productivity of crops (Tzanetakis, 2004). The genus *Crinivirus* contains viruses that belong to the family Closteroviridae, with linear RNA single-strands, renowned for their unusual capacity to multiply rapidly (Tzanetakis, 2004).

In Limpopo Province, Republic of South Africa, areas with high GHWF population densities have soil conditions that promote rapid reproduction and development of the root-knot nematodes (*Meloidogyne* species), particularly *M. incognita* races 2 and 4, along with *M. javanica* (Kleynhans *et al*., 1996). *Meloidogyne* species can cause substantial yield losses in watermelon (*Citrullus lanatus*) cultivars and oftentimes resulting into complete crop failure (Lamberti, 1979). In South Africa, total production of watermelon for export markets increased from 22 255 tons to 69 170 tons in 1980/81 and 2009/10 growing seasons,

respectively, with the likelihood of expansion since the country entered more the export watermelon markets in 1996 (Anon., 2011b).

Generally, crops are attacked singularly or simultaneously on different and/or same plant organ by various pathogens (Perry *et al*., 2009), with inflicted injuries being either additive or synergestic (Salisbury and Ross, 1992). Effects of disease-inducing organisms on each other may either be stimulatory, neutral or inhibitory (Chapter 5; Eisenback and Griffin, 1987). In most instances, most pest × plant interaction research focuses exclusively on a given pathogen in a particular plant organ, with interactions in a given plant also focusing on one plant organ. Examples for the latter include nematode × nematode (Eisenback and Griffin, 1987), nematode × fungus (Sikora and Carter, 1987), bacteria × fungus (Sikora and Carter, 1987), nematode × bacteria (Mashela and Nthangeni, 2002; Sikora and Carter, 1987), nematode × vesicular arbuscular mycorrhizal fungi (Smith, 1987) and nematode × *rhizobium* (Huang, 1987) interactions – all infecting roots. The approaches, although helpful in providing indispensable information with respect to these pathogens and the plant responses to the related inflicted injury in the roots are inadequate. The approaches provide scanty information as to how certain processes in plants would be affected if pests were simultaneously infecting complementary organs like roots and leaves, which are totally dependent upon each other for essential services.

Meloidogyne species and GHWF infect roots and leaves, respectively, with each having excessively high demand for photosynthates. Evidence exists that defence mechanisms in plants are dependent on the availability of secondary metabolites, with their anabolism having energy-dependent pathways (Inderjit and Malik, 2002; Inderjit *et al*., 1995). GHWF infestation was common in all trials inside the greenhouse, but its effect on nematode resistance is not documented. The objective of this study, therefore, was to determine whether simultaneous infection of leaves by GHWF and infection of roots by *M. javanica* might have any effect on nematode resistance in *C. africanus* seedling rootstocks.

7.2 Material and methods

Study locality and period

The experiment was conducted in the greenhouse at the Agricultural Research Council – Institute for Industrial Crops (ARC – IIC), Rustenburg, North West Province, South Africa (25°43'40"S, 27°17'30"E) in late spring (September–October 2010). Day/night ambient temperatures averaged 28/18^{o}C, with maximum temperatures controlled using thermostatically-activated fans. Fruit of *C. africanus* were collected from a local field, extracted, prepared for sowing and seedlings raised as described previously (Mafeo and Mashela, 2009).

The greenhouse was partitioned into 12 compartments using GHWF-proof nets, with each compartment serving as a 2.5 m^2 plot. Twenty-cm-diameter plastic pots, filled with 2 700 ml steam-pasteurised sand and Hygromix at 3:1 (v/v), were

placed on benches at 0.5 m inter-row and 0.6 m intra-row spacing. Each plant was fertilised using 3 g 2:3:2 (22) and 2 g 2:1:2 (43) per pot which provided the total essential nutrient elements described previously (Chapter 4). Uniform 3-week-old, nematode-free *Cucumis* seedlings were transplanted 1 day after irrigating the growing medium to field capacity with water having EC 2.6 dS m^{-1}.

Experimental design, inoculation and cultural practices

The 2 × 7 factorial experiment was laid out in a split-plot design with six replicates. Main plots constituted GHWF ($GHWF_1$; $GHWF_0$) infestations, whereas subplots comprised seven nematode inoculum levels, *viz.* 0, 200, 600, 1 000, 1 400, 1 800 and 2 200 eggs and second-stage juveniles (J2s). *Meloidogyne javanica* inoculum was prepared by extracting eggs and J2s from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) plants in 1% NaOCl (Hussey and Barker, 1973). One day after transplanting, pots were each infested by dispensing approximate numbers of nematode eggs and J2s using a 20-ml plastic syringe by placing into 2½-cm-deep holes on cardinal points of the stems per replication. Control plants received 20 ml filtrate (25-µm-mesh sieve) of nematode suspension to establish any microbes associated with *M. javanica* in their rhizosphere. Approximately 800 (range 600-1000) GHWF adults from tomato plants growing in a separate greenhouse were captured using a manual digitised suction pump and introduced into the appropriate compartments.

Four sets of Hadeco Moisture Meter (Hadeco Magic®, New Delhi, India) were inserted to 20-cm depths in randomly selected pots to monitor soil moisture tension. Plants were irrigated with 300 ml water dispersed through drip irrigation systems as soon as 50% of the moisture meters had average readings below 2 units. Weekly, plants in compartments without GHWF were slightly shaken, with any stray GHWF removed using the suction pump.

Data collection

Fifty-six days after initiating the treatments, chlorophyll content was measured on three fully matured leaves in the middle of the longest vine using a manual chlorophyll meter. Vine length was measured from the basal ends to the tips of various vines, with the sum constituting vine length per plant. Shoots were cut at the soil level and stem diameters measured 3 cm above the severed ends using a digital vernier caliper. Root systems were removed and gently immersed in water to free soil particles, blotted dry and weighed.

Root galls were assessed using the North Carolina Differential Scale (Taylor and Sasser, 1978). Nematodes were extracted from 5 g roots per plant by maceration in a blender for 30 sec in 1% NaOCl and then passed through a nested series of 150-, 45- and 25-µm-pore sieves (Hussey and Barker, 1973). Eggs and J2s from the 25-µm-pore sieve were poured into 100 ml plastic containers for counting using a stereomicroscope. Soil per pot was thoroughly mixed, a 250-ml soil sample was collected, with nematodes extracted using the sugar-floatation and

centrifugation method (Jenkins, 1964). Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 2 700 ml soil per pot to provide an estimated final nematode population density (Pf). The reproductive factor (RF) values, described as the proportion of the Pf to the initial nematode population density (Pi), were computed.

Shoots and the remaining roots were oven-dried at 70°C for 72 h and dry matter mass recorded. Mature leaves and roots were ground separately in a Wiley mill, passed through a 1-mm sieve, with Na^+ and K^+ quantified (Rhue and Kidder, 1983). A Haake chlorometer (Haake Buchler Instruments, Saddle Brook, NJ) was used to measure Cl^- concentration in leaf and root tissues. Glucose oxidase (Sigma) was used to analyse free glucose in the supernatant (Nelson, 1944). Soluble starch in supernatant and insoluble starch in pellet were analysed with glucose oxidase following a 48 h digestion using amyloglucosidase [Sigma, St. Louis, MO] (Smith, 1981). Arsenomolybdate (Sigma) was used to quantify reducing sugars (Roe *et al*., 1994), with resorcinol reagent used for ketone sugars (Smith, 1981).

Data analysis

Where interaction between seasons for the two trials was not significant ($P \leq 0.05$), data were then pooled ($n = 12$) and subjected to analysis of variance through the SAS software (SAS Institute, Inc., Cary, NC., U.S.A., 2008) to

determine the interactive effects of the main plots ($GHWF_1$; $GHWF_0$) and subplots (Pi). However, treatments were subjected to two sample t-test in order to emphasis the effect of nematodes under GHWF relative to nematodes in the absence of GHWF per level of inoculation.

Reproductive factor (RF) values (y-axis) and the $\log_{10}(Pi + 1)$ values (x-axis) were subjected to lines of the best fit. Responses of RF values to increasing Pi levels were modelled by regression curve estimations resulting to a quadratic equation: $Y = b_2x^2 + b_1x + a$, where Y = RF values, $x = \log_{10}(Pi + 1)$, with $x = -b_1/2b_2$ being the population nematode density where RF was at the maximum and minimum levels for convex and concave quadratic relationships, respectively.

Integrated nematode levels under $GHWF_0$ and $GHWF_1$ treatments were compared using a two sample t-test to determine the relative penetration index (RPI), which is a proportion of nematodes in roots to those in soil (Pofu and Mashela, 2011). Also, males and females were integrated across the nematode levels to determine the sex ratios. Similarly, non-structural carbohydrates and the osmoticum levels (K^+, Na^+, Cl^-) were separately integrated and compared. Unless otherwise stated, the nematode treatment with ($GHWF_1$) and without ($GHWF_0$) the GHWF were compared and discussed at the probability level of 5%. The degrees of freedom and their associated sum of squares were partitioned

(Appendices 7.1 – 7.4) to determine the percentage contribution of sources of variation to the total treatment variation.

7.3 Results

Under GHW_1 nematode infection increased the Pf values from 160% to 1 328% across the six levels of nematode inoculation (Table 7.1). Under $GHWF_0$ and $GHWF_1$ treatments, RF values were below and above one, respectively (Figure 7.1). Also, when RF values were plotted against $\log_{10}(Pi + 1)$, the relationships under $GHWF_0$ and $GHWF_1$ had convex and concave quadratic curves, respectively (Figure 7.1). Nematodes under $GHWF_0$ and $GHWF_1$ accounted for 94% and 64% of the total treatment variation (TTV) of RF values, respectively. Under $GHWF_0$, the maximum RF value of 0.26 was achieved at $\log_{10}(Pi + 1) = x = 3.57$, whereas under $GHWF_1$, the minimum RF value of 0.71 was achieved at $x = 3.91$ (Table 7.2).

The ratios, J2s in root: J2s in soil were under each treatment greater than unity (Table 7.3). Relative to the J2s in roots and soil under $GHWF_0$ treatment, under $GHWF_1$ treatment the variables were higher by 2 275% and 2 329%, respectively. The nematode sex (male: female) ratios under $GHWF_0$ and $GHWF_1$ treatments were 1:1 and 3:1, respectively (Table 7.3). Relative to males and females under $GHWF_0$ treatment, the two variables under $GHWF_1$ were higher by 1 322% and 4 467%, respectively. The $GHWF_1$ treatment decreased the nematode sex ratio by 67%.

Table 7.1 Effect of the greenhouse whitefly (GHWF) on resistance of *Cucumis africanus* seedlings to *Meloidogyne javanica* at different levels of inoculation (Pi) at 56 days after inoculation (n = 12).

	Pf		Impact	
Pi	$GHWF_0$	$GHWF_1$	(%)[z]	P ≤
200	56	514	1 328	0.01
600	156	408	162	0.05
1 000	250	1 070	328	0.05
1 400	350	910	160	0.05
1 800	360	2 484	590	0.01
2 200	396	2 706	583	0.01

[z]Impact (%) = $[(Pf_{with}/Pf_{without} - 1) \times 100$.

Nematodes under $GHWF_1$ had no effect on starch in leaves, but reduced this variable in roots by 25% (Table 7.4). Also, nematodes reduced ketone sugars and increased reducing sugars in both leaves and roots. Under $GHWF_1$ treatment, nematodes had no effect on root Cl^- but decreased K^+ in roots and in leaves, along with Na^+ in roots, but increased Cl^- and Na^+ in leaves (Table 7.5). Nematodes under $GHWF_1$ treatment reduced dry shoot mass and dry root mass, resulting in increase of root/shoot ratios (Table 7.6). Impact of nematodes under $GHWF_1$ treatment on vine length was variable, but the treatment increased the number of root galls from 67% to 200%. $GHWF_1$ treatment reduced chlorophyll content from 63% in control plants to 89% in plants inoculated with nematodes (Table 7.7).

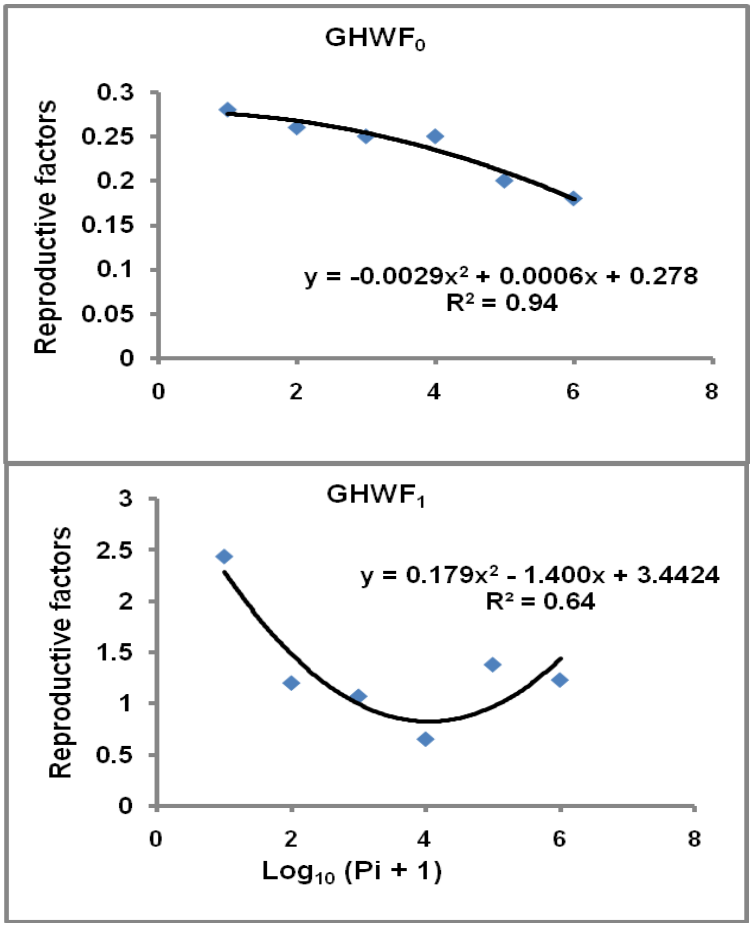

Figure 7.1 Relationship between the reproductive factor (RF) values of *Meloidogyne javanica* on *Cucumis africanus* without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly (GHWF) at 56 days after initiation of the treatments (n = 12).

Table 7.2 Estimates of maximum and minimum multiplication rate for *Meloidogyne javanica* on *Cucumis africanus* without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly under greenhouse conditions at 56 days after initiation of the treatments (n = 12).

Treatment	Model	R^2	x^y	a^z
$GHWF_0$	$y = –0.0029x^2 + 0.0006x + 0.278$	0.94	3.57	Maximum = 0.26
$GHWF_1$	$y = 0.179x^2 – 1.400x + 3.442$	0.64	3.91	Minimum = 0.71

[y]$x = – (b_1/2b_2)$, where in *C. africanus* $b_1 = 0.0006$ and $b_2 = 0.0029$.
[z]a = maximum multiplication rate, determined by substituting the value of x in the model.

Table 7.3 Soil: root ratio of second-stage juveniles (J2s) and sex ratio of *Meloidogyne javanica* on *Cucumis africanus* seedlings without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly (GHWF) at 56 days after inoculation with nematodes (n = 12).

Source of variation	Greenhouse whitefly (GHWF)		Impact[z]
	$GHWF_0$	$GHWF_1$	(%)
Soil J2s	4	95	22.75**
Root J2s	7	170	23.29**
J2s root: soil ratio	1.75	1.79	2.29**
Male	9	128	13.22**
Female	3	137	44.67**
Male: female ratio	3:1	1:1	–67**

[z]Impact (%) = [(with/without) – 1] × 100.

**Impact significant at P ≤ 0.05.

Table 7.4 Non-structural leaf and root carbohydrate of *Cucumis africanus* under *Meloidogyne javanica* infection without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly at 56 days after initiation of the treatments (n = 12).

Organ	Non-structural carbohydrate	Greenhouse whitefly (GHWF)		Impact[x]
		$GHWF_0$	$GHWF_1$	(%)
Leaf	Starch	1.56	1.61	3ns
	Ketone sugars[y]	2.64	2.43	–8**
	Reducing sugars[z]	0.67	1.17	75**
Root	Starch	2.82	2.11	–25**
	Ketone sugars	2.92	2.78	–5*
	Reducing sugars	0.80	0.94	18**

[x]Impact (%) = [(with/without) – 1] × 100; [y] Ketone sugars = sucrose + fructose + fructans; [z] Reducing sugars = fructose + glucose + others; ns = not significant at P ≤ 0.10, * = significant at P ≤ 0.05, ** = significant at P ≤ 0.01.

Table 7.5 Effect of *Meloidogyne javanica* on osmoticum ions on *Cucumis africanus* without ($GHFW_0$) and with ($GHFW_1$) the greenhouse whitefly at 56 days after infection (n = 12).

	Potassium		Chloride		Sodium	
	Root	Leaf	Root	Leaf	Root	Leaf
$GHFW_0$	1.83	1.96	1.22	0.14	0.65	0.15
$GHFW_1$	1.40	1.16	1.15	0.23	0.36	0.32
Impact (%)	–23**	–41**	-6^{ns}	64**	–45**	113**

ns = not significant at P ≤ 0.10, * = significant at P ≤ 0.05, ** = significant at P ≤ 0.01.

Table 7.6 Effect of the greenhouse whitefly (GHWF) on dry shoot mass, dry root mass and root galling of *Cucumis africanus* inoculated with seven inoculum levels (Pi) of *Meloidogyne javanica* at 56 days after the treatments (n = 12).

Pi	Dry shoot mass (g)			Dry root mass (g)			Root/shoot ratio		
	$GHWF_0$	$GHWF_1$	Impact[z] (%)	$GHWF_0$	$GHWF_1$	Impact (%)	$GHWF_0$	$GHWF_1$	Impact (%)
0	68.2	15.6	–77**	6.7	4.1	–38**	0.10	0.26	160**
200	72.9	10.1	–86**	6.0	2.7	–55**	0.08	0.27	238**
600	71.5	10.3	–86**	6.1	3.7	–40**	0.09	0.35	289**
1 000	66.6	14.7	–78**	6.7	3.5	–47**	0.10	0.24	140**
1 400	70.9	11.7	–84**	6.2	4.2	–32**	0.09	0.36	300**
1 800	65.4	10.2	–84**	7.8	3.5	–55**	0.12	0.34	183**
2 200	64.1	9.2	–86**	7.0	2.8	–60**	0.11	0.30	173**

[z]Impact (%) = $[(GHWF_1/ GHWF_0) – 1] \times 100$.

**Treatment means within the same row are significantly different at $P \leq 0.01$ using two sample t-test.

Table 7.7 Impact of the greenhouse whitefly on root galls, vine length and chlorophyll content of *Cucumis africanus* at seven levels of *Meloidogyne javanica* at 56 days after treatments (n = 12).

Nematode	Root gall (scale)			Vine length (cm)			Chlorophyll content		
Level	$GHWF_0$	$GHWF_1$	Impact[z] (%)	$GHWF_0$	$GHWF_1$	Impact (%)	$GHWF_0$	$GHWF_1$	Impact (%)
0	0	1.0	0	107.6	155.3	44**	34.6	28.5	–18**
200	1	1.7	67**	143.2	145.5	2[ns]	39.9	27.7	–31**
600	1	1.8	83**	146.0	127.3	–13[ns]	38.5	31.9	–17**
1 000	1	2.8	183**	139.5	140.2	1[ns]	35.3	28.3	–20**
1 400	1	2.8	183**	126.0	131.1	4[ns]	34.0	30.1	–11ns
1 800	1	3.0	200**	130.9	131.5	0[ns]	39.8	32.0	–20**
2 200	1	3.0	200**	126.3	111.9	–11[ns]	35.3	29.7	–16**

[z]Impact (%) = $[(GHWF_1/ GHWF_0) – 1] \times 100$

**Treatment means within the row were different at P ≤ 0.01 using two sample t-test, ns = treatment means were not different at P ≤ 0.10.

7.4 Discussion

Biological entities respond to extrinsic or intrinsic factors through quadratic relationships (Chen *et al*., 2005; Salisbury and Ross, 1992). Under controlled environments, RF values of various *Meloidogyne* species on $\log_{10}(Pi + 1)$ under *C. africanus* and *C. myriocarpus* cultivation confirmed the quadratic relationships, with curves having characteristics of nematode resistance (Chapter 3,4,5; Pofu and Mashela, 2011). In these curves, RF values started increasing as Pi increased, reached a plateau and then decreased with further increases in Pi, which translated into convex quadratic curves. On the basis of density-dependent relationships (Ferris, 1985), when Pi is already high, RF curves would start by decreasing, reaching a minimum and then increasing, resulting into concave quadrant curves.

Lines of the best fit are important in helping to interpret nematode-plant interactions in terms of the appropriate levels of the inoculum level (Chapter 3,4). Generally, positive and negative linear relationships suggest that the inoculum levels are below and above the Seinhorst (1967) equilibrium points, respectively. Either information is helpful since it helps in the adjustments of inoculum levels to cover points below and above the equilibrium level. Convex and concave quadratic curves are indicators of density-dependent growth patterns (Chen *et al*., 2005). Convex quadratic curve suggests that the maximum multiplication rate (a) shifted further away from the zero Pi, whereas the concave quadratic curve suggests that the maximum multiplication rates shifted backward towards the zero Pi. In this study, since the minimum multiplication rate was 0.71, the a value

was obviously above one, which suggested that *C. africanus* lost its resistance to *M. javanica* under GHWF, whereas the convex quadratic curve in the absence of GHWF suggested that *C. africanus* retained its previously observed status of being resistant to this nematode species.

Plant resistance to phyto-nematodes is a function of plant age, nematode species, biotic and abiotic factors (Seinhorst, 1967). With the exception of biological nematode races which were previously shown to break down the *Mi* gene-resistance in tomato (Ammanti *et al*., 1985; Eddaoudi *et al.*, 1997; Lopèz-Pèrez *et al*., 2006; Roberts and Thomason, 1986; Roberts *et al.,* 1990), limited studies had shown that abiotic factors might reduce resistance to plant-parasitic nematodes. For instance, in citriculture, Mashela *et al*. (1992a) demonstrated that cyclic salinity in the rhizosphere increased populations of the citrus nematode (*Tylenchulus semipenetrans*) in both nematode-susceptible and nematode-resistant citrus seedlings. Dropkin (1969) demonstrated that the *Mi* gene-resistance in tomato was temperature-sensitive and broke down at soil temperatures above 28°C. Most probably, this is the first report demonstrating that a honeydew-inducing insect can break nematode resistance. In addition to GHWF, common honeydew-inducing insects include aphids (family Aphidodae), mealybugs (family Pseudococcidae) and scales (family Coccidae).

The mechanism involved in breaking nematode resistance in *Cucumis* × GHWF inter-relation is not clear. However, honeydew-inducing insects feed by extracting

photosynthates from the phloem, in the process, injecting saliva and reducing the overall turgor pressure in plant cells (Campbell, 1990). Generally, GHWF damage is speedily exacerbated as population densities increase; with aggravated damage occurring as honeydew starts to leak out through infected organs, resulting in the proliferation of epiphytic sooty mould on affected organs Celix *et al*., 1996; Cohen *et al*., 1992; Duffus *et al*., 1996a,b; Tzanetakis, 2004; Winter *et al*., 1992; Wisler *et al*., 1998). Sooty mould might help to explain the incident of reduced chlorophyll content in plants infected with GHWF. Generally, sooty mould reduces photosynthesis rates in affected plants (Agrios, 2005). Also, in plants infected with GHWF, low non-structural carbohydrates might have been due to (i) leakage through honeydew, (ii) ingestion by GHWF and nematodes, (iii) reduced photosynthesis rates due to leaves that were covered with sooty mould and/or (iv) the internal control mechanism of turgor pressure in cells due to increased organic osmolytes.

In their classical study, Mashela and Nthangeni (2002) provided detailed explanation of dynamics involved in mechanisms whereby organic osmolytes affected the distribution of inorganic osmolytes (Na^+, K^+, $Cl^{-)}$ in citrus seedlings in terms of osmotic potential regulation in cells using *T. semipenetrans*, girdling of stems and removal of half-root systems. Reduction of ketone sugars (sucrose + fructose + fructans) in GHWF-infected plants confirmed observations under girdled plants where roots had the lowest of this variable (Mashela and Nthangeni, 2002), suggesting that the variable was related to quantities of

photosynthates arriving at the root system. In various crops, infection by honeydew-inducing insects results in loss of organic materials which include sucrose, which is the form in which carbohydrates are translocated from leaves to roots (Salisbury and Ross, 1992). Inevitably, the loss of sucrose through honeydew and ingestion by the two pests invariably reduced the total available non-structural carbohydrates. Similarly, reduced concentration of starch agreed with the observation that under GHWF there were reduced ketone sugars, more especially sucrose, which is an organic osmolyte, and therefore, there was no need to convert sucrose to starch. In contrast, the increase in reducing sugars (fructose + glucose + other sugars) suggested that GHWF-infected plants were under stress to meet the minimum metabolic requirements, where more glucose had to be produced for metabolic processes. Reduction of K^+ in both roots and leaves might be due to mechanisms involved in osmoregulation, since less sucrose had to be converted to starch (Mashela and Nthangeni, 2002). In contrast, reduced K^+ in the entire GHWF-infected plants might also provide some explanation towards the reduced starch in roots, since K^+ ions are required in the activities of starch synthase, which converts unmetabolised sugars to starch. Also, under GHWF treatment, root/shoot ratios increased, resulting in the altered physiology of the affected plants (Mashela and Nthangeni, 2002), where growth substances were directed towards the regrowth of shoots, which unfortunately, resulted in further loss due to infection by GHWF. Overall, the mechanism involved in breaking down resistance to *M. javanica* in *C. africanus* might include the disruption of organic and inorganic osmolytes required to run both the

primary and secondary metabolic pathways (Inderjit and Malik, 2002; Inderjit *et al*., 1995).

Penetration indices (J2s root: shoot ratios) that were above unity in GHWF-infected *C. africanus* seedlings suggested that since there were more juveniles inside the roots than in the soil, the pre-infectional resistance form observed previously (Chapter 5,6; Pofu and Mashela, 2011), was no longer in existent. Generally, both pre- and post-infectional nematode resistance forms are active processes (Kaplan and Keen, 1980; Veech, 1981), which are dependent on secondary metabolites and energy (Klocke *et al*., 2010; Thurau *et al*., 2010). Under normal circumstances, when food quality is not limiting and infection occurs, the sex ratio in *Meloidogyne* species is less than one, with the skewness towards femaleness (Ferraz and Brown, 2002). High populations of GHWF, which eliminated nematode resistance, resulted in the sex ratio being one, whereas in plants without GHWF, the sex ratio was higher than one, with the skewness towards maleness. The latter is common when J2s of *Meloidogyne* species encounter resistance (Fassuliotis, 1970; Pofu and Mashela, 2011).

The impact of the reduced carbohydrates on nematode resistance in plants may be appreciated if one views structures and pathways through which secondary metabolites are synthesised. Plants in the family Cucurbitaceae contain a total of 12 cucurbitacins, which are secondary metabolites (Chen *et al*., 2005). Cucurbitacin B, which occurs in *C. africanus* (Jeffery, 1978), is a terpenoid used

in plant defence against pathogens (Van Wyk *et al*., 1997). All terpenoids originate from the mevalonic pathways (Inderjit and Malik, 2002; Inderjit *et al*., 1995), which have high demand for energy, carbon and enzymes. Apparently, when sugars are lost through honeydew infection, less becomes available for plant metabolism and therefore, anabolism of secondary metabolites, which are essential in plant defence mechanisms.

7.5 Conclusion

High populations of GHWF broke resistance of *C. africanus* to *M. javanica*, resulting in high population densities of nematodes, imbalancing of organic and inorganic osmolytes and reduced plant yield. Consequently, the successful use of nematode resistance to suppress *Meloidogyne* species using *Cucumis* seedling rootstocks in watermelon production would be dependent upon the successful management of population densities of the greenhouse whitefly. The serious damage of GHWF to melons regardless of rootstock or nematode status is an impetus to manage the pest. This is encouraging for successful employment of resistance.

CHAPTER 8
SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RESEARCH AND CONCLUSION

8.1 Summary

The study focused on five major areas, *viz*. (1) host-status and host-sensitivity of wild watermelon (*Cucumis africanus*) and wild cucumber (*C*. *myriocarpus*) seedlings using a series of inoculation levels of *Meloidogyne incognita* races 2 and 4 and *M. javanica*, which are predominant in South Africa, (2) host-status and host-sensitivity of *C. africanus* and *C*. *myriocarpus* seedlings using a series of inoculation levels of *M. incognita* race 2 under multi-nematode infestations to establish whether the observed nematode resistance was sustainable when the plant was attacked by various pests at the root system level, (3) compatibility of inter-generic grafting of *Citrullus* and *Cucumis* seedlings to determine the potential uses of *Cucumis* species in olericulture and (4) influence of the greenhouse whitefly (*Trialeurodes vaporariorum*) infestation on resistance of *C. africanus* to *Meloidogyne* species to establish whether the observed nematode resistance was sustainable when the plant was attacked by various pests on complementary organs. Summary of the findings are as follows.

8.1.1 *Cucumis*-nematode interactions

The study was designed in such a way that the limitations of using the reproductive factor (RF = Pf/Pi), which is a proportion of final (Pf) and initial (Pi) nematode population densities was minimized. In a total of 17 experiments conducted under diverse environments, RF values of *M. incognita* races 2 and 4

and *M. javanica* invariably demonstrated that *C. africanus* and *C. myriocarpus* seedlings were non-hosts since neither reproduction nor development of nematodes occurred. Also, in the 17 trials, infection of the two *Cucumis* species by the test nematodes did not reduce yield or yield components. On the basis of empirical evidence it was, therefore, concluded that *C. africanus* and *C. myriocarpus* seedlings were resistant hosts to *M. incognita* races 2 and 4 and *M. javanica,* which are widely distributed in the Republic of South Africa (Kleynhans *et al.,* 1996). However, since the ring nematode (*Criconema mutabile*) and the spiral nematode (*Helicotylenchus dihystera*) reproduced on the two *Cucumis* species, without inflicting any damage, the two test plants are, therefore, described as being tolerant to the two exo-parasitic nematode species. Consequently, the two *Cucumis* species should be used with circumspection in managing population densities of *Meloidogyne* species when these species co-exist with the two exo-parasitic nematodes.

8.1.2 Mechanisms of resistance

RF values versus $\log_{10}(Pi + 1)$ of *Meloidogyne* species on the two *Cucumis* species suggested that the relationships were primarily quadratic, which are characteristic of density-dependent growth patterns in biological systems (Salisbury and Ross, 1992). After adjusting Pi levels of inoculation, relationships were primarily depicted by concave quadratic curves, with maximum multiplication rates of below unity, which is an empirical evidence of being non-host (Ferris, 1985). Using the relative penetration index (RPI), *C. africanus* had

mainly pre-infectional resistance form to *Meloidogyne* species, whereas *C. myriocarpus* had post-infectional resistance forms. In *C. africanus*, the potent chemical compound, cucurbitacin B, which is insoluble in water (Chen *et al*., 2005), was actually expected to accord post-infectional resistance, whereas cucurbitacin A in *C. myriocarpus*, which is the only cucurbitacin that is soluble in water (Chen *et al*., 2005), was expected to accord pre-infectional resistance through exudation into the rhizosphere. Nonetheless, resistance in *C. myriocarpus*, which is post-infectional, qualifies for use in introgression plant-breeding programmes for resistance against *Meloidogyne* species.

8.1.3 *Citrullus*-*Cucumis* inter-generic grafting

Watermelon (*Citrullus lanatus*) grafted on *C. africanus* and *C. myriocarpus* constituted inter-generic grafting since the two plants are from different genera, within the family Cucurbitaceae. Inter-generic grafting is characterized by having compatibility limitations, with high mortalities. In this study, inter-generic grafting had initially resulted to 64% mortalities, which was caused by different stem diameters in *Citrullus* cultivars and *Cucumis* species. The limitation was resolved through using seedling trays of different sizes. *Cucumis* and *Citrullus* seedlings were raised in 160-hole and 200-hole seedling trays, respectively, with *Cucumis* planted seven days before primed *Citrullus* seeds. This technique equated the stem diameters of the two genera, resulting into 100% survival of the grafts, which was an improvement of 178%. Also, after grafting, *Cucumis* seedling rootstocks retained their ability to suppress numbers of *Meloidogyne* species.

Under field conditions, *Cucumis* rootstocks induced early flowering and increased both fresh fruit yield and dry shoot mass of watermelon cultivars.

8.1.4 Breaking nematode resistance in *Cucumis* species

The primary insect associated with *Cucumis* species in the greenhouse trials was *T. vaporariorum*, which in trials broke down resistance of *M. javanica* in *C. africanus*. The mechanism was reduced to a complex interference of this pest with non-structural carbohydrates, which in turn resulted to imbalances of osmoticum ions in roots and leaves. Consequently, *T. vaporariorum* population densities must be managed if *Cucumis* species are to be sustainably used as an alternative to methyl bromide in suppression of *Meloidogyne* species in watermelon production.

8.2 Significance of findings

Use of nematode resistance in plants is the most eco-friendly tactic of managing plant-parasitic nematodes. Using adapted indigenous plants like *C. africanus* and *C. myriocarpus* seedlings in South Africa as rootstocks for watermelon cultivars would have enormous and sustainable economic benefits for watermelon growers. Endemic plant species, when they possess nematode resistance to local biological nematode races may be sustainable since this resistance might have developed over many years against existing *Meloidogyne* species and their biological races. Thus, there is little possibility of the material inducing resurgence of virulent pathogens or the resistance being broken by nematode

races. Results of this study also demonstrated that the two *Cucumis* species have different mechanisms of resistance. Although traditional methods of introgressing post-infectional resistance into nematode-susceptible watermelon cultivars had limitations, this study identified post-infectional resistance in *C. myriocarpus*, which could further be assessed for introgression in watermelon cultivars. The technique used to equate the stem sizes at the graft unions, with modifications for other genera, would enhance the use of inter-generic grafting technique in olericulture.

8.3 Future research

Firstly, cucurbitacins accumulate in roots and fruits, particularly in seeds. Since in *Citrullus-Cucumis* the rootstocks retained their nematode resistance capabilities to *Meloidogyne* species, it is necessary to investigate whether cucurbitacins, particularly the highly water-soluble cucurbitacin A, do not accumulate in watermelon fruits, to the detriment of fruit quality. Secondly, producing uniform nematode-resistant *Cucumis* seedlings through tissue culture technique could facilitate the commercialisation of the rootstocks for watermelon production, with the feasibility of establishing watermelon nurseries. Thirdly, introgression of genes from *C. myriocarpus* into watermelon requires the identification of resistance genes in *C. myriocarpus* and further introgression studies, followed by evaluation of nematode resistance. Fourthly, since the study demonstrated that the two *Meloidogyne*-resistant *Cucumis* seedling rootstocks were tolerant to *C. mutabile* and *H. dihystera,* monoculture of watermelons on the two *Cucumis*

rootstocks may build populations of these nematode species to pathogenic levels. Thus, it is essential to investigate the damage threshold of these exo-parasitic nematodes in order to allow for the determination of crop rotational cycles in areas with these nematodes. Fifthly, since there are more than 118 genera within the family Cucurbitaceae, inter-generic grafting using the new procedure developed in this study should be expanded to other genera. Sixthly and finally, further studies between *Meloidogyne* species and the greenhouse whitefly are necessary to provide damage threshold levels for proper management of the two pests.

8.4 Conclusion

Indigenous *Cucumis africanus* and *C. myriocarpus* were resistant to *Meloidogyne* species that occur in watermelon-producing regions of South Africa. Also, as shown by the survival percentages, early flowering, increased fruit yield, increased dry shoot mass and unhindered accumulation of essential nutrient elements in leaves, *C. lanatus* cultivars, the *Cucumis* rootstocks appeared to be highly compatible with *Citrullus* cultivars. Consequently, the two indigenous *Cucumis* species have the potential for use as commercial nematode-resistant rootstocks for the watermelon industry in South Africa.

REFERENCES

ACEDO, J.R., DROPKIN, V.H., and V.D. LUEDDERS. 1984. Nematode population attribution and histopathology of *Heterodera glycines* soybean associations*. Journal of Nematology* 16:48-57.

AGRIOS, G.N. 2005. Plant Pathology. Academic Press: New York.

AMMANTI, M., THOMASON, I.J., and P.A. ROBERTS. 1985. Screening *Lycopersicon* spp. for new genes imparting resistance to root-knot nematodes (*Meloidogyne* spp.). *Plant Disease* 69:112-115.

ANON., 2011a. Abstract of Agricultural Statistics, Department of Agriculture, Forestry and Fisheries. Government Printers: Pretoria.

ANON., 2011b. The U.S. Watermelon Industry, Seed-quest, Central Information. http://www.ers.usda.gov/.

ARAO, T., TAKEDA, H., and E. NISHIHARA. 2008. Reduction of cadmium translocation from roots to shoots in eggplant (*Solanum melongena*) by grafting onto *Solanum torvum* rootstock. *Soil Science and Plant Nutrition* 54:555-559.

BALES, C.L., MARTYN, R.D., and H.D. WILSON. 1989. Iso-enzymes and general protein from various watermelon cultivars and tissue types. *HortScience* 24:810-812.

BALLVORA, A., HESSELBACH, J., NIEWOHNER, J., LEISTER, D., SALAMINI, F., and C. GEBHARDT. 1995. Marker enrichment and high-resolution map of the segment of tomato chromosome VIl harbouring the nematode resistance gene Grol*. Molecular and General Genetics*

249:82-90.

BERENSON, M.L., and D.M. LEVINE. 1996. Basic Business Statistics: Concepts and Applications. Prentice-Hall: Englewood Cliffs, New Jersey.

BIRD, A.F. 1974. Plant response to root-knot nematodes. *Annual Review of Phytopathology* 12:69-85.

BOERMA, H.G., and R.S. HUSSEY. 1992. Breeding plants for resistance to nematodes*. Journal of Nematology* 24:242-252.

BOHN, H.L., McNEAL L., and G.A. O'CONNOR. 1985. Soil Chemistry. Wiley: New York.

BRUESKE, C.H. 1980. Phenylalanine ammonia-lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*. *Physiology* and *Plant Pathology* 16:409-414.

CAMPBELL, N.A. 1990. Biology. Benjamin/Cummings Publisher: Redwood City.

CASTAGNONE-SERENO, P. 2002. Genetic variability of nematodes: A threat to the durability of plant resistance genes? *Euphytica* 124:193-199.

CASWELL, E.P., and L.B. ROBERT. 1991. Ecological Management of Plant-parasitic Nematodes. University of California: Riverside.

CELIX, A., LOPEZ-SESE, A., ALMARZA, N., GOMEZ-GUILLAMON, M., and E. RODRIGUEZ-CEREZO. 1996. Characterization of cucurbit yellow stunting disorders virus, a *Bemisia tabaci*-transmitted Closterovirus. *Journal of Phytopathology* 86:1370-1376.

CHEN, J.F., and J. ADELBERG. 2000. Interspecific hybridization in *Cucumis*: progress, problems and perspectives. *HortScience* 35:11-15.

CHEN, J.C., CHIU, M.H., NIE, R.L., CORDELL, G.A., and S.X. QIU. 2005. Cucurbitacins and cucurbitane glycosides: Structures and biological activities. *Natural Products Report* 22:386-399.

CHITWOOD, D.J. 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agricultural Research Services. *Pest Management Science* 59:748-753.

COHEN, J., FRANK, A., VETTEN, H.J., LESEMANN, D.E., and G. LOEBENSTEIN. 1992. Closterovirus-like particles associated with a whitefly-transmitted disease of sweet potato. *Annals of Applied Biology* 121:257-268.

COHN, E. 1976. Reports on Investigations on Nematode of Citrus and Subtropical fruit crops in South Africa. Report Citrus Subtropical Fruit Resource Institute, Nelspruit.

COLBRAN, R.C. (1964). Studies of plant and soil nematodes. 7. Queensland records of the Order Tylenchida and genera *Trichodorus* and *Xiphinema*. *Queensland Journal of Agricultural Sciences* 21:77-123.

COLLA, G., ROUPHAEL, Y., CARDARELLI, M., WEIGHTA, D., SALERNO, A., and E. REA. 2006a. Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions. *Journal of Horticultural Science and Biotechnique* 81:146-152.

COLLA, G., ROUPHAEL, Y., CARDARELLI, M., and E. REA. 2006b. Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants. *HortScience* 41:622-627.

COOK, R., and K. EVANS. 1987. Resistance and Tolerance. *in:* Brown, R.H., and B.R. Kerry (eds.), Principles and Practice of Nematode Control in Crops. Academic Press: New York.

CORBETT, C.J., MONTES-SANCHO, M.J., and D.A. KIRSCH. 2005. The financial impact of ISO 9000 certification in the United States: An empirical analysis. *Management Sciences* 51:1046-1059.

COTTEN, J., and J.D. HAYES. 1969. Genetic resistance to the cereal cyst nematode (*Heterodera avenae*). *Heredity* 24:596-601.

CRAMMER, C.L., WEISSENBORN, D., COTTINGHAM, C.K., DENBOW, C.J., EISENBACK, J.D., RADIN, D.N., and X. XU. 1993. Regulation of defence-related gene expression during plant pathogen interactions. *Journal of Nematology* 25:507-518.

CUSHMAN, K. 2006. Grafting techniques for watermelons. Florida Cooperatives Extension Services, IFAS, HS1075. University of Florida: Gainesville.

DA COSTA, C.P., and C.M. JONES. 1971. Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis sativus* (L.). *Science* 172:1145-1146.

DANGL, J.L., DIETRICH, R.A., and M.H. RICHBERG. 1996. Death doesn't have

no mercy: Cell death programs in plant-microbe interactions. *Plant Cell* 8:1793-1807.

DAVIS, R.F. 2005. Effect of the Southern Root-knot Nematode on Watermelon Yield. *in*: Kelly, W.T., and D.R. Langston (eds.), Georgia Vegetable Research-Extension Report 2004. University of Georgia: Tifton, GA.

DAVIS, A.R., PERKINS-VEAZIE, P., HASSELL, R., LEVI, A., KING, S.R., and X. ZHANG. 2008. Grafting effects on vegetable quality. *HortScience* 43:1670-1672.

DEVRAN, Z., and M.A. SOGUT. 2011. Characterising races of *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria* in the West Mediterranean region of Turkey. *Crop Protection* 30:451-455.

DE WAELE, D., and A. ELSEN. 2007. Challenges in tropical plant nematology. *Annual Review Phytopathology* 45:457-485.

DI VITO, M., GRECO, N., and A. CARELLA. 1983. The effect of population densities of *Meloidogyne incognita* on the yield of cantaloupe and tobacco. *Nematologia Mediterranea* 11:169-174.

DRAPER, R., and H. SMITH. 1981. Applied Regression Analysis. Wiley: New York.

DROPKIN, V.H. 1969. Cellular responses of plants to nematode infections. *Annual Review Phytopathology* 7:101-122.

DUFFUS, J.E., LIU, H.Y., and G.C. WISLER. 1996a. Tomato infectious chlorosis virus: A new clostero-like virus transmitted by *Trialeurodes vaporariorum*. *European Journal of Plant Pathology* 102:219-226.

DUFFUS, J.E., LIU, H.Y., WISLER, G.C., and R.H. LI. 1996b. Lettuce chlorosis virus: A new whitefly-transmitted Closterovirus. *European Journal of Plant Pathology* 102:591-596.

DUNCAN, L.W. 2005. Nematode Parasites of Citrus. *in*: Luc, M., Sikora, R.A., and J. Bridge (eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI, Wallingford.

DUNCAN, L.W. 2009. Managing Nematodes in Citrus Orchards. *in*: Ciancio, A., and K.G. Mukerji (eds.), Integrated Management of Fruit Crops and Forest Nematodes. Springer Verlag.

DUNCAN, L.W., and R. McSORLEY. 1987. Modelling Nematode Populations. *in*: Veech, J.A., and D.W. Dickson (eds.), Vistas on Nematology. Society of Nematologists: Hyattsville, Maryland.

DUNCAN, L.W., MASHELA, P.W., FERGUSON, J., GRAHAM, J., ABOU-SETTA, M., and M. EL-MORSHEDY. 1995. Estimating crop loss in orchards with patches of mature citrus trees infected by *Tylenchulus semipenetrans*. *Nematropica* 25:43-51.

DUNN, R.A., and G.M. SYDENHAM. 2003. Plant Nematode Biology and Root-knot Nematode Symptoms. University of Florida, IFAS Extension. Gainesville, USA.

EDDAOUDI, M., AMMATI, M., and H. RAMMAH. 1997. Identification of resistance breaking populations of *Meloidogyne* on tomato in Morocco and their effect on new sources of resistance. *Fundamental and Applied Nematology* 20:285-289.

EDELSTEIN, M., BEN-HUR, M., COHEN, R., BURGER, Y., and I. RAVINA. 2005. Boron and salinity effects on grafted and non-grafted melon plants. *Plant Soil* 269:273-284.

EDELSTEIN, M., and M. BEN-HUR. 2006. Use of grafted vegetables to minimize chemicals usage and damage to plant growth and yield quality under irrigation with marginal water. *Acta Horticulturae* 699:159-167.

EISENBACK, J.D., and G.D. GRIFFIN. 1987. Interactions With Other Nematodes. *in*: Veech, J.A., and D.W. Dickson (eds.), Vistas on Nematology. Society of Nematologists: Hyattsville, Maryland.

ELSTON, D.A., PHILLIPS, M.S., and D.L. TRUDGILL. 1991. The relationship between initial population density of potato cyst nematode *Globodera pallid*a and the yield of partially resistant potatoes. *Revue de Nèmatologie* 14:213-219.

ENDO, B.Y. 1965. Histological response of resistant and susceptible soybean varieties, and backcross progeny to entry and development of *Heterodera glycines*. *Phytopathology* 55:375-381.

FAGHIHI, J., VIERLING, R.A., HALBRENDT, J.M., FERRIS, V.R., and J.M. FERRIS. 1995. Resistance genes in a 'Wasiams 82' x 'Hartwig' soybean cross to an inbred line of *Heterodera glycine. Journal of Phytopathology* 61:395-399.

FASSULIOTIS, G. 1977. Self-fertilization of *Cucumis metuliferus* Naud. and its cross-compatibility with melon (*Cucumis melo* L.). *Journal of the American Society for Horticultural Science* 102:336-339.

FASSULIOTIS, G. 1970. Resistance of *Cucumis* spp. to the root-knot nematode, *Meloidogyne incognita acrita*. *Journal of Nematology* 2:174-177.

FERRAZ, L.C.C.B., and D.J.F. BROWN. 2002. An Introduction to Nematodes: Plant Nematology. Pensoft: Sofia-Moscow.

FERRIS, H. 1981. Mathematical Approaches to the Assessment of Crop Damage. *in*: Zuckerman, B.M., and R.A. Rohde (eds.), Plant-Parasitic Nematodes. Academic Press: New York.

FERRIS, H. 1985. Density-dependent nematode seasonal multiplication rates and overwinter survivorship: A critical point model. *Journal of Nematology* 17:93-100.

FERRIS, H., and J.W. NOLING. 1987. Analysis and Prediction as a Basis for Management Decisions. *in*: Brown, R.H., and B.R. Kerry (eds.), Principles and Practices of Nematode Control in Crops. Academic Press: New York.

FERRIS, H., and L.T. WILSON. 1987. Concepts and Principles of Population Dynamics. Division of Nematology, University of California: Davis, CA 95616.

FERRIS, H., CARLSON, H.L., VIGLIERCHIO, D.R., WESTERDAHL, B.B., WU, F.W., ANDERSON, C.E., JUURMA, A., and D.W. KIRBY. 1993. Host-status of selected crops to *Meloidogyne chitwoodii*. *Supplement to the Journal of Nematology* 25:849-857.

FOUCHE, P.S., BESTER, D.H., and G.H., VELDMAN. 1977. The influences of potassium applications and nematicides on the potassium nutrition of

Valencia orange trees on replant citrus soils. *Journal of American Horticultural Science* 102:546-547.

FOURIE, H. 1997. The Influence of Plant-Parasitic Nematodes on Soybean Production in South Africa With Special Reference to Root-knot Nematodes (*Meloidogyne* species). M.Sc. Dissertation Submitted to Potchefstroom University for Christian Higher Education, Potchefstroom, South Africa.

FOURIE, H., and A.H. McDONALD. 2003. Integrated Nematode Control. Annual Research Report of the Agricultural Research Council (ARC) – Grain Crops Institute (GCI), Potchefstroom.

FLORES, F.B., SANCHEZ-BEL, P., ESTAN, M.T., MARTINEZ-RODRIGUEZ, M.M., MOYANO, E., MORALES, B., CAMPOS, J.F., GARCIA-ABELLAN, J.O., EGEA, M.I., FERNANDEZ-GARCIA, N., ROMOJARO, F., and M.C. BOLARÍN. 2010. The effectiveness of grafting to improve tomato fruit quality. *Scientia Horticulturae* 125:211-217.

FRANKLIN, M.T. 1965. A root-knot nematode, *Meloidogyne naasi* sp., on field crops in England and Wales. *Nematologica* 11:9-86.

GANAL, M.W., SIMON, R., BROMMONSCHENKEL, S., PHILLIPS, M.S., TANKSLEY, S.D., and A. KUMAR. 1995. Genetic mapping of a wide spectrum nematode resistance gene (Hero) against *Globodera rostochiensis* in tomato. *Molecular Plant-Microbe Interactions* 8:886-891.

GIEBEL, J. 1974. Biochemical mechanisms of plant resistance to nematodes: A review. *Journal of Nematology* 6:175-184.

GOMMERS, F.J., and D.J.M. VOOR IN'T HOLT. 1976. Chemotaxonomy of compositae related to their host suitability for *Pratylenchus penetrans*. *Netherlands Journal of Plant Pathology* 82:1-8.

GOMEZ, K.A., and A.A. GOMEZ. 1984. Statistical Procedures for Agricultural Research. Wiley: New York.

GRIFFIN, G.D., and W.W. WAITE. 1971. Attraction of *Ditylenchulus dipsaci* and *Meloidogyne hapla* by resistant and susceptible alfalfa seedlings. *Journal of Nematology* 3:215.

GRIFFITHS, H., and M.A.J. PARRY. 2002. Plant responses to water. *Annals of Botany* 89:801-802.

GRUBBEN, G.J.H., and O.A. DENTON. 2004. Plant Resources of Tropical Africa 2. Vegetables. Backhuys Publishers: PROTA Foundation, Wageningen, Netherlands.

HAYNES, R.L., and C.M. JONES. 1976. Effects of the *Bi* locus in cucumber on reproduction, attraction and response of the plant to infection by the southern root-knot nematode. *Journal of the American Society for Horticultural Science* 101:422-424.

HAMMOND-KOSACK, K.E., ATKINSON, H.J., and D.J. BOWLES. 1989. Systemic

accumulation of novel proteins in the apoplast of the leaves of potato plants following root invasion by the cyst nematode *Globodera rostochiensis*, physiological molecular. *Plant Pathology* 35:495-506.

HAMMOND-KOSACK, K.E., and J.D.G. JONES. 1996. Resistance gene-dependent plant defence responses. *Plant Cell* 8:1773-1791.

HARBORNE, J.B. 1999. The comparative biochemistry of phytoalexin induction in plants. *Biochemistry System Ecology* 27:335-367.

HARTMANN, K.M., and J.N. SASSER. 1985. Identification of *Meloidogyne* Species on the Basis of Differential Host Test and Perineal Pattern Morphology. *in*: Barker, K.R., Carter, C.C., and J.N. Sasser (eds.), An Advance Treatise on *Meloidogyne,* Biology and Control. North Carolina State University Graphics: Raleigh.

HIXSON, A.C., CROW, W.T., McSORLEY, R., and L.E. TRENHOLM. 2005. Saline irrigation affects *Belonolaimus longicaudatus* and *Hoplolaimus galeatus* on seashore paspalum. *Journal of Nematology* 37:37-44.

HO, J.Y., WEIDE, R., MA, H., VAN WORDRAGEN, M.F., LAMBERT, K.N., KOORNNEEF, M., ZABEL, P., and V.M. WILLIAMSON. 1992. The root-knot nematode resistance gene (*Mi*) in tomato: Construction of a molecular linkage map and identification of dominant Cdna markers in resistant genotypes. *Plant Journal* 2:971-982.

HSIAO, T.C., and L.K. XU. 2000. Sensitivity of growth of roots versus leaves to water stress: Biophysical analysis and relation to water transport.

Journal of Experimental Botany 51:1595-1616.

HUANG, J.S. 1987. Interaction of Nematodes With Rhizobia *in:* Veech, J.A., and D.W. Dickson (eds.), Vistas on Nematology. Society of Nematologists: Hyattsville, Maryland.

HUANG, S.P. 1986. Penetration, development, reproduction, and sex ratio of *Meloidogyne javanica* in three carrot cultivars. *Journal of Nematology* 18:408-412.

HUANG, C.S. 1985. Formation, Anatomy and Physiology of Giant Cells Induced by Root-knot Nematodes. *in*: Sasser, J.N., and C.C. Carter (eds.), An Advanced Treatise of *Meloidogyne* Species. University Graphics: Raleigh, USA.

HUSSEY, R.S., and K.R. BARKER. 1973. A comparison of methods of collecting inocula of *Meloidogyne* species, including a new technique. *Plant Disease Reporter* 42:865-872.

HUSSEY, R.S., and F.M.W. GRUNDLER. 1998. Nematode Parasitism of Plants. *in:* Perry, R.N., and D.T. Wright (eds.), The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes. CABI, Wallingford.

IBRAHAM, I.K.A., KHALIL, H.A.A., and M.A. REZK. 1980. Development and pathogenesis of *Meloidogyne javanica* in cotton roots*. Nematologia Mediterranea* 8:29-33.

INDERJIT, K., and A.U. MALIK. 2002. Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems. Birkhauser-Verlag: Berlin.

INDERJIT, K., DAKSHINI, K.M.M., and F.A. EINHELLIG. 1995. “Allelopathy: Organisms, Processes and Applications. ACS Symposium Series 582. American Chemical Society: Washington, DC.

JARRET, B., BILL, R., TOM, W., and A. GARRY. 1996. Cucurbits Germplasm Report. Watermelon National Germplasm System, Agricultural Services, United States Department of Agriculture: Washington, DC.

JEFFERY, C. 1978. Cucurbitaceae. *in*: Launert, E. (ed.), Flora Zambesiaca Managing Committee: London, UK.

JENKINS, W.R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.

JOHNSON, A.W., and C.J. NUSBAUM. 1970. Interactions between *Meloidogyne incognita*, *Meloidogyne hapla* and *Pratylenchus brachyurus* in tobacco. *Journal of Nematology* 2:334-340.

JONES, J.B.1997. Plant Nutrition Manual, Boca Raton. CRC Publications: FL, USA.

KALOSHIAN, I., WILLIAMSON, V.M., MIYA., O., LAWN, D., and B.B. WESTERDAHL. 1996. Resistance breaking nematodes identified in California tomatoes. *California Agriculture* 50:18-19.

KAPLAN, D.T., and E.L. DAVIS. 1987. Mechanisms of Plant Incompatibility With Nematodes. *in*: Veech, J.A., and D.W. Dickson (eds.), Vistas on Nematology. Society of Nematologists*:* Hyattsville, Maryland.

KAPLAN, D.T., and N.T. KEEN. 1980. Mechanisms conferring plant incompatibility to nematodes. *Revue de Nèmatologie* 3:123-134.

KELLER, G., and B. WARRACK. 2003. Statistics For Management and Economics. Curt Hinrichs: USA.

KIRNAK, H., KAYA, C., TAS, I., and D. HIGGS. 2001. The influence of water deficit on vegetative growth, physiology, fruit yield and quality in eggplants. *Plant Physiology* 27:34-46.

KHAN, H., AHMAD, R., AKHTAR, A.S., MAHMOOD, A., BASIT, T., and T. NIAZ. 2000. Effect of inoculum density of *Meloidogyne incognita* and plant age on severity of root-knot disease in tomato. *International Journal of Agriculture and Biology* 2:360-363.

KLEIN-LANKHORST, R.M., SALENTIJN, E.M., DIRKSE, J., ARENSDEB, W.G., REUVER, M., and W.J. STIEKEMA. 1994. Construction of a YAC library from a *Beta vulgaris* fragment addition and isolation of a major satellite DNA cluster linked to the beet cyst nematode resistance locus. *Theoretical Journal of Applied Genetics* 89:426-434.

KLEMENT, Z., FARKAS, G.L., and L. LOVREKOVICH. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in tobacco leaf. *Phytopathology* 54:474-477.

KLEYNHANS, K.P.N., VAN DER BERG, E., SWART, A., MARAIS, M., and N.H. BUCKLEY. 1996. Plant Nematodes in South Africa. Handbook No. 8. Plant Protection Research Institute: Pretoria.

KLOCKE, E., NOTHRAGEL, T., and G. SCHUMANN. 2010. Vegetables. *in*: Widholm, J.M., Lorz, H., and T. Nagata (eds.), Biotechnique in Agriculture and Forestry. Springer Heidelberg: New York.

KRAUS-SCHMIDT, H., and S.A. LEWIS. 1981. Dynamics of concomitant populations of *Hoplolaimus columbus*, *Scutellonema brachyurum*, and *Meloidogyne incognita* on cotton. *Journal of Nematology* 13:41-48.

KREIKE, C.M., DEKONING, J.R.A., VINKE, J.H., VAN OOIJEN, J.W., GEBHARDT, C., and W.J. STIEKEMA. 1993. Mapping of loci involved in quantitatively inherited resistance to the potato cyst-nematode *Globodera rostochiensis*. *Pathotype Ro 1. Theoretical and Applied Genetics* 87:464-470.

KREIKE, C.M., DEKONING, J.R.A., VINKE, J.H., VAN OOIJEN, J.W., and W.J. STIEKEMA. 1994. Quantitatively-inherited resistance to *Globodera pallida* is dominated by one major locus in Solanum spegazzinii. *Theoretical and Applied Genetics* 88:764-769.

KRISTKOVA, E., LEBEDA, A., VINTER V., and O. BLAHOUSEK. 2003. Genetic resources of genus *Cucumis* and their morphological description. *HortScience* 30:14-42.

KURATA, K. 1994. Cultivation of grafted vegetables: Development of grafting Robots in Japan. *HortScience* 29:810-812.

KURATA, H. 1976. Studies on the sex expression of flowers induced by day-length and temperature in pumpkin and watermelon. *Memba Faculty of Agriculture, Kagawa University* 29:1-49.

KWAYE, R.G., MASHELA, P.W., SHIMELIS, H., and N. MAPOPE. 2008.

Determination of *Tylenchulus semipenetrans* biotype in Zebediela and Champagne, Republic of South Africa*. Plant Disease* 92:639-641.

LAMBERTI, F. 1979. Chemical and Cultural Control. *in:* Lamberti, F., and C.E. Taylor (eds.), Root-knot Nematodes (*Meloidogyne* species) Systematics, Biology and Control. Academic Press, London.

LAMBERT, K., HUSSEY, R.S., and K.R. BARKER. 1990. A comparison of methods of collecting inocula of *Meloidogyne* species including a new technique. *Plant Disease* 42:865-872.

LAMBRIDES, C.J., and J.F. MILLER. 1998. Inheritance of root-knot nematode resistance in a source of MC29 maize germplasm. *Journal of Crop Science* 34:1225-1230.

LEE, J.M. 1994. Cultivation of grafted vegetable. 1. Current status, grafting methods and benefits. *HortScience* 29:235-239.

LEE, J.M. 2003. Advances in vegetable grafting. *Chronica Horticulturae* 43:13-19.

LEEDY, P.D., and J.E. ORMROD. 2005. Practical Research: Planning and Design. Pearson Education, New Jersey.

LITTLE, T.M., and F.J. HILLS. 1981. Statistical Methods in Agricultural Research. University of California: Davis.

LIU, D.L., AN, M., JOHNSON, I.R., J.V. LOVETT. 2003. Mathematical modelling of allelopathy. III. A model for curve-fitting allelochemical dose responses. *Nonlinearity in Bio.Tox. Med.,* 1: 37-50.

LOPÈZ-PÈREZ, J.A., LE STRANGE, M., KALOSHIAN, I., and A.T. PLOEG. 2006. Differential response of *Mi* gene-resistant tomato root-stocks to root-knot nematodes (*Meloidogyne incognita*). *Journal of Crop Protection* 25:382-388.

MAFEO, T.P. 2006. Propagation, Fertilization and Irrigation of *Cucumis myriocarpus.* Masters Dissertation Submitted to the University of Limpopo, Sovenga, South Africa.

MAFEO, T.P., and P.W. MASHELA. 2009. Responses of germination in tomato, watermelon and butternut squash to a C*ucumis* bio-nematicide. *Journal of Agricultural and Environmental Sciences* 6:215-219.

MAFEO, T.P., MASHELA, P.W., and M.S. MPHOSI. 2011. Sensitivity of selected alliaceae seedlings to crude extract of *Cucumis myriocarpus* fruits. *African Journal of Agricultural Research* 6:158-164.

MAMPHISWANA, N.D., MASHELA, P.W., and L.K. MDEE. 2010. Distribution of total phenolics and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*. *African Journal of Agricultural Research* 5:2570-2575.

MASHELA, P.W. 2007. Undefeatable Enemies: Answering Questions With Questions. Inaugural Lecture, 19 March 2007. University of Limpopo Press, Sovenga.

MASHELA, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in micro-plots. *Nematropica* 32:13-19.

MASHELA, P.W. 1992. Interactions of *Tylenchulus semipenetrans*, Citrus Rootstocks and Salinity. Thesis Submitted to the University of Florida. Gainesville, Florida.

MASHELA, P.W., and T. MORUDU. 2009. Feasibility Study for Establishment of Two Fresh Produce Depot Facilities Per Province in South Africa. Commissioned by National Agricultural Marketing Council.

MASHELA, P.W., and M.E. NTHANGENI. 2002. Osmolyte allocation in response to *Tylenchulus semipenetrans* infection, root pruning and stem girdling. *Journal of Nematology* 34:273-277.

MASHELA, P.W., SHIMELIS, H.A., and F.N. MUDAU. 2008. Comparison of the efficacy of ground wild cucumber fruits, aldicarb and fenamiphos on suppression of the root-knot nematode in tomato. *Journal of Phytopathology* 156:264-267.

MASHELA, P.W., DUNCAN, L.W., and R. McSORLEY. 1992a. Salinity reduces resistance to *Tylenchulus semipenetrans*. *Nematropica* 22:7-12.

MASHELA, P.W., DUNCAN, L.W., GRAHAM, J.W., and R. McSORLEY. 1992b. Leaching soluble salts increase population densities of *Tylenchulus semipenetrans*. *Journal of Nematology* 24:103-108.

MATSUO, S.D., ISHIUCHI, D., and T. KOYAMA. 1985. Breeding for new bottle gourd cultivar ‘Renshi’ for use as a rootstock for watermelon. *Bulletin for Vegetable and Ornamental Crops Research Station* 8:1-21.

MAQBOOL, A., AURANGZEB, R.A., HABIB, N.B., and I. KHAN. 1987. Nutritional

and organoleptic evaluation of wheat rotis supplemented with soybean flour. *Pakistan Journal of Science and Industry Research* 38:615-618.

McMAHON, M.J., KOFRANEK, A.M., and V.E. RUBATZKY. 2005. Hartmann's Plant Science: Growth, Development and Utilisation of Cultivated Plants. Prentice Hall, New Jersey.

McSORLEY, R., and R.N. GALLAHER. 1991. Nematode population changes and forage yields of six corn and sorghum cultivars. *Supplement to the Journal of Nematology* 23:673-677.

McSORLEY, R., and R.N. GALLAHER. 1993. Population dynamics of plant-parasitic nematodes on cover crops of corn and sorghum. *Journal of Nematology* 25:446-453.

MILLER, J.F., and T.J. GUYLA. 1987. Inheritance of resistance to *Meloidogyne incognita* race 2 in maize*. Journal of Crop Science* 27:210-212.

MPHOSI, M.J. 2004. Influence of *Bacillus* Species and *Cucumis myriocarpus* on *Meloidogyne incognita* Suppression and Tomato Productivity. Master Dissertation Submitted to University of the North. Sovenga, South Africa.

MOFOKENG, M.A. 2005. Host-status of *Cucumis myriocarpus* to *Meloidogyne incognita*. Master Mini-Dissertation Submitted to the University of Limpopo. Sovenga, South Africa.

MOTJAHEDI, H., VAN DER BEEK, J.G., SANTO, G.S., and C.R. BROWN. 1998.

Re-assessment of host race concept for Columbia root-knot nematode, *Meloidogyne chitwoodi*. *Journal of Nematology* 30:507.

MONTALVO, A.E., and J. ESNARD. 1994. Reaction of ten cultivars of watermelon (*Citrullus lanatus*) to a Puerto Rican population of *Meloidogyne incognita*. *Journal of Nematology* 26:640-643.

MOTSINGER, R.E., MOODY, E.H., and C.M. GAY. 1977. Reaction of certain French marigolds (*Tagetes patula*) cultivars to three *Meloidogyne* species. *Journal of Nematology* 9:278.

MUEDI, H.T.H., MASHELA, P.W., and M.C. MATHABE. 2005. Bioactivity of wild Cucumber fruit extracts on plant-parasitic nematodes. *Proceedings of Nematological Society of Southern Africa* 17:27.

MULLER, K.O. 1959. Hypersensitivity. Volume 1. *in*: Horsfall, J.G., and A.E. Dimond (eds.), Plant Pathology: An Advanced Treatise. Academic Press, New York.

NELSON, N. 1944. A photometric adaptation of the somoyi method for the determination of glucose. *Journal of Biological Chemistry* 153:375-380.

NGOBENI, G.L., FOURIE, H., MC DONALD, A.H., and P.W. MASHELA. 2011. Host suitability of selected South African maize genotypes to the root-knot nematode species *Meloidogyne incognita* race 2 and *Meloidogyne javanica. South African Journal of Plant and Soil* 28:49-54.

NORTON, J.D., and D.M. GRANBERRY. 1980. Characteristics of progeny from

an interspecific cross of *Cucumis melo* with *Cucumis metuliferus. Journal of the American Society for Horticultural Science* 105:174-180.

ODA, M., OKADA, K., SASAKI, K., AKAZAWA, S., and M. SEI. 1997. Growth and yield of eggplant grafted by newly developed robot. *Horticultural Science* 32:848-849.

OPPERMANN, C.H., DONG, K., and S. CHANG. 1994a. Genetic Analysis of the Soybean-*Heterodera glycines* Interaction. *in*: Lamberti, F., De Giorgi, C., and D.M. Bird (eds.), Advances in Molecular Plant Nematology. Plenum Press, New York.

OPPERMANN, C.H., ACEDO, G.N., SARAVITZ, D.M., SKANTAR, A.M., SONG, W., TAYLOR, C.G., and M.A. CONKLING. 1994b. Bioengineering Resistance to Sedentary Endoparasitic Nematodes. *in*: Lamberti, F., De Giorgi, C., and D.M. Bird (eds.), Advances in Molecular Plant Nematology. Plenum Press, New York.

ORNAT, C., VERDEJO-LUCAS, S., and F.J. SORRIBAS. 2001. A population of *Meloidogyne javanica* from Spain virulent to the *Mi* resistance gene in tomato. *Plant Disease Reporter* 85:271-276.

PAULSON, R.E., and J.M. WEBSTER. 1972. Ultrastructure of the hypersensitive reaction in roots of tomato, *Lycopersicon esculentum* L., to infection by the root-knot nematode, *Meloidogyne incognita*. *Physiology of Plant Pathology* 2:227-234.

PERRY, R., MOENS, M., and J.L. STARR. 2009. Root-knot Nematodes.

CABI, Wallingford.

PITRAT, M., CHAUVET, M., and C. FOURY. 1999. Diversity, history and production of cultivated cucurbits. *Acta Horticulturae* 492:21-28.

PLOEG, A.T., and P.C. MARRIS. 1999. Effect of temperature on suppression of *Meloidogyne incognita* by *Tagetes* cultivars. *Journal of Nematology* 31:709-714.

PLOEG, A.T., and M.S. PHILLIPS. 2001. Damage to melon (*Cucumis melo* L.) cv. 'Durango' by *Meloidogyne incognita* in Southern California. *Journal of Nematology* 3:151-158.

POFU, K.M. 2008. Reproductive and Damage Potential of *Meloidogyne javanica* on Selected Hemp Cultivars. Masters Dissertation Submitted to the University of Limpopo. Sovenga, South Africa.

POFU, K.M., and P.W. MASHELA. 2011. Using relative penetration and maleness indices in *Meloidogyne incognita* to establish resistance type in *Cucumis myriocarpus*. *African Journal of Biotechnique* 10:390-393.

RABE, E., and L.A. VON BROEMBSEN. 1991. Production Guidelines for Export Citrus. Volume 1, Citriculture Establishment, Co-operative Citrus Exchange Technical Division, Nelspruit, South Africa.

RAJA, A., and D.R. DASGUPTA. 1986. Enhanced synthesis of messenger RNA in relation to resistance expression in cowpea (*Vigna unguiculata*) infected with the root-knot nematode, *Meloidogyne incognita. Journal*

of Nematology 9:35-49.

RHODE, R.A. 1972. Expression of resistance in plants to nematodes. *Annual Review Phytopathology* 10:233-252.

RHUE, R.D., and G. KIDDER. 1983. Procedures Used by IFAS Extension Soil Testing Laboratory and Interpretation of Results. Circular no. 596. Florida Co-operative Extension Services, IFAS. University of Florida, Gainesville.

RICE, S.L., STONE, A.R., and B.S.C. LEADBEATER. 1987. Changes in cell structure in roots of resistant potatoes parasitized by potato cyst nematodes. 2. Potatoes with resistance from *Solanum vernei*. *Physiological and Molecular Plant Pathology* 31:1-14.

RICHARD, D.A., and A.W. DUPREE Jr. 1978. The effectiveness of ten kinds of marigolds and five other treatments for control of four *Meloidogyne* species. *Journal of Nematology* 4:296-297.

RIMINGTON, P. 1938. Medicinal and Poisonous Plants of Southern and Eastern Africa. University of Natal Press, Pietermaritzburg.

RIZVI, S.J.H., and V. RIZVI. 1992. Allelopathy: Basic and applied aspects. Chapman and Hall, London.

ROBERTS, P.A. 1992. Current status of the availability, development, and use of host plant resistance to root-knot nematodes. *Journal of Nematology* 24:213-227.

ROBERTS, P.A. 1993. The future of nematology: Integration of new and

improved management strategies. *Journal of Nematology* 25:383-394.

ROBERTS, P.A., and I.J. THOMASON. 1986. Variability in reproduction of isolates of *Meloidogyne incognita* and *Meloidogyne javanica* on resistant tomato genotypes. *Plant Disease* 70:547-551.

ROBERTS, P.A., and I.J. THOMASON. 1989. A review of variability in four *Meloidogyne* spp. measured by reproduction on several hosts including *Lycopersicon*. *Agricultural Zoology Reviews* 3:225-252.

ROBERTS, P.A., DALMASSO, A., GAB, G.B., and P. CASTAGNONE-SERENO. 1990. Resistance in *Lycopersicon peruvianum* to isolates of Mi gene compatible *Meloidogyne* populations. *Journal of Nematology* 22:585-589.

ROBERTSON, L., and M.A. DIEZ-ROJO. 2008. New host races of *Meloidogyne arenaria*, *Meloidogyne incognita*, and *Meloidogyne javanica* from horticultural regions of Spain. *Plant Disease* 93:180-184.

ROE, M.W., PHILIPSON, L.H., FRANGAKIS, C.J., KUZNETSOV, A., MERTZ, R.J., LANCASTER, M.E., SPENCER, B., WORLEY, J.F., and I.D. DUKES. 1994. Defective glucose-dependent endoplasmic reticulum Ca^{2+} sequestration in diabetic mouse islets of Langerhans. *Journal of Biological Chemistry* 269:18279-18282.

ROUPHAEL, Y., SCHWARZ, D., KRUMBEIN, A., and G. COLLA. 2010. Impact of grafting on product quality of fruit vegetables. *Scientia Horticulturae* 127:172-179.

ROUPHAEL, Y., CARDARELLI, M., REA, E., and G. COLLA. 2008. Grafting of cucumber as a means to minimize copper toxicity. *Environmental Experimental Botany* 63:49-58.

ROY, T.K. 1981. Biochemical aspects of host-parasite relationships in plant parasitic nematodes. *Proceedings of Indian National Science Academy B* 47:919-936.

SAKATA, Y., TAKAYOSHI, O., and S. MITSUHIRO. 2007. The history and present state of the grafting of cucurbitaceous vegetables in Japan. *Acta Horticulturae* 731:159-170.

SALISBURY, F.B., and C.W. ROSS. 1992. Plant Physiology. Wadsworth, Belmont California.

SANTO, G.S., and W.J. BOLANDER. 1977. Separate and concomitant effects of *Macroposthonia xenoplax* and *Meloidogyne hapla* on concord grapes. *Journal of Nematology* 9:282-283.

SASSER, J.N., CARTER, C.C., and K.M. HARTMAN. 1984. Standardization of Host Suitability Studies and Reporting of Resistance to Root-Knot Nematode. Raleigh, USA.

SATOH, S. 1996. Inhibition of flowering of cucumber grafted on rooted squash rootstocks. *Journal of Physiology of Plants* 97:440-444.

SAVVAS, D., PAPASTAVROU, D., NTATSI, G., ROPOKIS, A., OLYMPIOS, C., HARTMANN, H., and D. SCHWARZ. 2009. Interactive effects of grafting and Mn-supply level on growth, yield and nutrient uptake by tomato. *HortScience* 44:1978-1982.

SEINHORST, J.W. 1967. The relationship between population increase and population density in plant-parasitic nematodes. 3. Definition of the terms host, host-status and resistance. 4. The influence of external conditions on the regulation of population density. *Nematologica* 13:429-442.

SEINHORST, J.W. 1965. The relationship between nematode density and damage to plants. *Nematologica* 11:137-154.

SHAKWANE, S.K., MOKGALONG, N.M., and P.W. MASHELA. 2005. Responses of *rhizobium* nodulation to the root-knot nematode, ground wild cucumber fruits, castor bean fruits and fever tea leaves. *Proceedings of Nematological Society of Southern Africa* 17:8.

SIDDIQUI, M.A., and M.M. ALAM. 1988. Effect of root exudates of Neem and Persian lilac on plant-parasitic nematodes. *Journal of Plant Science* 62:33-35.

SIKORA, R.A., and C. CARTER. 1987. Nematode Interactions With Fungal and Bacterial Plant Pathogens: Fact or Fantasy. *in*: Veech, J.A., and D.W. Dickson (eds.), Vistas on Nematology. Society of Nematologists. Hyattsville, Maryland.

SIKORA, R.A., MALEK, R.B., TAYLOR, D.P., and D.I. EDWARDS. 1979. Reduction of *Meloidogyne naasi* infection of creeping bentgrass by *Tylenchorhynchus arri* and *Paratrichodorus minor*. *Nematologica* 25:179-183.

SIKORA, R.A., and E. FERNÀNDEZ. 2005. Nematode Parasites of Vegetables.

in: Luc, M., Sikora, R.A., and J. Bridge (eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI, Wallingford.

SIGUENZA, C., SCHOCHOW, M., TURINI, T., and A. PLOEG. 2005. Use of *Cucumis metuliferus* as a rootstock for melon to manage *Meloidogyne incognita*. *Journal of Nematology* 37:276-280.

SMITH, G.S. 1987. Interactions of Nematodes With Mycorrhizal Fungi. *in*: Veech, J.A., and D.W. Dickson (eds.), Vistas on Nematology. Society of Nematologists: Hyattsville, Maryland.

SMITH, D. 1981. Removing and Analysing Total Non-structural Carbohydrates From plant Tissues. Publication R-2107. University of Wisconsin: Madison.

SORIA, C., GOMEZ-GUILLAMON, M.L., ESTEVA, J., and F. NUEZ. 1990. Ten inter-specific crosses in the genus *Cucumis:* A preparatory study to seek crosses resistant to melon yellowing disease. *Cucurbit Genetics Cooperation Report* 13:31-33.

SPETH, J.G. 2004. Red Sky at Morning: America and the Crisis of the Global Environment. Yale University Press, New Haven.

STARR, J.L., COOK, R., and J. BRIDGE. 2002. Plant Resistance to Parasitic Nematodes. Biddles, Guildford UK.

STEELE, A.E., and H. SAVITSKY. 1981. Resistance of trisomic and diploid hybrids of *Beta vulgaris, B. procumbens* to the sugar beet nematode, *Heterodera schachtii*. *Journal of Nematology* 13:352-357.

STEYN, A.G.W., SMIT, C.F., DU TOIT, S.H.C., and C. STRASHEIM. 2003. Modern Statistics in Practice. Van Schaik, Pretoria.

STIRLING, G.R. 1991. Biological Control of Plant Parasitic Nematodes. Problems, Progress and Prospects. CABI, Wallingford.

TAYLOR, A.L., and J.N. SASSER. 1978. Biology, Identification and Control of Root-knot Nematodes *(Meloidogyne* species*)*. North Carolina State University Press, Raleigh.

TIEDERMANN, R. 1989. Graft union development and symplastic phloem contact in the heterograft *Cucumis sativus* and *Cucurbita ficifolia*. *Plant Physiology* 134:427-440.

THIES, J.A., and A. LEVI. 2006. Resistance of *Citrullus lanatus* var. Citroides germplasm to root-knot nematodes. *Journal of Nematology 38:258-303*.

THIES, J.A., and A. LEVI. 2007. Characterisation of watermelon (*Citrullus lanatus* var. citroides) germplasm for resistance to root-knot nematodes. *HortScience* 42:1530-1533.

THIES, J.A., KOUSIK, S., and R. HASSEL. 2009. Evaluation of Rootstocks for Managing Root-knot Nematodes in Grafted Watermelon. National Watermelon Association Research Project, United States Department of Agriculture.

THIES, J.A., ARISS, J., HASSELL, R., KOUSIK, C.S., OLSEN, S., and A. LEVI. 2010. Grafting for managing southern root-knot nematode, *Meloidogyne incognita*, in watermelon. *Plant Disease* 94:1195-1199.

THOMASON, I.J., and H.E. McKINNEY. 1959. Reaction of some Cucurbitaceae to root-knot nematodes *(Meloidogyne* spp.). *Plant Disease Reporter* 43:448-450.

THURAU, T., YE, W., and D. CAI. 2010. Insect and Nematode Resistance. *in:* Widholm, J.M., and T. Nagata, (eds.), Biotechnique in Agriculture and Forestry. Springer-Verlag, Heidelberg Berlin.

TRAKA-MAVRONA, E., KOUTSIKA-SOTIRIOU, M., and T. PRITSA. 2000. Response of squash (*Cucurbita* spp.) as rootstock for melon *(Cucumis melo* L.). *Scientia Horticulturae* 83:353-362.

TRUDGILL, D.L. 1992. Resistance to and tolerance of plant-parasitic nematodes in plants. *Annual Review of Phytopathology* 29:167-192.

TRUDGILL, D.L. 1985. Concepts of Resistance, Tolerance and Susceptibility in Relation to Cyst Nematodes. *in*: Lamberti, F., and C.E. Taylor (eds.), Cyst Nematodes. Plenum Press, New York.

TSAMBANAKIS, J. 1984. Grafting cucumber hybrids on rootstock *Cucurbita ficifolia*. Proceedings of the Third Conference on Protected Vegetables and Flowers. Heraklion, Greece.

TZANETAKIS, I.E. 2004. Molecular Characterisation of Criniviruses and Ilarviruses infecting Strawberry. Ph.D. Dissertation Submitted at Oregon State University. Corvallis, USA.

VAN WYK, B.E., VAN OUDSHOORN, B., and N. GERICKE. 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria.

VEECH, J.A. 1981. Plant Resistance to Nematodes. *in*: Zuckerman, B.M., and

R.A. Rhode (eds.), Plant-parasitic Nematodes. Academic Press, New York.

WALLACE, H.R. 1971. Abiotic Influences in the Soil Environment. Vol. 1. *in*: Zuckermann, B.M, Mai, W.F., and R.A. Rhodes (eds.), Plant-parasitic Nematodes. Academic Press, New York.

WANG, M., and I.L. GOLDMAN. 1996. Resistance to root-knot nematode (*Meloidogyne hapla* Chitwood) in carrot is controlled by two recessive genes. *Journal of Heredity* 87:119-23.

WEBB, D.M., BALTAZAR, B.M., RAO-ARELLI, A.P., SCHUPP, J., CLAYTON, K., KELM, P., and W.D. BEAVIS. 1995. Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean P1437.654. *Theoretical and Applied Journal of Genetics* 91:574-581.

WEISCHER, B. 1982. Histopathological studies on grape species with different degrees of resistance to *Xiphenema index*. *Nematologica* 28:178-179.

WINTER, S., PURAC, A., LEGGETT, F., FRISON, E.A., ROSSEL, H.W., and R.I. HAMILTON. 1992. Partial characterization and molecular cloning of a closterovirus from sweet potato infected with the sweet potato virus disease complex from Nigeria. *Journal of Phytopathology* 82:869-875.

WISLER, G.C., DUFFUS, J.E., LIU, H.Y., and R.H. LI. 1998. Ecology and epidemiology of whitefly-transmitted closteroviruses. *Plant Disease* 82:270-280.

WINDHAM, G.L., and W.P. WILLIAMS. 1988. Reproduction of *M. javanica* on corn Hybrids and inbreeds. *Annals of Applied Nematology* 2:25-28.

WINSTEAD, N.N., and R.D. RIGGS. 1959. Reaction of watermelon varieties to root-knot nematodes. *Plant Disease Reporter* 43:909-912.

WU, Y., and D.J. COSGROVE. 2000. Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. *Journal of Experimental Botany* 51:1543-1553.

YAMAKAWA, B. 1983. Grafting. *in*: Nishi, K. (ed.), Vegetables Handbook. Yokendo Book Company, Tokyo.

YAMASAKI, A., YAMASHITA, M., and S. FURUYA. 1994. Mineral concentrations and cytokinin activity in the xylem exudates of grafted watermelons as affected by rootstocks and crop load. *Journal of Japanese Society of Horticultural Science* 62:817-826.

ZACHEO, G., ORLANDO, C., and T. BLEVE-ZACHEO. 1993. Characterisation of anionic peroxidases in tornado isolines infected by *Meloidogyne incognita*. *Journal of Nematology* 25:249-256.

APPENDICES

Appendix 3.1 Analysis of variance for the final nematode population densities (Pf) of *Meloidogyne incognita* race 2 on *Cucumis africanus* and *Cucumis myriocarpus* under greenhouse conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	2.223	25	2.41	0.26
TRT (B)	6	5.530	62	1.01	0.03
A*B	54	1.113	13		
TOTAL	69	8.866	100		
Cucumis myriocarpus					
REP (A)	9	1.063	10	1.14	0.36
TRT (B)	6	6.876	65	6.27	0.01
A*B	54	2.669	25		
TOTAL	69	10.608	100		

Appendix 3.2 Analysis of variance for the reproductive factor (RF) values of *Meloidogyne incognita* race 2 on *Cucumis africanus* and *Cucumis myriocarpus* under greenhouse conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	0.170	4	1.26	0.31
TRT (B)	6	3.185	84	2.01	0.01
A*B	54	0.463	12		
TOTAL	69	3.818	100		
Cucumis myriocarpus					
REP (A)	9	1.619	9	2.59	0.09
TRT (B)	6	12.826	70	17.08	0.001
A*B	54	3.754	21		
TOTAL	69	18.199	100		

Appendix 3.3 Responses of dry shoot mass of *Cucumis africanus* and *Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under greenhouse conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	8.492	8	0.72	0.61
TRT (B)	6	24.574	24	1.74	0.15
A*B	54	70.535	68		
TOTAL	69	103.601	100		
Cucumis myriocarpus					
REP (A)	9	15.825	11	3.36	0.58
TRT (B)	6	39.632	29	0.76	0.65
A*B	54	82.254	60		
TOTAL	69	137.711	100		

Appendix 3.4 Responses of vine length of *Cucumis africanus* and *Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under greenhouse conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	1582.560	33	3.63	0.07
TRT (B)	6	396.316	8	0.77	0.65
A*B	54	2825.430	59		
TOTAL	69	4804.306	100		
Cucumis myriocarpus					
REP (A)	9	2542.420	43	3.66	0.05
TRT (B)	6	598.133	10	1.18	0.35
A*B	54	2808.141	47		
TOTAL	69	5948.694	100		

Appendix 3.5 Analysis of variance for reproductive factor (RF) values of *Meloidogyne incognita* race 2 on *Cucumis africanus and Cucumis myriocarpus* under micro-plot conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	0.062	40	3.47	0.01
TRT (B)	4	0.021	14	2.69	0.05
A*B	36	0.072	46		
TOTAL	49	0.155			
Cucumis myriocarpus					
REP (A)	9	0.382	17	1.47	0.20
TRT (B)	4	0.834	37	7.23	0.01
A*B	36	1.038	46		
TOTAL	49	2.254	100		

Appendix 3.6 Analysis of variance for dry shoot mass of *Cucumis africanus and Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under micro-plot conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	13612.9	20	1.14	0.36
TRT (B)	4	6352.63	9	1.14	0.33
A*B	36	47589.0	71		
TOTAL	49	67554.6	100		
Cucumis myriocarpus					
REP (A)	9	861072	26	1.50	0.18
TRT (B)	4	251642	7	0.99	0.43
A*B	36	2289340	67		
TOTAL	49	3402053	100		

Appendix 3.7 Analysis of variance for dry root mass of *Cucumis africanus and Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under micro-plot conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	380.323	14	0.69	0.71
TRT (B)	4	159.858	6	0.65	0.63
A*B	36	2208.71	80		
TOTAL	49	2748.89	100		
Cucumis myriocarpus					
REP (A)	9	1959.06	39	2.56	0.02
TRT (B)	4	20.7688	1	0.06	0.99
A*B	36	3058.38	60		
TOTAL	49	5038.21	100		

Appendix 3.8 Analysis of variance for stem diameters of *Cucumis africanus and Cucumis myriocarpus* infested with *Meloidogyne incognita* race 2 under micro-plot conditions at 56 days after inoculation (n = 10).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	9	63.0461	20	1.25	0.30
TRT (B)	4	53.4797	17	2.38	0.07
A*B	36	202.244	63		
TOTAL	49	318.769	100		
Cucumis myriocarpus					
REP (A)	9	13.1041	21	1.19	0.33
TRT (B)	4	3.89155	6	0.79	0.54
A*B	36	44.1530	72		
TOTAL	49	61.1487	100		

Appendix 4.1 Reproductive factor (RF) values of *Meloidogyne incognita* race 4 in *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	11	19228.2	3	0.92	0.49
TRT (B)	5	373543	75	17.85	0.01
A*B	55	104618	22		
TOTAL	71	497389	100		
Cucumis myriocarpus					
REP (A)	11	17994.5	4	0.86	0.52
TRT (B)	5	386240	76	18.44	0.01
A*B	55	104754	20		
TOTAL	71	508989	100		

Appendix 4.2 Influence of male population densities of *Meloidogyne incognita* race 4 in roots of *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	11	46.0000	5	0.51	0.77
TRT (B)	5	490.667	50	5.44	0.01
A*B	55	451.333	45		
TOTAL	71	988.000	100		
Cucumis myriocarpus					
REP (A)	11	703.222	22	2.81	0.04
TRT (B)	5	1133.89	37	4.53	0.01
A*B	55	1251.44	41		
TOTAL	71	3088.56	100		

Appendix 4.3 Influence of second stage juveniles of *Meloidogyne incognita* race 4 on dry shoot mass of *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	11	630.524	7	0.58	0.72
TRT (B)	5	1411.19	16	1.08	0.40
A*B	55	6559.97	77		
TOTAL	71	8601.69	100		
Cucumis myriocarpus					
REP (A)	11	1794.85	5	0.34	0.88
TRT (B)	5	6442.41	16	1.01	0.43
A*B	55	31747.1	79		
TOTAL	71	39984.4	100		

Appendix 4.4 Influence of second stage juveniles of *Meloidogyne incognita* race 4 on dry root mass of *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	11	88.8393	15	1.35	0.27
TRT (B)	5	105.705	18	1.34	0.27
A*B	55	395.279	67		
TOTAL	71	589.823	100		
Cucumis myriocarpus					
REP (A)	11	8.70398	12	1.03	0.42
TRT (B)	5	15.2038	20	150	0.21
A*B	55	50.7157	68		
TOTAL	71	74.6235	100		

Appendix 4.5 Influence of male and female nematode population densities of *Meloidogyne javanica* in roots of *Cucumis africanus* at 56 days after inoculation (n = 12).

Male					
SOURCE	DF	SS	%	F	P
REP (A)	11	355.556	12	1.06	0.41
TRT (B)	5	855.556	30	2.55	0.05
A*B	55	1677.78	58		
TOTAL	71	2888.89	100		
Female					
REP (A)	11	347.222	12	1.33	0.28
TRT (B)	5	1180.56	42	4.53	0.01
A*B	55	1302.78	46		
TOTAL	71	2830.56	100		

Appendix 4.6 Influence of second stage juveniles of *Meloidogyne javanica* on stem diameters of *Cucumis africanus* and *Cucumis myriocarpus* at 56 days after inoculation (n = 12).

Cucumis africanus					
SOURCE	DF	SS	%	F	P
REP (A)	11	0.04405	10	0.93	0.48
TRT (B)	5	0.11286	26	1.98	0.10
A*B	55	0.28429	64		
TOTAL	71	0.44119	100		
Cucumis myriocarpus					
REP (A)	11	4.65548	9	0.72	0.61
TRT (B)	5	7.81667	15	1.01	0.44
A*B	55	38.8262	76		
TOTAL	71	51.2983	100		

Appendix 6.1 Rootstock-scion combinations of watermelon cultivars ‘Charleston Gray’ and ‘Congo’ with and without *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under field conditions at 56 days after grafting (n = 20).

‘Charleston Gray’					
SOURCE	DF	SS	%	F	P
REP (A)	19	0.05300	19	2.03	0.05
TRT (B)	7	0.03950	14	1.94	0.08
A*B	133	0.18300	67		
TOTAL	159	0.27550	100		
‘Congo’					
REP (A)	19	0.06113	20	2.10	0.04
TRT (B)	7	0.03488	12	1.54	0.17
A*B	133	0.20388	68		
TOTAL	159	0.29988	100		

Appendix 6.2 Reproductive factor (RF) values of *Meloidogyne incognita* race 2 on scion-rootstock combinations of watermelon cultivars ‘Charleston Gray’ and ‘Congo’ with and without *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks under greenhouse conditions at 56 days after grafting (n = 20).

‘Charleston Gray’					
SOURCE	DF	SS	%	F	P
REP (A)	19	0.01194	3	0.35	0.87
TRT (B)	7	0.30472	79	22.41	0.01
A*B	133	0.06798	18		
TOTAL	159	0.38464	100		
‘Congo’					
REP (A)	19	0.09352	16	1.22	0.37
TRT (B)	7	0.33233	58	10.87	0.01
A*B	133	0.15280	26		
TOTAL	159	0.57865	100		

Appendix 6.3 Vine length of watermelon cultivars ‘Charleston Gray’ and ‘Congo’ with and without *Cucumis africanus* and *Cucumis myriocarpus* nematode-resistant seedling rootstocks in pots infested with 1 000 juveniles and eggs of *Meloidogyne incognita* race 2 at 56 days after grafting (n = 20).

‘Charleston Gray’					
SOURCE	DF	SS	%	F	P
REP (A)	19	99453.8	78	9.64	0.01
TRT (B)	7	7432.44	6	1.80	0.21
A*B	133	20632.2	16		
TOTAL	159	127518	100		
‘Congo’					
REP (A)	19	36895.2	60	4.11	0.03
TRT (B)	7	6249.00	10	1.74	0.22
A*B	133	17966.3	29		
TOTAL	159	61110.5	100		

Appendix 6.4 Stem diameters of watermelon cultivars ‘Charleston Gray’ and ‘Congo’ with and without *Cucumis africanus* and *Cucumis myriocarpus* nematode-resistant seedling rootstocks in pots infested with 1 000 juveniles and eggs of *Meloidogyne incognita* race 2 at 56 days after grafting (n = 20).

‘Charleston Gray’ (Above)					
SOURCE	DF	SS	%	F	P
REP (A)	19	4.57778	52	2.74	0.08
TRT (B)	7	0.88111	10	1.32	0.31
A*B	133	3.33889	38		
TOTAL	159	8.79778	100		
‘Charleston Gray’ (Below)					
REP (A)	19	1.64000	8	1.04	0.45
TRT (B)	7	15.2233	76	24.11	0.01
A*B	133	3.15667	16		
TOTAL	159	20.0200	100		
‘Congo’ (Above)					
SOURCE	DF	SS	%	F	P
REP (A)	19	1.59167	18	0.67	0.66
TRT (B)	7	2.34333	27	2.46	0.12
A*B	133	4.77000	55		
TOTAL	159	8.70500	100		
‘Congo’ (Below)					
REP (A)	19	2.69111	18	1.32	0.33
TRT (B)	7	8.50778	56	10.40	0.01
A*B	133	4.09222	27		
TOTAL	159	15.2911	100		

Appendix 6.5 Dry shoot mass of watermelon cultivars ‘Charleston Gray’ and ‘Congo’ with and without *Cucumis africanus* and *Cucumis myriocarpus* nematode-resistant seedling rootstocks in pots infested with 1 000 juveniles and eggs of *Meloidogyne incognita* race 2 at 56 days after grafting (n = 20).

‘Charleston Gray’					
SOURCE	DF	SS	%	F	P
REP (A)	19	47.2670	39	1.66	0.23
TRT (B)	7	16.3715	14	1.43	0.28
A*B	133	57.0824	47		
TOTAL	159	120.721	100		
‘Congo’					
REP (A)	19	24.6882	16	0.39	0.84
TRT (B)	7	5.82484	4	0.23	0.80
A*B	133	125.632	80		
TOTAL	159	156.145	100		

Appendix 6.6 Responses of macro nutrient elements (Nitrogen, Phosphorus and Potassium) to inter-generic grafting in leaves of watermelon cultivar 'Congo' with and without *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks at 66 days after transplanting (n = 20).

Nitrogen (N)					
SOURCE	DF	SS	%	F	P
REP (A)	19	0.92250	40	1.44	0.25
TRT (B)	7	0.10770	5	0.67	0.52
A*B	133	1.27864	55		
TOTAL	159	2.30883	100		
Phosphorus (P)					
SOURCE	DF	SS	%	F	P
REP (A)	19	0.01659	21	0.53	0.81
TRT (B)	7	0.00096	01	0.06	0.94
A*B	133	0.06204	78		
TOTAL	159	0.07912	100		
Potassium (K)					
SOURCE	DF	SS	%	F	P
REP (A)	19	4.01490	67	4.34	0.01
TRT (B)	7	0.10054	02	0.43	0.65
A*B	133	1.84973	31		
TOTAL	159	5.96516	100		

Appendix 6.7 Responses of calcium, magnesium and zinc to inter-generic grafting in leaves of watermelon cultivar 'Charleston Gray' with and without *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks at 66 days after transplanting (n = 20).

Calcium (Ca)					
SOURCE	DF	SS	%	F	P
REP (A)	19	1.61259	17	0.44	0.88
TRT (B)	7	0.47356	05	0.52	0.61
A*B	133	7.34117	78		
TOTAL	159	9.42732	100		
Magnesium (Mg)					
SOURCE	DF	SS	%	F	P
REP (A)	19	0.12574	31	0.91	0.53
TRT (B)	7	0.00643	02	0.19	0.83
A*B	133	0.27724	67		
TOTAL	159	0.40941	100		
Zinc (Zn)					
SOURCE	DF	SS	%	F	P
REP (A)	19	1164.52	22	0.84	0.57
TRT (B)	7	1319.24	25	3.82	0.04
A*B	133	2760.76	53		
TOTAL	159	5244.52	100		

Appendix 6.8 Responses of boron, copper and iron to inter-generic grafting in leaves of watermelon cultivar 'Charleston Gray' with and without *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks at 66 days after transplanting (n = 20).

Boron (B)					
SOURCE	DF	SS	%	F	P
REP (A)	19	91.0741	35	1.09	0.42
TRT (B)	7	2.35185	01	0.11	0.89
A*B	133	167.481	64		
TOTAL	159	260.907	100		
Copper (Cu)					
SOURCE	DF	SS	%	F	P
REP (A)	19	18.0000	29	0.84	0.59
TRT (B)	7	1.05556	02	0.20	0.82
A*B	133	43.1111	69		
TOTAL	159	62.1667	100		
Iron (Fe)					
SOURCE	DF	SS	%	F	P
REP (A)	19	9408.73	31	0.92	0.53
TRT (B)	7	5.21407	01	0.00	0.10
A*B	133	20470.5	68		
TOTAL	159	29884.5	100		

Appendix 6.9 Responses of boron, copper and iron to inter-generic grafting in leaves of watermelon cultivar 'Congo' with and without *Cucumis africanus* and *Cucumis myriocarpus* seedling rootstocks at 66 days after transplanting (n = 20).

Boron (B)					
SOURCE	DF	SS	%	F	P
REP (A)	19	77.5000	47	2.18	0.09
TRT (B)	7	17.0556	10	1.92	0.18
A*B	133	70.9444	43		
TOTAL	159	165.500	100		
Copper (Cu)					
SOURCE	DF	SS	%	F	P
REP (A)	19	18.2963	48	2.04	0.12
TRT (B)	7	2.01852	05	0.90	0.43
A*B	133	17.9815	47		
TOTAL	159	38.2963	100		
Iron (Fe)					
SOURCE	DF	SS	%	F	P
REP (A)	19	15792.5	51	2.67	0.05
TRT (B)	7	3458.42	11	2.34	0.13
A*B	133	11837.1	38		
TOTAL	159	31088.1	100		

Appendix 7.1 Analysis of variance for the reproductive factor (RF) values of *Meloidogyne javanica* on *Cucumis africanus* without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly (GHWF) at 56 days after initiation of the treatments (n = 12).

		$GHWF_0$			
SOURCE	DF	SS	%	F	P
REP (A)	11	0.04593	9	0.57	0.72
TRT (B)	6	0.04501	9	0.56	0.73
A*B	66	0.39987	82		
TOTAL	83	0.49081	100		
		$GHWF_1$			
REP (A)	11	0.47222	4	0.70	0.63
TRT (B)	6	11.1389	74	16.57	0.01
A*B	66	3.36111	22		
TOTAL	83	14.9722	100		

Appendix 7.2 Analysis of variance for dry shoot mass of *Cucumis africanus* without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly (GHWF) at 56 days after initiation of the treatments (n = 12).

		$GHWF_0$			
SOURCE	DF	SS	%	F	P
REP (A)	11	665.673	11	0.78	0.57
TRT (B)	6	404.360	7	0.40	0.88
A*B	66	5110.10	82		
TOTAL	83	6180.13	100		
		$GHWF_1$			
REP (A)	11	303.912	34	4.88	0.01
TRT (B)	6	223.402	25	2.99	0.02
A*B	66	373.784	41		
TOTAL	83	901.098	100		

Appendix 7.3 Analysis of variance for vine length of *Cucumis africanus* without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly (GHWF) at 56 days after initiation of the treatments (n = 12).

		$GHWF_0$			
SOURCE	DF	SS	%	F	P
REP (A)	11	9567.45	31	3.72	0.01
TRT (B)	6	6250.18	20	2.03	0.09
A*B	66	15420.2	49		
TOTAL	83	31237.8	100		
		$GHWF_1$			
REP (A)	11	7097.64	14	1.20	0.33
TRT (B)	6	7015.38	14	0.99	0.45
A*B	66	35519.4	72		
TOTAL	83	49632.4	100		

Appendix 7.4 Analysis of variance for dry root mass of *Cucumis africanus* without ($GHWF_0$) and with ($GHWF_1$) the greenhouse whitefly (GHWF) at 56 days after initiation of the treatments (n = 12).

		$GHWF_0$			
SOURCE	DF	SS	%	F	P
REP (A)	11	9.04056	6	0.70	0.63
TRT (B)	6	62.2055	42	4.03	0.01
A*B	66	77.2318	52		
TOTAL	83	148.478			
		$GHWF_1$			
REP (A)	11	28.1270	32	3.57	0.01
TRT (B)	6	11.9018	14	1.26	0.31
A*B	66	47.3024	54		
TOTAL	83	87.3312	100		