Evaluation and verification of resistance in selected vegetable crops for sustainable root-knot nematode management in developing agriculture

By

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TABLE OF CONTENTS

ABSTRACT

Root-knot nematodes, (*Meloidogyne* species) are a major constraint in vegetable production systems. These parasites cause high yield losses, particularly in subsistence farming systems. This study was conducted to establish i) whether monospecific populations of *M. incognita* race 2 and *M. javanica* were used in these trials by means of molecular identification, ii) determine whether root-knot nematode-resistance is present in commercially available tomato, green bean, pumpkin and *Brassica* genotypes and to iii) verify resistance found. *M. incognita* race 2 and *M. javanica* were identified as monospecific using molecular techniques. Host suitability trials for the relevant vegetable crops were conducted in a greenhouse. Although various nematode parameters were used (the number of eggs and J2 per root system and per gram of roots, number of egg masses and egg-laying females (ELF) per root system), reproduction factors values [RF = final egg and J2 numbers (Pf)/initial egg and J2 numbers (Pi)] were used as the main criteria to select for root-knot nematode resistance. Although substantial variation existed among the relevant vegetable genotypes with regard to all parameters used, none of these genotypes were immune to either *M. incognita* race 2 or *M. javanica* since these parasites reproduced on all vegetable genotypes used in this study. However, three tomato and a range of *Brassica* genotypes had RF-values lower than 1, indicating resistance to *M. incognita* race 2. With regard to the verification of *M. incognita* race 2-resistance in tomato genotype Rhapsody relative to the susceptible Moneymaker in a microplot trial using a range of initial inoculation levels, strong relationship existed for both genotypes for the majority of nematode variables used. These relationships were best described by non-linear equations. Significantly lower numbers of eggs and J2 in roots, as well as J2 in soil were obtained for Rhapsody compared to Moneymaker. RF-values were inversely proportional to initial population density (Pi) for Rhapsody ($r = -0.3$), while it increased gradually to Pi for Moneymaker ($r = 0.94$). A range of Brassica genotypes were also identified resistance to *M. incognita* race 2 and *M. javanica*, respectively, but none of the green bean and pumpkin screened had RF-values ≤ 1, indicating susceptibility to both species.

UITTREKSEL

Knopwortelaalwurms (*Meloidogyne* spp.) is beperkende faktor in groenteproduksie en veroorsaak betekenisvolle oesverliese, veral in kleinboer-produksiestelsels. Gevolglik is hierdie studie onderneem om vas te stel of monospesifieke bevolkings van *M. incognita* ras 2 en *M. javanica* gebruik is in proewe deur middel van molekulêre identifikasie, knopwortelaalwurmweerstand teenwoordig is in plaaslik beskikbare tamatie, groenboon, pampoen en Brassica variëteite en om sogenaamde weerstand te verifier in 'n mikroplotproef. Evaluasie van die verskillende groente-variëteite vir weerstand teen die twee knopwortelaalwurmspesies is uitgevoer in verskeie glashuisproewe. Alhoewel verskillende aalwurmparameters, naamlik die hoeveelheid eiers en J2 per wortelstelsel en per gram wortels, die hoeveelheid eierpakkies en eierproduserende wyfies (ELF) per wortelstelsel gebruik is, is RF-waardes [RF = finale eier en J2 getalle (Pf) / inisiële eier en J2 getalle (Pi)]) as die primêre kriterium gebruik om knopwortelaalwurmweerstand te identifiseer. Substansiële variasie is verkry vir die verskillende groente variëteite met betrekking tot alle parameters wat gebruik is. Geeneen van hierdie variëteite is egter immuun teen *M. incognita* ras 2 of *M. javanica* nie, aangesien hierdie parasiete in wortels van al hierdie variëteite voortgeplant het. Drie tamatie variëteite het egter RF-waardes ≤ 1 gehad en is dus weerstanbiedend teen *M. incognita* ras 2. Weerstand in die tamatiekultivar Rhapsody is vervolgens gedoen deur gebruik te maak van 'n reeks Pi's. Betekenisvolle verwantskappe is vir Rhapsody en die vatbare kultivar Moneymaker aangedui in hierdie mikroplotproef en is beskryf deur nie-liniere modelle. Die aantal eiers en J2 in die wortels, sowel as in grondmonsters vanaf die risosfeer van Rhapsody was ook betekenisvol laer wanneer dit vergelyk is met die van Moneymaker. RF-waardes vir Rhapsody omgekeerd eweredig aan Pi ($r = -0.03$), terwyl dit geleidelik toegeneem het met Pi vir Moneymaker ($r = 0.94$). 'n Aantal Brassica variëteite is ook as weerstandbiedend geidentifiseer ten opsigte van *M. incognita* ras 2 en *M. javanica*, respektiewelik aangesien dit RF-waardes ≤ 1. Geen knopwortelweerstand is egter gei·dentifiseer in die groenboon- of pampoen variëteite wat in hierdie studie ge-evalueer is nie.

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 4

Figure 8: Relationships between initial (Pi) and final *Meloidogyne incognita* race 2 populations (Pf) in 50g tomato roots (A), 200ml soil (B) as well as for RF-values (C) at 86 days after inoculation (DAI) for a susceptible (Moneymaker) and a resistant (Rhapsody) tomato cultivar in a microplot trial at Potchefstroom.

LIST OF TABLES

CHAPTER 3

Chapter 1

1.1. General introduction

This study concerns the interaction between four vegetable crops namely tomato (*Lycopersicon esculentum* L. Mill.), varieties of green beans (*Phaseolus vulgaris L.)*, varieties of pumpkin (Cucurbita pepo L.Alef) and Brassica spp. (Nonnecke, 1989; Mc Creight, 1996) and root-knot nematode species, namely *Meloidogyne incognita* (Kofoid & White) and *M. javanica* (Treub, 1885) Chitwood, 1949. This introductory chapter focuses on the relevant vegetable crops, referring to its general background, classification, anatomy, agronomy and its economical and social importance. In terms of plant-parasitic nematodes, particularly root-knot nematodes, their life cycle, interaction with other organisms and control are emphasised. In addition, the concepts of host plant resistance, verification of resistance and establishment of damage threshold levels for root-knot nematodes are explained. Finally, molecular identification of root-knot nematodes and objectives of this study are outlined.

1.2. Vegetable crops

Vegetable forms an integral part of both resource-poor farming and commercial agriculture, since they are high-value cash crops and is the largest supplementary constituent of the human diet (Potter & Olthof, 1993, Sikora & Fernandez, 2005). A vegetable is roughly defined as any part of a herbaceous plant that is edible and commonly consumed by humans (Anon, 2006a). Vegetables include leaf (e.g. lettuce), stem (e.g. asparagus), root (e.g. carrot), flower (e.g. broccoli) and fruits such as cucumber, squash, pumpkin and capsicum, as well as pulses such as green beans and peas (Anon, 2006a). Each group of vegetables contributes to the human diet in its own way. Fleshy roots are high in energy value and are good sources of the vitamin B-group, while seeds are relatively high in carbohydrates and proteins (Sikora & Fernandez, 2005; Anon, 2006a). Vegetable leaves, stem and fruits are excellent sources of minerals, vitamin, water and roughage (Sikora & Fernandez, 2005; Anon, 2006a).

Vegetable production and consumption have expanded rapidly in most areas of the world, with a 32 % increase recorded in Africa from 1990 to 2002 (Sikora & Fernandez, 2005). Asia is the major vegetable producer in tropical countries, followed by Africa, South and Central America (Sikora & Fernandez, 2005). In terms of greenhouse production, total tomato production for France amounted to 892 545 metric tonnes (mt) (Anonymous, 2003f), Portugal produced an average of 100 000 mt of tomato (Anynomous, 2006g), the Mediterranean region of Europe cultivates 100 000 ha of vegetables, followed by Italy with 61 775 ha and Spain with 46 000 ha (Sikora & Fernandez, 2005). According to Hanan (1998), as reported by Sikora and Fernandez (2005) Japan, China and Turkey as well as as many countries in North Africa also have significant areas under controlled vegetable cultivation.

Although vegetable production is increasing, crop yield and quality are frequently reduced due to infection by insects, diseases and other pests. Those include various plant-parasitic nematode species, especially root-knot nematodes (*Meloidogyne* species) (Jensen, 1972; Potter & Olthof, 1993; Bridge, 1996; Agrios, 1997).

Estimated annual losses due to nematode infection of ± 14 % have been reported in vegetable production systems (Jensen, 1972; Agrios, 1997) in developing tropical countries of the world (Page & Bridge, 1993). Those losses are often higher in the tropics due to nematode genera and species diversity compared to those occuring in temperate countries (Page & Bridge, 1993). Furthermore, parasitism by plant-parasitic nematodes is also more severe in the tropics due to increased fecundity of the parasites as a result of shorter life cycles, higher environmental temperatures, longer growing seasons and absence of shorter winter/cold periods (Page & Bridge, 1993). Damage by plant-parasitic nematodes on vegetable crops has increased in economic importance due to quality and yield losses that occur in intensive vegetable production systems where monoculture is practised (Sikora & Fernandez, 2005). Intensive practices of monoculture, multiple intercropping and intercropping for long periods lead to severe crop damage, with catastrophic consequences for producers (Jensen, 1972).

1.2.1. *Lycopersicon esculentum*

The botanical name for tomato is *Lycopersicon esculentum*. Synonyms are *Lycopersicon lycopersicum* L. and *Solanum lycopersicon* L. Tomato is a tender, warm-season perennial, which was cultured into an annual crop (Pierce, 1987). In South Africa tomato is mainly produced on commercial scale in Limpopo, the Mpumalanga low- and middle-veld, the Pongala area of Kwazulu-Natal, the southern parts of the Eastern Cape and the Western Cape (Anon, 2006b).

1.2.1.1. Origin

Although the exact origin of tomato is unknown, wild-type tomato species are speculated to be native to Bolivia (Nonnecke, 1989; Peirce, 1987). The small domesticated and wild tomato was moved from its center of origin to Central America and eventually to Mexico, where the large-fruited, cultured fruit was developed (Pierce, 1987). Although tomato was introduced to Europe in 1544 (Nonnecke, 1989), northern Europeans first grew tomato in the 1650's (Nonnecke, 1989) and in the early 1800's tomato had been introduced into every region in the world where temperatures occurred that favoured its development, i.e. in fields and greenhouses (Nonnecke, 1989).

1.2.1.2. Classification (Nonnecke, 1989)

Kingdom: Plantae
Class: Dicotyledoneae
Order: Polemoniales
Family: Solanaceae
Genus: Lycopersicon
Species: *L. esculentum* L.

1.2.1.3. Anatomy

Tomato plants are typically growing 1-3m tall, with a weakly woody stem (Nonnecke, 1989). The fruit is an edible, brightly colored (usually red, as a result of the pigment lycopene) berry of 1-2 cm diameter in wild plants, but commonly much larger in

cultivated forms (Bridge, 1983). The root system of the tomato plant forms a deep taproot with extensive secondary root fibers (Nonnecke, 1989).

1.2.1.4. Agronomy

Tomato is classified as a widely adapted species, grown everywhere where environmantal conditions (a mean temperature of 24 °C with a range of 21 °C to 29 °C during the day and 18 °C to 20 °C during the night) favour its development (Pierce, 1987). It is sensitive to cold and tolerant to high temperatures (Nonnecke, 1989). The most temperature-sensitive period is during flowering. In most tomato cultivars flowers do not develop below 15 °C or above 35 °C (Nonnecke, 1989). The optimum temperature range for flowering is between 21 °C and 24 °C (Nonnecke, 1989). Seed germination does not occur in soil temperatures below 10 °C or beyond 35 °C (Nonnecke, 1989). The optimum range for germination occurs between mean soil temperatures of 15.5 °C and 29 °C (Nonnecke, 1989). High-quality yields are dependent on adequate soil moisture, optimum temperature and availability of sufficient soil nutrients (Nonnecke, 1989). Tomato yields generally range from 5-10 mt per hectare but could reach dimensions of 80-120 mt/ha in some areas (Chikwamba, 2002).

1.2.1.5. Economic and social importance

Vitamin A, B and C and lycopene (powerful antioxidants) are the major nutrients contained by tomato (Bridge, 1983). Since the end of the nineteenth century the crop has increased in popularity as one of important and life-sustaining food crop in the tropics and subtropics (Bridge, 1983; Laurie, 1996) and is produced for processing as well as as for the fresh market (Nonnecke, 1989).

Tomato is the second most important vegetable crop in South Africa (Laurie, 1996), exceeded only by potato (Pierce, 1987; Nonnecke, 1989). Globally tomato occupies 16 926 000 ha, with total production of 871 394 000 mt in tropical and subtropical regions namely, Africa, Central America, South America and Asia (Sikora & Fenandez, 2005). According to the Food and Agricultural Organisation (FAO, 2004) China is the leading tomato-producing country, with production of 30 000 000 mt,

while African countries account for 12 452 000 mt of the world's total production (Sikora & Fernandez, 2005). South Africa produces an average of 205 000 tonnes of tomato crop, which is less than 1 % of the total world production (Louw *et al.*, 2004).

1.2.2. *Phaseolus vulgaris*

1.2.1. Origin

Green bean *(Phaseolus vulgaris*, L.) also known as haricot, French, common, kidney, string, salad, runner or snap bean originated from Mexico between 2 300 and 4 000 BC (Sikora & Fernandez, 2005).

1.2.1.2. Classification (Nonnecke, 1989)
Kingdom: Plantae
Class: Dicotyledoneae
Order: Rosales
Family: Leguminosae
Genus: *Phaseolus*
Species: *P. vulgaris* L.

1.2.1.3. Anatomy

The common bean is a tender, warm-season, herbaceous, annual, dicotyledonous plant with an epigeal germination habit. Although considered an annual, the climbing types of *P. vulgaris* can be consireded perennials where frost does not occur. The petioled common-bean leaves are pinnately trifoliate, the self-fertile flowers occur in axillary racemes and vary in color from white to yellow to red or purple (Nonnecke, 1989). Common bean pods carry four to 12 kidney-shaped seeds (Michaels, 1991). Seed colours, markings and shapes vary widely among species and races (Michaels, 1991). Depending upon cultivars beans may either be true determinate or bushy types (reaching heights between 20-60 cm in height) or they may be indeterminate/semi-bush or climbing types (Michaels, 1991; Wortmann *et al.*, 2004). Like other members of the legume family, the bean plant is nitrogen-fixing. Bean plants develop a rather shallow root system, spreading its main feeder roots in the upper 20-30 cm of the soil, with a radius of about 45-70 cm. (Nonnecke, 1989).

1.2.1.4. Agronomy

Common bean, a major food crop in many parts of Africa is noted for its versatility and diversity. It is adapted to a vast range of climatic and agronomic conditions (Wortmann & Allen, 1994). Distribution of beans in Africa is heavily dependent on rural population densities and mean daily temperatures during the growing season (Wortmann & Allen, 1994). Beans germinate in about six days under optimum temperatures that range from 15.6 °C to 21.1 °C (Micheals, 1991). Germination does not occur below 0 °C or above 35 °C (Annecke, 1989) and is poor when soil temperature is less than 10 °C (Michaels, 1991). *P. vulgaris* grows readily wherever temperatures are suitable (minimum ± 10 °C and maximum ± 27 °C) and beyond the ravages of frost (Michaels, 1991). Although the crop has an extensive root system, the plant is quite sensitive to dry soils, particularly at flowering and pod setting. It also grows well in well-drained, sandy-loam, silt-loam or clay-loam soils, at a pH-range of 5.2 to 6.8 in soils containing a high organic matter content and when water supply is not limited (Michaels, 1991). Flower drop is a serious limiting factor that occurs when soil moisture is below 60 % of field capacity and/or air temperature is higher than 27 °C with a low relative humidity (Pierce, 1987; Michaels, 1991).

1.2.1.5. Economical and social importance

Legumes rank second to cereal crops in terms of nutritional importance for humankind (Sikora *et al.*, 2005). *P. vulgaris* species are, however, the most important legumes for direct human consumption (Sydenham *et al.*, 1977). Although most beans produced by small-scale farmers are for home use, marketing of this crop is also important. Beans also serve as an important source of cash for small-scale farmers in Africa, whether as part of their total income or providing a marketable product (Wortmann & Allen, 1994). This is true during critical times when farmers have nothing else to sell, such as before the maize crop is harvested (Wortmann & Allen, 1994). Grown for its edible, fleshy pods and as mature seeds, the crop provides a protein-rich food for many small-scale farmers (Parsons *et al.*, 1995; Mkandawire *et al.*, 2004). Both the immature and mature seeds of these food legumes constitute approximately 22 % of the important dietary-source proteins (Michaels, 1991). According to Pachico (1993) common bean is a major staple food in Eastern and

Southern Africa, where it is recognised as the second most important source of human dietary protein and the third most important source of calories, with consumption exceeding 50 kg per person per year. Immature pods contain significant amounts of vitamins A and C, whereas protein, carbohydrate and some of the minerals are major constituents of dry seed (Sikora *et al.*, 2005). In some parts of the tropics leaves are used as pot herbs and young leaves are also eaten. After beans are harvested its straw is used for fodder (Duke, 1983).

Phaseolus vulgaris is the most widely cultivated food legume and the most uniformly distributed crop in the world, with approximately 27 Mha of (Sikora *et al.*, 2005) production in year 2000 (Sikora *et al.*, 2005). Among the most widely grown *Phaseolus* species, namely *P. lunatus* L., *P. coccineus*, *P. acutifolius*, *P. vulgaris* is the most uniformily distributed (Michael, 1991; Sikora *et al.*, 2005). The species occupies more than 85 % of production area sown to all *Phaseolus* species in the world (Singh, 2001). It is of great importance and the main food legume in the Americas, especially in Brazil, Mexico and the USA (Sikora *et al.*, 2005), as well as in many parts of Africa (Wortmann & Allen, 1994). More than 77 % of the world's bean production occurs in tropical, developing countries (Sydenham *et al.*, 1997). In Asia the haricot bean is extensively cultivated in India, consisting of 36 % of the world acreage (Sikora *et al.*, 2005). Only 2 % of the world legume acreage is produced in Europe (Sikora *et al.*, 2005). In Africa an estimated 3 741 million ha of different sorts of beans are sown annually (Wortmann & Allen, 1994) with the main producers being Burundi, Cameroon, Congo, Ethiopia, Rwanda, Tanzania and Uganda (Sikora *et al.*, 2005). Southern African countries account for approximately 32 % of bean production in Africa (Wortmann & Allen, 1994).

1.2.3. Cucurbitaceae

1.2.3.1. Origin

Cultivated pumpkin is believed to have originated from Central America. Seeds from related plants found in Mexico date back to 5 500 B.C (Nonnecke, 1989; Pierce, 1987).

Pumpkin belongs to the family Cucurbitaceae (gourd family), which includes cucumbers, melons, squashes and gourds (Anon, 2006d). It grows as a fruit from a trailing vine. Pumpkins are cultivated in North America and continental Europe. Species include *Cucurbita pepo* L. (true pumpkin), *C. maxima* Duchesne (true squashes), *C. mixta* Pangalo or *C. moshata* (Duchesne) Duchesne ex Poir. (McCreight, 1996)

1.2.3.2. Classification (Nonnecke, 1989)

Kingdom: Plantae

Class: Dicotyledoneae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Cucurbita*

Species: *C. pepo* L.

1.2.3.3. Anatomy

Cucurbita species have shallow, extensive root systems, vigorous growth and monoecious flowering habits. The leaves of *C. pepo* are spiculate and the vining determinate (or bushy), with five-to eight-sided peduncles. The flowers are large and interspecific crosses are possible. Pumpkin fruits vary greatly in form, sometimes being nearly globular but more frequently oblong or ovoid in shape. The rind varies in colour from orange to yellow (Nonnecke, 1989; McCreight, 1996).

1.2.3.4. Agronomy

Cucurbit plants prefer high temperatures and require relatively long, frost-free growing periods (Nonnecke, 1989; McCreight, 1996). Optimum soil temperature for seed germination ranges between 21 °C to 35 °C. Temperatures below 13.5 °C suppress cucurbit seed germination. Seedling emergence and plant growth are terminated at 10 °C. The optimum temperature for plant growth is 18 °C to 24 °C. Although commercial production is limited to specific regions, this group of plants thrives as garden vegetables. A soil pH-range of 6.5 to 7.5 provide the best nutrient balance for *C. pepo* varieties and due to their large leaf area, regular or supplemental

irrigation is essential for these crops if optimum yields are to be attained (Nonnecke, 1989).

1.2.3.5. Economical and social importance

Immature and mature fruits are produced both for fresh markets and processing (Pierce, 1987). Production of cucurbits has increased significantly from 5 176 000 (Nonnecke, 1989) to 28 737 000 metric tonnes (mt) (Sikora *et al.*, 2005) since the last three decades. China, India and Ukraine are the leading pumpkin producers in the world, with production of 5 600 000 mt, 3 500 000 mt and 900 000 mt, respectively, (FAO, 2004). According to the FAO (2004) pumpkin is the sixth highest produced crop after grapefruit, maize, castor beans, pears and lupins in South Africa, with approximately 340 000 mt of pumpkin produced locally (FAO, 2004). Pumpkin is low in calories, rich in fiber, potassium, riboflavin, vitamin C and E. This crop is also a particularly good source of essential fatty acids, potassium and magnesium (Nonnecke, 1989). Pumpkin also contains large amounts of lutein and alpha- and beta-carotene (McCreight, 1996). The seeds are also an important source of oil and protein in parts of Africa, Asia and Latin America (McCreight, 1996).

1.2.4. *Brassica oleraceae* L. var. *capitata*

1.2.4.1. Origin

B. oleracea L. var. capitata (cabbage) is derived from wild sea kale (Pierce, 1987). Well-known varieties of the species include broccoli, Brussels sprouts, cauliflower, kohlrabi, broccoli raab and Chinese cabbage. Of all these cole crops, cabbage is the most important, followed by broccoli, cauliflower and Brussels sprouts (Pierce, 1987).

1.2.4.2. Classification (Nonnecke, 1989)
Kingdom: Plantae
Class: Dicotyledonae
Order: Papaverales
Family: Cruciferae
Genus: *Brassica*

Species: *B. oleracea*

Cultivar group: *B. oleraceae* L. var. *capitata*

1.2.4.3. Anatomy

It is herbaceous, biennial and a dicotyledonous, flowering plant with leaves that form a characteristic compact cluster (Anon, 2006c). In early developmental stages the cabbage plant shows no tendency to head. As the leaves become large and growth accelerates, new leaves arise from the short stem, curve and cup inward, overlapping to cover the growing point and develop inside to become crumpled and densely packed as the head develops. Head size develops slowly at first, accelerating during mid to late growth until the head constitutes over one-half of the plant's total weight at harvest (Pierce, 1987). Different head shapes are evident for the cabbage crop, i.e. pointed, conical, oblong, round, ball-shaped or drumhead shaped (more flattened than spherical). The leaves may be varying shades of green or purplish red and they may be very smooth or crimped (Nonnecke, 1989). The cabbage plant has a strong tap root system, supported by a wide network of fibrous and finely branched feeder roots that are located in the top 22 cm of soil. The greatest concentration of these roots is just below the soil surface (Nonnecke, 1989).

1.2.4.4. Agronomy (Nonnecke, 1989)

Cabbages are well adapted to cool temperatures. However, exposure to prolonged periods below 10 °C or above 25 °C brings about deleterious physiological changes in the plants. Although cabbage is not sensitive to photoperiods, prolonged exposure (four to six weeks) to cool temperatures during the juvenile stage will retard and finally terminate normal vegetative development and subsequently trigger flowering. Conversely, warmer temperatures prolong the growing period in cabbage. This wide range of growing conditions in which cabbage can be grown, makes it possible to produce cabbage for the fresh market throughout the year. The ideal soil pH to grow cabbage ranges from 6.0 to 7.5.

1.2.4.5. Economical and social importance

Cabbage is high in nutrition since it consists of proteins, carbohydrates, minerals calcium, iron (Tiwari *et al.*, 2003), vitamin C and fiber (Hunt, 2000). The cabbage head is widely consumed raw or cooked or preserved in a great variety of dishes (Anon, 2006).

Cruciferous are some of the economically most important crops worldwide (Hooks & Johnson, 2003). Although the USA is the largest producer of fresh-market cabbage (Dillard *et al.*, 2004), China and India are the leading producers of cabbages in the world, with an estimated production of 32 000 000 and 6 000 000 mt, respectively (FAO, 2004). A total of 871 394 000 mt of cabbages and 581 117 000 mt of cauliflower are produced in large tropical and subtropical regions (Sikora & Fernandez, 2005), while 1 485 000 mt of cabbages are produced in Africa (Sikora & Fernandez, 2005).

1.3. Plant-parasitic nematodes

Nematodes belong to the kingdom Animalia and comprise a large phylum (Nematoda) that encompasses plant, animal and human parasites as well as free-living species. Plant-parasitic nematodes are grouped in two classes, namely the Adenophora and Secernentea, comprising the two orders Dorylaimida and Tylenchida (Maggenti, 1981). The latter order represents the majority of plant-parasitic nematode genera (Maggenti, 1981), constituting approximately 20 % of the described species within the phylum Nematoda (Ferraz & Brown, 2002).

Plant-parasitic nematodes are obligate, biotrophic organisms that obtain nutrients only from the cytoplasm of living plant cells. Although these tiny, unsegmented organisms are small (approximately 300µm to 4 000µm long and 15µm to more than 35µm wide) and barely visible the naked eye, they are easily observed under a microscope (Agrios, 1997). Nematodes are generally wormlike in shape and contain no appendages. However, mature females of some genera (*Meloidogyne*, *Heterodera*, *Nacobbus*, etc.) have swollen, saccate bodies (Agrios, 1997).

Plant-parasitic nematodes are generally separated into two major groups according to their feeding habits, namely ectoparasites and endoparasites, which can both be migratory or sedentary (Boerma & Hussey, 1992). Ectoparasitic nematodes generally remain outside the host tissue and feed on epidermal plant cells, using their stylets (Boerma & Hussey, 1992). Conversely, migratory endoparasites enter, migrate and feed inside host plant tissue and generally cause considerable tissue destruction (Boerma & Hussey, 1992). Sedentary endoparasites have evolved highly specialised feeding relationships with their hosts and depend on modified host cells for the provision of nutrients in order to develop and reproduce optimally (Hussey & Williamson, 1998). Most plant-parasitic nematodes have a hollow stylet with which they inject enzymatic secretions in plant cells they damage and with which they subsequently ingest cytoplasmic cell contents (Ferraz & Brown, 2002). Approximately 4 100 plant-parasitic nematode species have been identified to date (Decraemer & Hunt, 2006) as important parasites of crops, inflicting yield and quality losses in agriculture and horticulture (Decraemer & Hunt, 2006).

1.3.1. Plant-parasitic nematodes associated with tomato

In addition to a wide range of pests and diseases, including fungi, bacteria, viruses, viroids mycoplasma-like organisms, insects and mites that attack tomato, plant-parasitic nematodes are also an important destructive pest of tomato (Tigchelaar, 1991). According to Keetch and Buckley (1984) and Overman, (1991) plant-parasitic nematodes associated with tomato include *Aphelenchus avenae, Belonolaimus* spp. *Criconemoides* spp., *Aphelenchoides bicaudatus, Ditylenchus* spp., *Globodera rostochiensis, Helicotylenchus dihystera, Hemicycliophora corbetti, Malenchus tantulus, Meloidogyne acronea, M. arenaria, M. hapla, M. incognita, M. javanica, Merlinius brevidens, Pratylenchus* spp., *Quinisulcius capitatus, Radopholus* spp., *Rotylenchus unisexus, Rotylenchulus* spp., *Scutellonema africanum, S. brachyurum, S. labiatum, S. magniphasmum, S. truncatum, Trichodorus* spp., *Paratrichodorus* spp. and *Xiphinema neobasiri.* Stress induced by these nematodes may directly or indirectly influence tomato yield and plant survival by damaging roots and reducing plant size and vigour (Overman, 1991).

Root-knot nematodes are, however, the predominant nematode parasites of tomato world-wide (Jacquet *et al.*, 2005). Although *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* are the four predominent and devastating root-knot nematode species reported to infect tomato in the tropics (Johnson & Fassuliotis, 1984; Nono-Womdim *et al.*, 2002), *M. graminicola, M. hapla, M. kikuyensis, M. partityla, M. vandervegtei*, etc. also parasitise this crop in warmer climates (Kleynhans, 1991). These species occur in many soils types, but cause greatest economic loss in warm sandy soils (Overman, 1991). Root-knot nematode-infected plants may suffer severe yield losses depending on the nematode population level present (Jacquet *et al.*, 2005). Tomato crops are occasionally completely lost as result of root-knot nematode infection. An estimated yield loss of 29 % in tomato could be experienced under root-knot nematode infestations in the tropics (Bridge, 1983). However, depending on biotic and abiotic factors the overall impact of root-knot nematode infection on tomato is highly variable (Nono-Womdim *et al.*, 2002).

1.3.2. Plant-parasitic nematodes associated with beans

In addition to fungal, bacterial, viral and non-infectious diseases, plant-parasitic nematodes are among the most destructive pests that severely restrain bean production globally (Hall, 1991; Sikora *et al.*, 2005). The range of plant-parasitic nematodes associated with beans include *Aphelenchus avenae, Belonolaimus longicaudatus, Cricinemoides spp., Ditylenchus* spp., *Dolichodorus spp., Helicotylenchus dihystera, H. microcephalus, Hoplolaimus pararobustus, Longidorus brevicaudatus, Meloidogyne acronea, M. arenaria, M. hapla, M. incognita, M. javanica, Heterodera glycines, Pratylenchus brachyurus, P. zeae, P. penetrans, Radopholus similis, Rotylenchulus variabilis, R. reniformis, Scutellonema labiatum, Paratrichodorus* spp., *Longidorus spp., Tylenchorhynchus* spp., *Xiphinema* spp. and *X. sandellum* (Keetch & Buckley, 1984; Abawi *et al.*, 1991). However, only species of *Meloidogyne* and *Pratylenchus* are frequently encountered in bean roots, causing considerable damage to this crop when present in high numbers (Abawi *et al.*, 1991).

Meloidogyne species are one of the economically most important plant-parasitic nematode groups encountered in beans (Johnson & Fassuoliotis, 1984; Abawi *et al.*, 1991). Due to its faster reproduction rate high populations cause significant yield

losses that range from 50 % - 90 % (Abawi *et al.*, 1991). *M. incognita, M. javanica and M. arenaria* are generally the most damaging root-knot nematode species on beans (Sikora *et al.*, 2005). *M. incognita* and *M. javanica* are also the root-knot nematode species that are the most prevalent nematodes on beans in tropical and subtropical regions (Michaels, 1991), with up to 60 % yield losses ascribed to them in Kenya (Ngundo & Taylor, 1974). Reaction of bean roots to infection by root-knot nematodes is extremely variable, ranging from no galling to severe galling responses (Johnson & Fassuolitis, 1984). Above-ground symptoms exhibited by *Meloidogyne*-infected plants do not permit a positive diagnosis for root-knot nematode infection. Roots must therefore be lifted to identify knots/galls due to root-knot nematodes infection. Severely infected plants may, however, have chlorotic, stunted, necrotic and wilted appearances (Michaels, 1991).

1.3.3. Plant-parasitic nematodes associated with cucurbits

Fungi, bacteria, viruses, viroids, insects, parasitic spermatophytes and plant-parasitic nematodes are pests and diseases of cucurbits that reduce the quality and quantity of fruit (Zitter, 1996). Plant-parasitic nematodes associated with *Cucurbit* spp. in southern Africa include *Belonolaimus longicaudatus*, *Ditylenchus* spp., *Helicotylenchus* spp., *Hemicycliophora* spp., *Hoplolaimus* spp., *Longidorus* spp., *Meloidogyne javanica, M. incognita, M. hapla*, *Pratylenchus vulnus, P. thornei, Radopholus* spp., *Xiphinema elongatum, X. variabile, Rotylenchus reniformis*, *Paratylenchus* spp., *Paratrichodorus spp.* and *Trichodorus* spp. (Keetch & Buckley, 1984; Thies, 1996). Although these nematode species parasitise these crops, *M. incognita, M. javanica* and *M. arenaria* are the predominant and the most damaging nematode group associated with cucurbits (Thies, 1996). Damage inflicted by this nematode group is greatest in warm regions where these nematode species most commonly occur (Thies, 1996). Root-knot nematode-infected symptoms are most severe in light soils where drought stress occurs. Plants may exhibit chorotic, stunted, yellowing of foliage, reduced leave size, wilting and poor fruit quantity (Thies, 1996). Pumpkin plants may eventually die before producing marketable fruits (Johnson & Fassuliotis, 1984).

1.3.4. Plant-parasitic nematodes associated with *Brassica* spp.

Numerous plant-parasitic nematodes are associated with cabbage in southern African countries, namely *Aphelenchoides* spp., *Aphelenchus avenae, Helicotylenchus dihystera, H. egyptiensis, H. nannus, Heterodera* spp., *Meloidogyne arenaria, M. incognita* and *M. javanica, Nothocriconema mutabile, Paratrichodorus minor, Scutellonema* spp. and *Trichodorus* spp. (Keetch & Buckley, 1984; Kleynhans *et al.*, 1996).

However, two genera of plant-parasitic nematodes, namely *Heterodera* (cyst nematodes) and *Meloidogyne* (root-knot nematodes) are considered to be the predominant nematode pests of cabbage. Cyst nematodes (*H. cruciferae*) frequently occur in cabbage in regions of California and can reduce yields and/or delay crop maturity (Sikora & Fernandez, 2005). Root-knot nematodes, particularly *M. incognita* and *M. javanica* occur globally on cabbage crops and could significant yield reductions (Anon, 2003c; Sikora & Fernandez, 2005). Root-knot nematode-infected plants are severely stunted and chlorotic and most of the older leaves die off (Johnson & Fassuliotis, 1984).

1.4. Root-knot nematodes

Although a wide spectrum of plant-parasitic nematodes are associated with vegetable crops, root-knot nematodes (Sikora & Fernandez, 2005) are economically the most important group on vegetable crops, followed by the reniform nematode, *Rotylenchulus reniformis* (Roberts *et al.*, 2005), *Cactodera, Ditylenchus dipsaci, Globodera rostochiensis, Heterodera cruciferae, H. schachtii, Nacobbus aberrans, N. bolivianus, N. dorsalis, Paratrichodorus minor* (Sikora & Fernandez, 2005).
Root-knot nematodes have been identified as parasites of vegetable crops since 1855, when Berkeley in England first described their symptoms as "vibrios-forming excrescences on cucumber roots" (Johnson & Fassuliotis, 1984). More than 90 *Meloidogyne* species have been described to date, with four species being of particular economic importance in global vegetable production, namely *Meloidogyne arenaria, M. hapla, M. incognita and M. javanica M. incognita* (Sikora & Fernandez, 2005).

This study focused on root-knot nematodes because *Meloidogyne arenaria, M. incognita and M. javanica M. incognita* in particular account for most of the crop losses due to root-knot nematode infection (Xu *et al.*, 2001) and accounts for approximately 99 % of populations collected from cultivated plant species (Abawi *et al.*, 1991). Most of the plants, including vegetables that account for the majority of human and animals food, are susceptible to one or more of these root-knot nematode species (Taylor & Sasser, 1978). Some crops are generally invaded by more than one root-knot nematode species at the same time (Jensen, 1972). *Meloidogyne incognita* is the most important root-knot nematode species on vegetables worldwide (Lamberti, 1979; Castagnone-Sereno *et al.*, 1993) and together with *M. javanica*, are the most prevalent and economically important nematode species in Africa (Bridge, 1995). *M. javanica* is, however, the predominant species in southern African countries (Nono-Womdim *et al.*, 2002). Both *M. incognita* and *M. javanica* pose a serious threat to vegetable cropping systems and are one of the major obstacles that hamper production of adequate food supplies in many developing countries (Hussey & Janssen, 2002), particularly in subsistence farming systems where vegetable crops serve as a primary food source (Jensen, 1972). In South Africa these nematodes are described as the most common root-knot nematode parasites of plants (Kleynhans *et al.*, 1996) causing greater economic damage than other plant-parasitic nematodes (Van der Wal, 1999b). They were also reported to be predominantly associated with vegetable crops in South African home, community and small-scale farming systems (Fourie & Mc Donald, 2000). *M. arenaria,* on the other hand occur more commonly in the subtropics but also is found sporadically in the tropics. (Fargette *et al.*, 1996; Sikora & Fernandez, 2005) and *M. hapla*, a species common in temperate regions, is occasionally found in the cooler upland tropics (Sikora & Fernandez, 2005).

1.4.1. Life cycle

Root-knot nematodes are obligate, sedentary endoparasites (Kleynhans, 1991) that complete most of their life cycle within the roots/tubers/pods of it host plant. When environmental conditions are favourable it may also survive on a range of weeds, particularly broadleaf species (Overman, 1991). Root-knot nematodes survive in soil as eggs and also as anhydrobiotic, second-stage juveniles (Sikora & Fernandez, 2005). *Meloidogyne javanica* and *M. incognita* reproduce by mitotic parthenogenesis (Xu *et*

al., 2001; Sikora & Fernandez, 2005) and have a strong reproductive potential to produce multiple generations per season. The life cycle duration of both these root-knot nematodes is generally the same, namely ± 21 days at 26 °C (Taylor & Sasser, 1978). This usually depends upon genetic qualities of the host plant (species and cultivar) and environmental conditions (soil temperature, etc), which influence both the nematode and the plant and which in total constitute the host-nematode inter-relationship complex (DeGuiran & Ritter, 1979; Johnson & Fassuliotis, 1984).

First-stage juveniles (J1) moult once within the eggs and hatch as fully developed and functional second-stage juveniles (J2). This infective, motile J2 migrates through soil and penetrates relevant tissue of a suitable host plant. The J2 subsequently establishes a permanent feeding site by thrusting its stylet into plants cells surrounding its head (Dropkin, 1980, Ferraz & Brown, 2002). Feeding also allows the vermiform J2 to enlarge and undergo morphological changes to become a sedentary, sausage-shaped parasitic J2. Without feeding it then moults three times into the third- and fourth-stage juveniles and finally moults into an adult, pear-shaped female (Ferraz & Brown, 2002). A third-stage male juvenile undergoes the fourth and final moulting stages and emerges from roots as wormlike, adult male, which becomes free-living in the soil, without feeding on host plants (Agrios, 1997).

Mature root-knot nematode females are embedded inside roots and are generally visible as a swelling in the root (Ferraz & Brown, 2002). Several hundreds of eggs are deposited into a gelatinous matrix called an egg sac or egg mass, which protects the eggs from dehydration on the tissue surface (Heyns, 1971). Continuous feeding by root-knot nematode females adversely affects normal physiological processes of the host plant, which include hampering of water and nutrient uptake and transport (Sikora & Fernandez, 2005). Furthermore, root-knot nematode infection results in the formation of characteristic root galls (Sikora & Fernandez, 2005), which vary in size and shape depending on the host plant, nematode population levels and root-knot nematode species present in the soil (Sikora & Fernandez, 2005). Feeding by these root-knot nematode females also induces the formation of giant cells (Xu *et al.*, 2001). Above-ground symptoms of root-knot nematode-infected plants might include

stunting, wilting, yellowing, general unthrifty appearance of plants, reduced yield and yield quality as well as premature death (Cerkauskas, 2004).

1.4.2. Interactions with other organisms

The interaction of plant-parasitic nematodes with other organisms is an important constraint to global agriculture (Page & Bridge, 1993). Association of root-knot nematodes with their host-plants is often accompanied by infection of other pathogens, usually bacteria and fungi, e.g. *Ralstonia solanacearum* (bacterial wilt), *Sclerotium rolfsii* (southern blight), *Fusarium* spp. and *Rhizoctonia* spp. (Cerkauskas, 2004), resulting in the development of disease complexes (Jensen, 1972). These complexes are formed because root-knot nematodes damage plant tissue during feeding, forming wound sites through which other micro-organisms can enter (Abawi & Chen, 1998). These interactions of root-knot nematodes with other pathogens increase the severity of damage or predispose host plants to a more rapid or severe expression of other diseases, affecting the host in different physiological ways (Johnson & Fassuliotis, 1984).

Although yield losses of 5–34 % have been reported as a result of synergistical interactions between root-knot nematodes and other plant pathogens (Ibrahim & Ibrahim, 2000), it is complicated to determine the role that *Meloidogyne* species play in such crop losses. The latter scenario is common in tropical regions, since crops are simultaneously attacked by fungi, insects, other pests and plant-parasitic nematodes (Sikora & Fernandez, 2005). Suppression of such disease complexes in vegetable crops by controlling of root-knot nematodes is fundamental to control of the disease complex and could increase yields significantly (Sikora & Fernandez, 2005; Hussey & McGuire, 1987).

1.4.3. Control

Crops highly susceptible to plant-parasitic nematodes may rapidly maintain high nematode numbers, even if low initial population densities occurred at planting time (Keetch & Milne, 1982). Therefore, nematode control measures are designed to reduce populations of plant-parasitic nematodes so that limited number of nematodes

will be present in soil when follow-up crops are planted early in the growing season (Ferraz & Brown, 2002). These control measures, however, vary with paedoclimatic conditions, the socio-economic situation, economics of the crop, availability of registered nematicides, availability of resistant cultivars and feasibility of certain agricultural practices (Lamberti, 1979).

Control strategies aimed at reducing plant-parasitic nematodes effectively are categorised into to two major groups, namely cultural and classical (chemical) control (Bridge, 1996; Ferraz & Brown, 2002). A condensed synopsis of these control strategies follows.

1.4.3.1. Cultural control

Cultural nematode control strategies are applied in both commercial and subsistence agricultural systems. Particularly small-scale farmers in developing countries (Madulu *et al.*, 1994) use various integrated farming practices, namely:

(i) Prevention and spread of plant-parasitic nematodes using nematode-free planting material (Bridge, 1996). Preventing the establishment of these parasites is the first, crucial step towards successful nematode control (Jensen, 1972) since nematode infestations are promoted by the absence of proper nematode- and disease-free planting material (Sikora & Fernandez, 2005). Subsistence farmers usually produce their own planting material infected with plant-parasitic nematodes, resulting in poor quality seedlings or tubers (Sikora & Fernandez, 2005).

(ii) The use of direct, non-chemical, cultural and physical control methods:

Fallow is one of management strategies often used to reduce plant-parasitic nematode populations and is based on the fact that nematode populations would decline rapidly to levels below the damage threshold for crop damage without food residues available for a given period of time (Ferraz & Brown, 2002).

Flooding is another nematode control strategy and is used in areas where water is abundant and fields are level (Johnson & Fassuliotis, 1984). It is sometimes possible

to control nematodes by flooding land to a depth of 10 cm of water or more for several months (Johnson & Fassuliotis, 1984). However, this method is not economically feasible for sustainable subsistence-agriculture as abundant water supply is not always available in resource-poor areas (Ferraz & Brown, 2002).

Use of trap crops is also a method to control particularly endoparasitic nematodes (Keetch & Milne, 1982) and entails that a highly susceptible, quick-growing crop is planted on a field and allowed to grow for a short time, after which it is plowed under or otherwise destroyed. Control is based on the principle that the endoparasitic nematodes become sedentary and are subsequently destroyed together with the plant before they are able to reproduce. Use of this method involves careful timing. If the crop is left for too long the nematode populations might increase as reproduction of these parasites occur (Johnson & Fassuliotis, 1984). Although the method is effective to reduce plant-parasitic nematode populations it is not always economically feasible because productivity is lost during that period (Jensen, 1972). It also has additional expenses, e.g. planting and growing a crop that is destroyed and therefore a producer gets no financial return (Keetch & Milne, 1982).

Crop rotation is another method that is widely used to reduce plant-parasitic nematode numbers and has often been applied successfully to minimise nematode problems (Brown, 1982). Nematode species with a narrow host range are most effectively controlled by the effective use of rotation crops (Webster, 1972; Kleynhans et al., 1996). Crop rotation allows sufficient time intervals after each host crop to allow nematode populations to return to lower levels before follow-up host crops are planted (Brown, 1982). The main aim of crop rotation is to reduce nematode population densities to acceptable levels by using a non-host crop (either resistant or immune) before planting a susceptible, follow-up crop (Oostenbrink, 1972). Various crop sequences that effectively control root-knot nematodes have been reported. Some crops and varieties such as castor (*Ricinus communis*), velvet bean (*Mucuna deeringina*), Mississippi Silver cowpea (*Vigna unguiculata*), American joint vetch (*Aeschynomene americana*), Deltapine 51 cotton (*Gossypium hirsutum*) and SX-17 sorghum-sudangrass (*Sudan bicolor* x *S. sudanense*) were effective as rotational crops in maintaining low population densities (<12/100 cm^3 soil) of *M. incognita* race 1 (McSorley & Dickson, 1995).

(iii) Encouragement of naturally-occuring biological agents and effecient use of soil amendments (Bridge, 1996).

Biological agents that are natural enemies of plant-parasitic nematodes, e.g. bacteria, fungi, arthropods, protozoa and nematodes are abundant in most soils (Ferraz & Brown, 2002). Those agents can contribute to nematode control as part of an intergrated control system in conducive environments (Webster, 1972), since they modify the ecological environment of the pest in order to restrict its activities below damage threshold levels (Webster, 1972). On the other hand, soil amendments such as cattle and chicken manure, oilseed cake, etc. generally improve the nutrient and water-holding capacity of soil, improving plant growth (Keetch & Milne, 1982). A higher organic matter content also stimulates microbial activity thus increasing the activity of beneficial micro-oraginisms (i.e. fungi, bacteria, etc.) that are antagonistic to nematodes (Bridge, 1996). Some of the well-documented examples of effective biological control methods of root-knot nematodes are cow dung and urine (Abubakur *et al.*, 2004) as well as chicken manure (Kaplan & Noe, 1993; Ibrahim & Ibrahim, 2000) and *Pasteuria penetrans* (Sekhar & Gill, 1990, Weibelzahl-Fulton *et al.*, 1996). However, the effectiveness of biological control agents under harsh environmental conditions (i.e. dry and irrigated land in southern Africa) has not been proven to date.

Other nematode control strategies, e.g. early planting, cover crops, antagonistic plants, etc., are also available to reduce plant-parasitic nematode numbers but are not discussed because of limited possibilities of application.

1.4.3.2. Classical control

Nematicides have been used extensively since the 1900's (Ferraz & Brown, 2002) as the major nematode control strategy to reduce plant-parasitic nematode numbers in high-value crops such as vegetables (Netscher & Sikora, 1993), legumes (Sikora & Greco, 1993) and a range of other crops (Luc *et al.*, 1993). Since the use nematicides are declining (Ferraz & Brown, 2002) environmentally-friendly, cost-effective nematode control methods are becoming increasingly important, particularly for subsistance farming systems (Bridge, 1996). Use of nematicides will, however, probably always play an important role in protecting crops from plant-parasitic

nematodes (Ferraz & Brown, 2002; Sikora & Fernandez, 2005). Inclusion of this strategy in an integrated nematode control system is therefore of utmost importance.

1.4.3.3. Host-plant resistance

Principles and practices of root-knot nematode management is essential to reduce and maintain the pest damage below threshold levels, thus increasing and/or maintaining the quantity as well as the quality of vegetable crops (Johnson & Fassuliotis, 1984). Host-plant resistance is one of the most popular, environmentally friendly and cost-effective nematode control strategies in both commercial and subsistence farming systems (Bridge, 1996a; Starr *et al.*, 2002). Nematode-resistant cultivars play a key role as one of the most useful means to manage root-knot nematodes in a range of agricultural crops (Starr *et al.*, 2002).

Resistance or susceptibility on the one hand and tolerance or sensitivity on the other hand are defined as independent, relative qualities of a host plant's reaction to nematode infection, based on comparison between a susceptible and resistant cultivar/genotype (Bos & Parlevliet, 1995). A susceptible host plant has a complex of characteristics that are favourable for nematode development and reproduction. Such a host plant is unable to impede the growth and development of the nematode (Bos & Parlevliet, 1995). However, host-plant resistance entails a range of mechanisms to resist nematode penetration, establishment and spread of a nematode within a host (Bos & Parlevliet, 1995). A highly resistant cultivar will support little nematode reproduction (< 10 % compared to a susceptible cultivar), while a moderately resistant cultivar will support an intermidiate level of nematode reproduction relative to a susceptible cultivar (Hussey & Janssen, 2002).

Non-preference, antibiosis and tolerance are considered the three main mechanisms of resistance (Painter, 1951; Cook & Evans, 1987). Non-preference is a property exhihited by a host plant that denotes a nematode's response to plants that lack characteristics to serve as host. The nematode will therefore avoid a plant or have a negative reaction to the plant during its search for food, penetration sites or shelter (Painter, 1951; Cook & Evans, 1987). Antibiosis on the other hand, includes all

adverse effects exerted by the host plant on the nematode's biology, e.g. its survival, development and reproduction (Painter, 1951; Cook & Evans, 1987). Tolerance includes all responses by the host plant that result in the ability to withstand nematode infecion and to support nematode populations and crop yield which would otherwise severely damage susceptible plants (Oostenbrink, 1972; Roberts, 2002). However, tolerant plants are of limited value in subsistence farming system as nematode reproduction may be sufficient for population densities to reach the damage threshold level (Cook & Starr, 2006).

Subsistence farmers, who represent a significant part of the world's agriculture, have limited resources available to deal with adverse conditions (environment, crop, cultivar, etc.) and to produce sufficient food to sustain family and community needs (Bridge, 1987). This includes the serious threat posed by difficult-to-control root-knot nematodes (Fourie & Mc Donald, 2000; Starr *et al.*, 2002). Ineffective root-knot nematode control in these farming systems leads to greater crop losses, as these farmers usually lack collaboration with relevant role players, i.e. government, public enterprises, etc. (Sikora & Fernandez, 2005).

Due to adverse effects associated with the use of chemical nematicides, plant resistance is an attractive solution for controlling root-knot nematodes (Jacquet *et al.*, 2005; Sikora & Fernandez, 2005). According to Epps *et al.* (1981) and Young (1992) resistant cultivars without nematicide treatment generally yield as much as high-yielding, susceptible cultivars treated with nematicides. Use of resistant cultivars is, therefore, the most economical and environmentally friendly method of controlling root-knot nematodes (Castagnone-Sereno *et al.*, 1993). It may be one of the few viable practical management tactics for subsistence farmers (Roberts, 1992) as it does not require special application techniques, skills or equipment and there is no additional cost to the grower (Cook & Evans, 1987). Perfomance of resistant varieties may, however, vary according to species, subspecies or biotypes of the nematode population present, growing conditions of the crop and bacterial or fungal disease complexes involved, etc. (Jensen, 1972).

1.4.3.3.1. Host plant resistance to root-knot nematodes in vegetable crops

A summary is given below in terms of root-knot nematode resistance present in vegetable crops used in this study, i.e. tomato, green beans, *Brassicas* and pumpkins.

Resistance to root-knot nematodes in tomato was first identified in the wild species *Lycopersicon peruvianum* (L.) Mill and was later introgressed into *L. esculentum* (Johnson & Fassuliotis, 1984). The majority of tomato cultivars with root-knot resistance currently available are derived from this source (Medina-Filho & Tanksley, 1983). Although the exact number of genes involved in this resistance is unknown (Roberts *et al.*, 1990), the resistance is suspected to be controlled by a single dominant gene, namely the "*Mi*–gene" (Gilbert & McGuire, 1956). This gene confers resistance to *M. incognita*, *M. javanica* and *M. arenaria* in tomato (Sikora & Fernandez, 2005). *Mi*-resistance in tomato is currently extensively used on a worldwide basis to control root-knot nematodes, both at a commercial level and in home gardens (Sikora & Fernandez, 2005). Di Vito *et al.* (1991) evaluated susceptible and resistant tomato cultivars and found negligible reduction in plant growth, yield, fruit size as well as decreased *M. incognita* populations in the soil in rhizosphere of a resistant cultivar DISA N compared to a susceptible cultivar, Ventura. Mani and Zidgali (1995) also reported that, out of the 21 tomato genotypes screened for resistance against *M. incognita,* Mont Carle exhibited moderate resistance while all the others were highly susceptible. Charchar *et al.* (2003) also observed that no yield losses were observed in resistant tomato cultivars Nemadoro, Itaparica and Del Rey evaluated against a mixed population of *M. incognita* race 1 and *M. javanica* compared to susceptible Rio Grande, Europeel and Calipso. Although successful use of host-plant resistance has been reported by several authors previously, this resistance, however, tends to be ineffective at high soil temperature (> 28 °C) and it does also not confer resistance to geographically isolated populations of root-knot nematodes (Overman, 1991). Dropkin (1969) showed that the resistant tomato cultivar Nematx became susceptible to *M. incognita*, *M. arenaria* and *M. javanica* as the temperature increased from 29 °C to 33 °C. He also noted that elevated temperatures during only the first two to three days after inoculation adversely affected the resistance exhibited at lower temperatures.

Host-plant resistance to *M. incognita* in green beans is present in the snap bean cultivar Nemasnap (Sikora *et al.*, 2005), while several *Brassica* cultivars with resistance to an Australian population of *M. javanica* have been reported (Stirling & Stirling, 2003). These include mustard (cv. Nemafix), canola (cv. Dunkeld) and rape (cvs. Rangi, Nemcon and Striker). Little research has, however, been done in the development of pumpkin varieties with resistance to root-knot nematodes on a world-wide scale (Brust *et al.*, 2003).

1.4.3.3.2. Verification of host plant resistance

Once root-knot nematode resistance has been identified in crop cultivars, it should be verified under natural environmental conditions for sustainability (Hussey & Jansen, 2002). Although microplot screening has advantages and disadvantages, it is a valuable tool for verification of resistant sources (Hussey & Janssen, 2002). Yield data could be obtained and root-knot nematode populations could be established in resistant cultivars under near-field conditions (Hussey & Janssen, 2002). Charchar *et al.* (2003) demonstrated that levels of *M. incognita* race 1 and *M. javanica* in resistant cultivars Nemadoro, Itaparica and Del Rey were maintained in both greenhouse and in field trials. The advanced tomato breeding lines ARP 365-2, ARP 367-1 and ARP 367-2, also showed resistance under greenhouse and field conditions (Nono-Womdim *et al.*, 2002).

1.4.3.3.3. Establishment of damage threshold levels for root-knot nematodes

The presence of nematodes in a field does not necessarily imply that crop damage and yield loss will occur since nematode populations may be below damage threshold levels for a specific set of conditions (Brown, 1987). Yield losses are usually related to the status of a nematode population at planting although the level at which they occur varies with the nematode species involved, the soil type, the climate and the crop (Brown, 1987). The relationship between root-knot nematode initial populations (Pi) and final population (Pf) as well as between Pi and crop yield is crucial for being able to decide whether to plant a susceptible crop and whether additional management measures are required (Ferris, 1978a & b). Di Vito *et al.* (1991) reported good relationships between Pi of *M. incognita* and the damage caused to resistant as well as

susceptible tomato crops. The latter author observed that stunting on susceptible tomato cv. Ventura at the inoculum level of 128 eggs/cm^3 soil while the resistant cultivar cv. DISA N showed negligible reduction in growth at the same inoculum level. Flowering and fruiting were also poor for Ventura, resulting in small and unmarketable fruits compared to the resistant cultivar in the same trial. Also, the percentage marketable yield of susceptible tomato was significantly affected and was approximately 90 % at the lowest Pi (Pi = 0.031). The final population densities (Pf) of *M. incognita* increased at Pi between 0.031 and 32 eggs and juveniles/cm^3 soil and decreased at Pi ≥ 64 eggs/cm^3 soil in microplots planted with susceptible tomato (Di Vito *et al.*, 1991).

1.5. Molecular identification of root-knot nematodes

DNA-based diagnostics provide an attractive solution for the detection and identification of plant-parasitic nematodes that are considered to be economically important. Not only are qualitative differences among nematodes important for diagnosis (i.e. which genus, species, and race is present in a sample), but nematode populations must also be quantified for preventative management decisions (Roberts, 1994).

The extensive morphological variation among and within root-knot nematode species complicates their identification (Hartman & Sasser, 1985). Accurate and reliable identification of root-knot nematodes are fundamental requirements before research programmes or proper management strategies could be implemented, particularly where quarantine organisms are concerned (Piotte *et al.*, 1992; Zijlstra, 2000). Furthermore, accurate species identification of *Meloidogyne* is important for proper selection of non-host crops for rotation purposes or for the use of a resistant cultivar when available. Identification and variability of a root-knot nematode species significantly aid in the development of resistant cultivars and control through the use of crop rotation (Thomason & Caswell, 1987).

Available methods to identify plant-parasitic nematodes that are based on morphological characteristics (Jepson, 1987), require a lot of skill and are often

inconclusive for individual, because they are often vary considerably within a population (Zijlstra *et al.*, 2000). Extensive morphological variation among and within root-knot nematodes further complicates their identification (Hartman & Sasser, 1985; Piotte *et al.*, 1992) as well as the fact that morphological features of these parasites are subject to phenotypical variations (Franklin, 1979).

Since host plant resistance contribute to the reduction of plant-parasitic nematodes in crop rotation systems (Roberts, 1992), accurate, reliable and timely identification of root-knot nematodes. This is fundamental for research programmes and proper management strategies to be implemented, since it could improve progress and crop management decisions (Powers & Harris, 1993; Zijlstra, 2000).

DNA-based diagnostics provide attractive solutions for clear, reliable identification. (Ziljstra et al., 2000). DNA analysis have been successfully used in the past by researchers (Powers et al., 1986; Castegnone-Sereno et al., 1991; Piotte et al., 1992; Xue et al., 1992; Fargette et al; 1996). The polymerase chain reaction offers both sensitivity and genetic specifity for DNA amplification and identification (Powers & Harris, 1993) (Roberts, 1994) and PCR-based methods require much less DNA as compared to other DNA techniques, for example RFLP (Zijlstra et al., 2000). However, the SCAR-PCR technique is more sensitive than other existing molecular techniques and enables detection of species present in mixed populations in proportions of less than 1% (Fourie et al., 2000).

1.6. Rationale and aims of the present study

The main objectives of the study were i) to verify the identity of two root-knot nematode species (*M. incognita* and *M. javanica*) used in trials by means of molecular techniques, ii) to evaluate locally available tomato, green bean, pumpkin and Brassica cultivars for resistance to *M. incognita* race 2 and *M. javanica*, respectively, in separate trials and iii) to verify the resistance under more natural conditions.

Chapter 2

Identification of *Meloidogyne incognita* and *M. javanica* using SCAR-PCR assays.

2.1. Introduction

Root-knot nematodes are among the most destructive plant parasites, particularly in subsistance small-scale farming systems (Powers & Harris, 1993), where they may reduce vegetable yield and quality up to 100% if timely and effective control strategies have not been applied (Fassuliotis, 1991; Sikora & Fernandez, 2005).

Accurate identification of root-knot nematodes is essential and is the foundation for successful management practices, which could be optimised if species are correctly identified (Piotte *et al.*, 1992). Available methods to identify root-knot nematodes are generally based on morphological characters (Jepson, 1987), they require a lot of skill and are often inconclusive for individuals because they can vary considerably within a population (Zijlstra *et al.*, 2000).

On the other hand, DNA-based diagnostics provide attractive solutions for reliable identification of root-knot nematodes (Ziljstra *et al.*, 2000) and have been successfully used in the past by several researchers (Powers *et al.*, 1986; Castagnone-Sereno *et al.*, 1991; Piotte *et al.*, 1992; Xue *et al.*, 1992; Fargette *et al.*, 1996, Ziljstra *et al.*, 2000). Therefore, the two root-knot nematode species used for inoculum purposes in the present study, namely *M. incognita* race 2 and *M. javanica*, were subjected to SCAR-PCR analysis to confirm it as monospecific populations.

2.2. Materials and methods

2.2.1. DNA extraction

Fifteen root-knot nematode females of *M. incognita* race 2 and *M. javanica*, respectively, were randomly removed with a scalpel from galls on tomato roots infected with the appropriate species from *in vivo* mass rearing populations in the green house. This procedure was conducted using a stereo-microscope. Single root-

knot nematode females of the appropriate species were each placed in a micro-centrifuge tube and crushed with a disposable pipette tip. DNA was isolated from each single root-knot nematode female by adding 50µl cell lysis buffer (50mM TrisHCl pH 8.0; 100 mM NaCl; 10 mM EDTA; 1 % SDS (v/v) to each tube and 2µl of proteinase K (10 mg/ml) added. The latter solution was incubated at 65 °C for 1 hour. The DNA was extracted using a GeneClean Kit (Bio101) according to the manufacturer's instructions. Three volumes of sodium iodide (NaI) solution (150µl) and 2µl glassmilk were added, mixed and incubated at room temperature (25 °C - 27 °C) for five minutes. The solution was then centrifuged at 10 000 rpm for five seconds and the supernatant decanted. The pellet (glassmilk with bound DNA) remaining at the bottom of the tube was resuspended in 200µl NEW Wash and centrifuged at 10 000 rpm for another five seconds. This process was repeated two more times. The liquid supernatant was removed and the pellet dried at room temperature, resuspended in 20µl TE buffer and centrifuged for 30 seconds at 10 000 rpm. Two microlitres of root-knot nematode DNA was subsequently used for SCAR-amplification reactions.

2.2.2. SCAR amplification

The polymerase chain reaction (PCR) was carried out in a total volume of 20 µl, containing 1-2 µl DNA, 1 x GoTaq Green Master Mix (Promega) containing GoTaq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer at optimal concentrations, 5 pmol each of *M. incognita* forward and reverse primers or 5 pmol each of *M. javanica* forward and reverse primers, respectively. DNA amplification for *M. incognita* race 2 was done in a ThermoHybaid Thermal Cycler programmed as follows: denaturation at 94 °C for two minutes (one cycle), followed by 40 cycles of denaturation at 94 °C for 30 seconds, annealing at 54 °C for 30 seconds and extension at 72 °C for one minute, followed by a final extension step at 72 °C for five minutes. The same programme was used for *M. javanica* with the exception of the annealing temperature being at 64 °C (Zijlstra, 2000; Zijlstra *et al.*, 2000).

Female root-knot nematode DNA products resulting from the amplification process were analysed by electrophoresis in a 3 % (m/v) agarose gel with 1x TBE running buffer (89 mM Tris-borate, 2.5 mM EDTA, pH 8.3). Ethidium bromide (1 µg/ml)

was added to the gel and samples were visualised under UV-light. One blank reaction (no DNA), one reference sample and Lambda DNA restricted with HindIII and EcoRI as molecular weight markers were loaded on both sides of the comb to establish the size of the DNA bands for each root-knot nematode species. The gel containing DNA products of the respective females was electrophoresed for 2 hours at 80 V and banding patterns were visualised with ultraviolet (UV) illumination. A photograph was taken of each gel containing the DNA-banding patterns.

2.3. Results

PCR with the *M. incognita* specific SCAR-primers resulted in amplification of the *M. incognita* 1 200 bp SCAR-fragment for the *M incognita* race 2 populations used in greenhouse and a microplot trial during this study (Fig. 1). The same result was obtained for the *M. incognita* population from Marble Hall (Mpumalanga Province) that was used as a reference population in this regard.

In terms of the *M. javanica* population used in this study, the 670 bp *M. javanica* SCAR fragment was amplified during PCR-reactions with the *M. javanica*-specific SCAR marker (Fig. 2). However, less intensive DNA bands were visible for the *M. javanica* female DNA used in lanes 6, 7, 8, 9, 11, 12, 14 & 15.

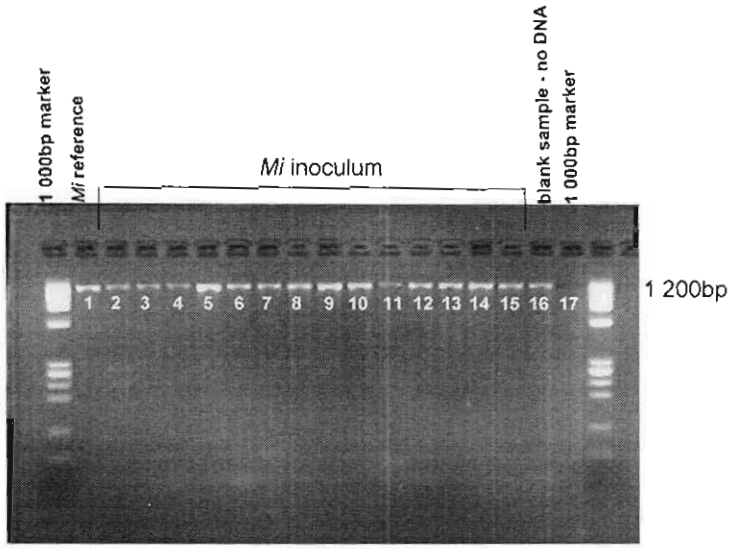


Figure 1. Amplification products of PCR reactions using *Meloidogyne incognita* specific primers and template DNA of *Meloidogyne* species populations. (*Mi* reference = *M. incognita* reference population; *Mi* inoculum = *M. incognita* race 2 used as incoculum).

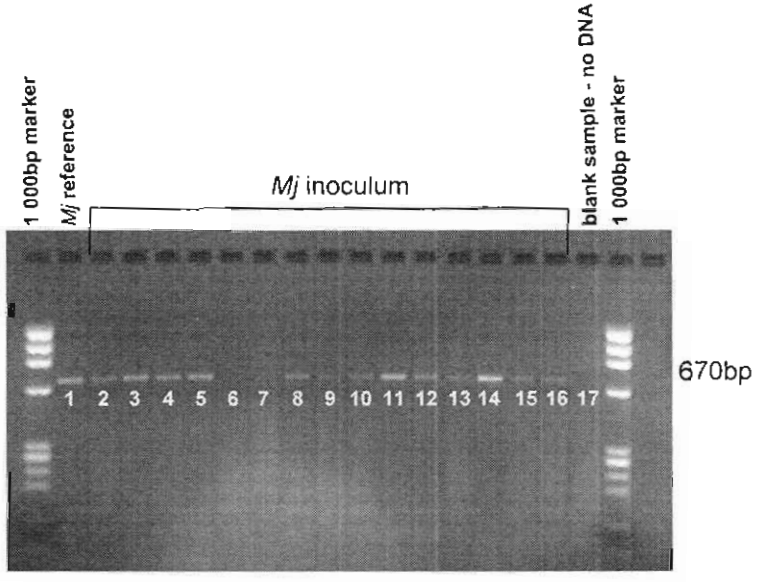


Figure 2. Amplification products of PCR reactions using *Meloidogyne javanica* specific primers and template DNA of *Meloidogyne* species populations. (Mj reference = M. *javanica* reference population; Mj inoculum = *M. javanica* used as incoculum).

2.4. Discussion

The DNA-based identification was valuable in verifying and confirming the identity of both these two economically important root-knot nematode species and race.

Results from SCAR-PCR reactions indicated that monospecific populations of *M. incognita* race 2 and *M. javanica* were mass-reared and subsequently used as inoculum in greenhouse and microplot trials during this study. Molecular identification of the two respective root-knot nematode species was thus valuable with regard to identification of host suitability to a relevant species and/or race, as well as in terms of the verification of resistance to *M. incognita* race 2 in a microplot trial. The SCAR-PCR method used in this study proved to a reliable tool for routine diagnostic identification of root-knot nematodes, since it was used with success by various other researchers (Blok *et al.*, 1997; Fourie *et al.*, 2001; Zijlstra, 2000; Zijlstra *et al.*, 2000).

Chapter 3

Host suitability of vegetable crops to *Meloidogyne incognita* race 2 and *M. javanica.*

3.1. Introduction

Root-knot nematodes (*Meloidogyne* species) are serious pests of vegetable crops worldwide (Roberts *et al.*, 2005), particularly in developing countries (Lamberti, 1997). According to Fourie and Mc Donald (2000) these nematodes parasitise various vegetable crops in home and community gardens as well as in small-scale farming systems in South Africa, with devastating effects on yield and quality of crops.

Use of resistant vegetable crop varieties remains one of the most economical and environmentally-friendly options to limit crop yield and quality losses as a result of parasitism by plant-parasitic nematodes. This is particularly the case for small-scale farmers with limited infrastructure and financial resources (Nono-Womdim *et al.*, 2002). The primary objective of the present study was to evaluate locally available tomato (*Lycopersicon esculentum*), green bean (*Phaseolus vulgaris*), pumpkin (*Cucurbita* species) and *Brassica* spp. genotypes for host suitability to *M. incognita* race 2 and *M. javanica* in separate greenhouse trials.

3.2. Materials and methods

3.2.1. Vegetable germplasm

Local, commercially available tomato (21), green bean (13), Brassica (24) and pumpkin (18) genotypes were evaluated for host suitability to *M. incognita* race 2 and *M. javanica* in separate greenhouse trials during 2004-2006. Genotypes of each crop were obtained from local seed companies, namely Mayford, Hygrotech, Kirchhoffs, Starke Ayres, Voorspoed Saad and SeedCor.

A susceptible standard cv. Moneymaker (Hadisoeganda & Sasser, 1982; Nono-Womdim *et al.*, 2002) was included in both tomato trials. FA 593 was used as the resistant standard, since it was reported as resistant to root-knot nematodes by the

seed company Mayford and it is adapted to local climatic conditions (Anon, 2005). Due to the unavailability of FA 1454 tomato seed, cultivar Rodade was used instead in the *M. javanica* trial.

Green bean genotypes included garden, runner and stem bean, while pumpkin genotypes included marrow, squash and traditional pumpkin. Due to limited seed supply, pumpkin genotype Early Jarrah was unavailable for evaluation against *M. javanica*. Brassica crops included broccoli, Brussels sprouts, cabbage and cauliflower. For these latter three vegetable crops no information was locally available with regard to resistance or susceptibility.

Black plastic pots (4 000 cm^3) were filled with a methyl bromide-fumigated (1,162g a.i./2 m^3 soil) and steam-pasteurised sandy loam soil (3.9 % clay, 93.6 % sand, 1.9 % silt & 0.6 % organic matter content). The soil pH (H_20) was 6.55. Nutrients were added according to a soil nutrient analysis, namely 0.88g potassium chloride (KCl), 7.9g super phosphate (10 % phosphorus) and 0.11g limestone ammonium nitrate (LAN) (28 % nitrogen). Two seeds of each genotype for each crop were planted per pot and seedlings were thinned to one per pot two weeks later. Root-knot nematode eggs and second-stage juveniles (J2) were used as inoculum and were reared as described in paragraph 3.2.2. Nematode inoculation was performed at fourteen days after planting (DAP) as described in paragraph 3.2.3. Plants were watered three times a week and removed 56 days after inoculation (DAI). Fifty-six days (DAI) trials were terminated and plant roots were rinsed free of adhering soil and debris. The number of root-knot nematode egg masses per root system was subsequently counted as described in paragraph 3.2.4. Root-knot nematode-infected root systems were subsequently weighed and eggs and J2 were extracted using the adapted NaOCl-method of Riekert (1995) as described in paragraph 3.2.5. Eggs and J2 were counted and reproduction factors (RF-values) calculated as described in paragraph 3.2.5. Nematode data were submitted to analyses of variance (Statgraphics 5 Plus for Windows).

3.2.2. *In vivo* mass rearing of *M. incognita* race 2 and *M. javanica* populations, respectively, on tomato.

M. incognita race 2 and *M. javanica* populations were maintained separately *in vivo* in potted tomato plants (cv. Moneymaker) in two separate greenhouses. Temperature regimes of 19-27 °C and a 14:10 hour photoperiod were maintained in each greenhouse for the duration of the respective trials. Plastic pots (25 000 cm^3 capacity) were filled with a methyl bromide-fumigated (1,162g a.i./2 m^3 soil) and steam-pasteurised soil (4.5 % clay, 92.8 % sand, 2.4 % silt and 0.3 % organic matter content). The soil pH (H_20) was 4.35. Nutrients were added according to soil nutrient analyses, namely 24g dolomitic lime, 12g zinc (Zn), 1.68g 2:3:2 (NPK), 0.78g potassium chloride (KCl) and 14.04g super phosphate (10 % phosphorus). The *M. incognita* race 2 populations used during this study were originally obtained from Jan Kempdorp in the Northern Cape Province, while the *M. javanica* populations were obtained from Mount Edgecombe in Kwazulu-Natal Province. The above-mentioned species were identified by means of molecular techniques (Chapter 1) and were subsequently reared to obtain large numbers of eggs and J2.

3.2.3. Root-knot nematode inoculation

Root-knot nematode eggs and J2 of the respective species were extracted from roots of infected tomato plants using Riekert's (1995) modified NaOCl-method (described in paragraph 3.2.5.). Nematode eggs and J2 were collected in tap water in a glass flask that was placed on a magnetic stirrer and kept in suspension throughout the inoculation process.

Ten aliquots of 10ml each were collected with a pipette, poured into a counting dish and counted to check for at least a 95 % accuracy level. When this accuracy level was not achieved the whole procedure was repeated. The contents of the 10 aliquots were poured back into the glass flask. The desired number of nematodes to be inoculated per plant was always suspended in 10ml aliquots of tap water. This was achieved by diluting the nematode suspension to a volume that contained ± 5 000 root-knot nematode eggs and J2 per 5ml water of the appropriate species, namely *M. incognita* race 2 or *M. javanica*.

When the number of root-knot nematode eggs and J2 in the original suspension was insufficient to allow inoculation of all the plants in a trial at the same time, more infected tomato roots were obtained and eggs and J2 extracted. When the concentration of nematode eggs and J2 was too high in the original suspension, it was diluted until the desired concentration of eggs and J2 were obtained.

3.2.4. Nematode reproduction assessment

Fifty-six days after nematode inoculation, root systems of plants from the relevant vegetable crops were rinsed free of adhering soil and debris with running tap water. These root systems were blotted on towel paper, weighed and the number of egg masses per root system counted. Staining of egg masses to facilitate counting was done by immersion of roots in a 0.1 % phloxine B solution for 20 minutes (Hussey & Boerma, 1981). Each root system was cut into approximately 1 cm pieces and transferred to a rectangular, white plastic container with more or less 200ml tap water. Root pieces were individually inspected and the red-stained egg masses counted using a commercial magnifying glass. Egg masses coming loose from the root pieces during the staining and counting process were collected using a Pasteur pipette and were also counted and extracted. Subsequently the ELF-index was rated according to the method of Hussey and Boerma (1981) on a 0-5 scale where 0 = no egg masses; 1 = 1-2 egg masses; 2 = 3-10 egg masses; 3 = 11-30 egg masses; 4 = 31-100 egg masses and 5 = more than 100 egg masses per root system. Eggs were extracted using Riekert's (1995) modified NaOCl-method and counted under a stereo microscope. The reproductive potential of the root-knot nematodes was determined using Oostenbrink's reproduction factor (RF) (Windham & Williams, 1987), where RF = final egg and J2 numbers (Pf) / initial egg and J2 numbers (Pi). The host status of the relevant vegetable genotypes was classified according to RF-values using the index of Windham and Williams (1988) as explained in Table 1. Vegetable genotypes were also grouped as resistant (R), moderately resistant (MR), susceptible (S) and very susceptible (VS) to root-knot nematodes according to the ELF-index of Murray *et al.* (1986) as indicated in Table 2. Finally, the number of eggs/g of fresh roots was determined by the following equation: (1g roots / actual weight of root system) x Pf.

Table 1. RF-values and classification of the host status of tomato genotypes (Windham & Williams, 1988).

CATEGORY	RF-value	Host status
1.	< 1	Resistant / poor host
2.	1 to 5	Good hosts
3.	> 5	Excellent hosts

Table 2. ELF-index, egg masses per plant and resistance categories according to Murray *et al.* (1986).

CATEGORY	ELF-rating index	Egg masses/plant	Classification of cultivar *
1.	0	0	R
2.	1.0-3.2	1-15	MR
3.	3.3-3.7	16-25	S
4.	3.8-5.0	26-100+	VS

* R = resistant; MR = moderately resistant; S = susceptible and VS = very susceptible

3.2.5. Extraction of root-knot nematode eggs and second-stage juveniles using the adapted NaOCl-method of Riekert (1995).

A 50-g root sample was cut into 1-cm pieces and mixed thoroughly. The root sample was then shaken in 800ml of a 1 % NaOCl solution for four minutes. The weak bleach solution breaks down the gelatinous matrix surrounding the eggs and release them from the roots. The mixture containing the root-knot nematode eggs and J2 was subsequently decanted through a range of nested sieves, consisting of a 710-µm, 250-µm, 75-µm, 63-µm, 45-µm, 25-µm and a 10-µm sieve in order from top to bottom. This arrangement of sieves ensures less clogging on the bottom sieve. A vacuum pump was connected to the 10-µm sieve to enhance the passing of water through the sieve. Washing through the sieves each sample of root fragments continued for five minutes. Nematode eggs and larvae were collected on the 10-µm sieve by washing it from this sieve into a 100-ml sample bottle. Counting of eggs and J2 and calculation of RF-values were done subsequently, as described in paragraph 3.2.4.

3.2.6. Experimental design and data analysis

Randomised complete block designs were used for all the respective trials, with six replicates per entry (Figures 3, 4, 5 & 6). Nematode data from each trial were subjected to an analysis of variance (Statgraphics Plus 5 for Windows). Means were separated by the Tukey test of significance ($P \leq 0.05$) and degrees of freedom (error) > 18 (Van Ark, 1981) were always persued. Correlation coefficients were calculated to indicate the relationship between the number of egg masses and ELF, respectively, when compared with RF-values for each vegetable crop screened against each root-knot nematode species using a Multiple Variable Analysis (Statgraphics Plus 5 for Windows).

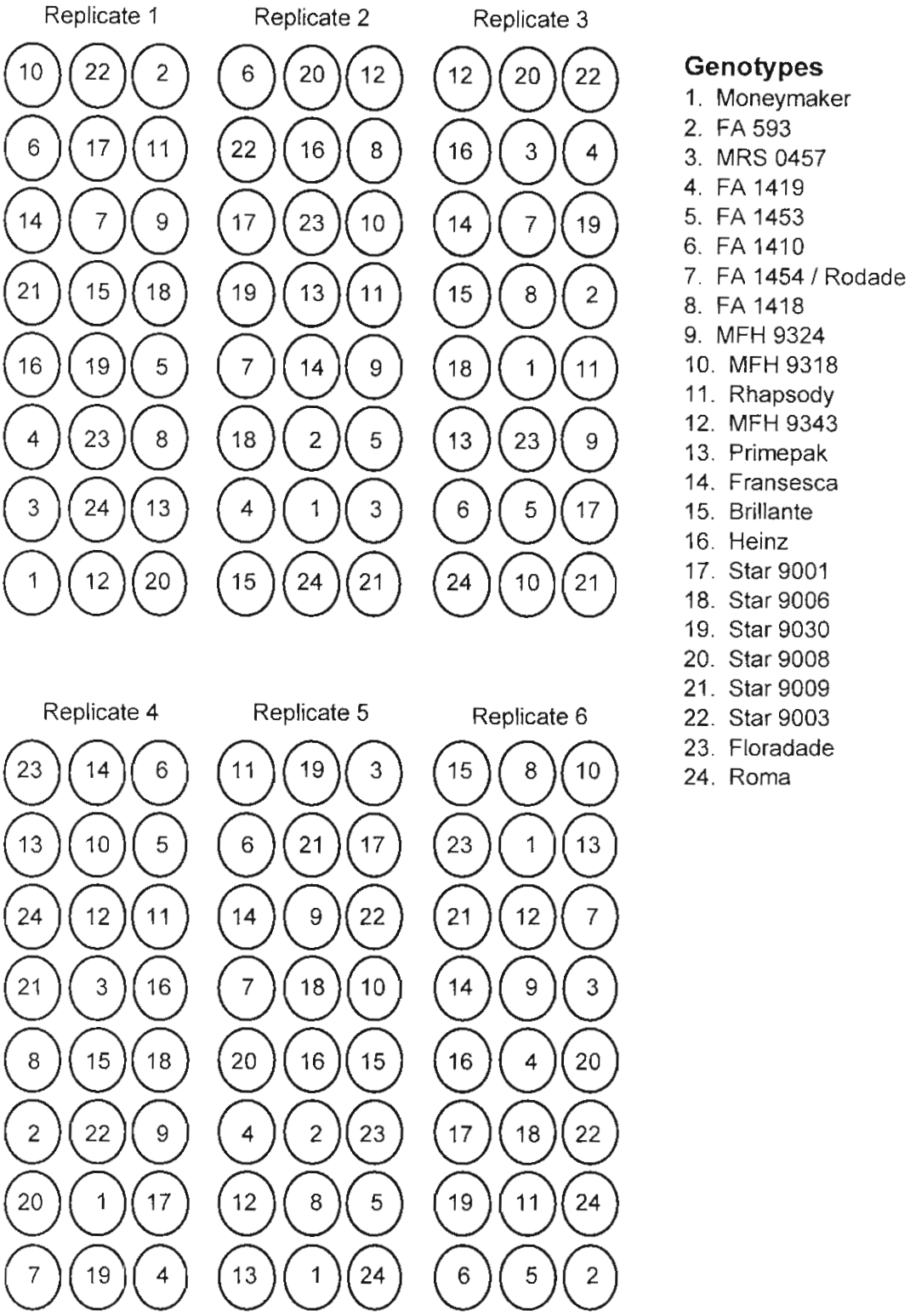
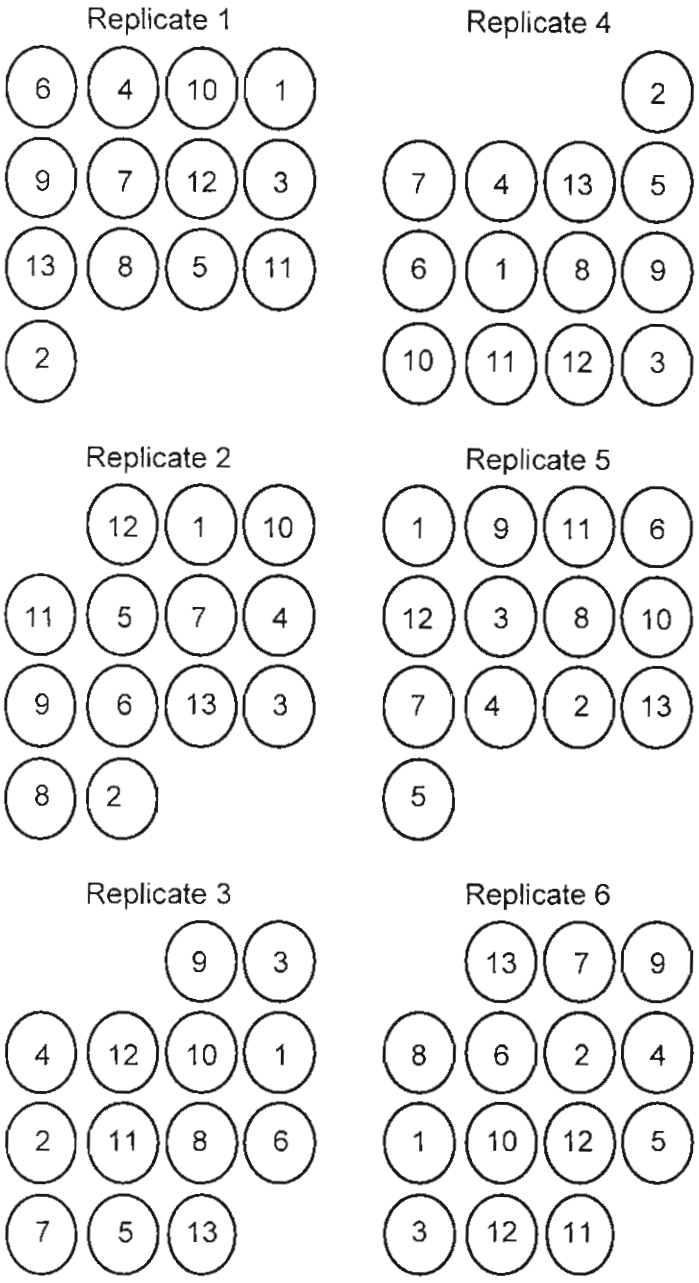


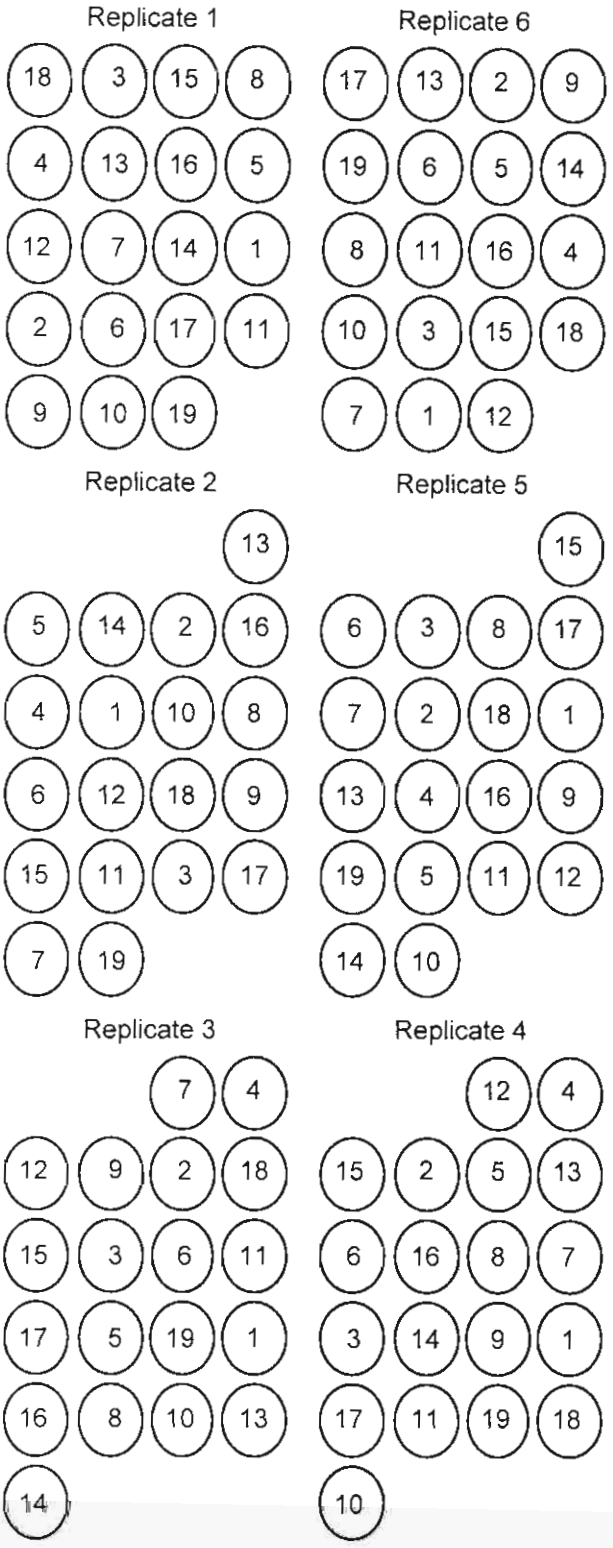
Figure 3. Experimental design and randomization used to evaluate twenty-four local tomato genotypes for host suitability to *Meloidogyne incognita* (race 2) and *Meloidogyne javanica*, respectively, in two separate greenhouse trials.



Genotypes

1. Espada (stem bean)
2. Provider (garden bean)
3. Contender (garden bean)
4. Nelson (garden bean)
5. Quteniqua (dwarf garden bean)
6. Tokai (dwarf garden bean)
7. Winter Green (garden bean)
8. Tongati (dwarf garden bean)
9. Star 2000 (garden bean)
10. Witsa (runner bean)
11. Newton (garden bean)
12. Top Crop (Bush garden bean)
13. Lazy Housewife (runner bean)

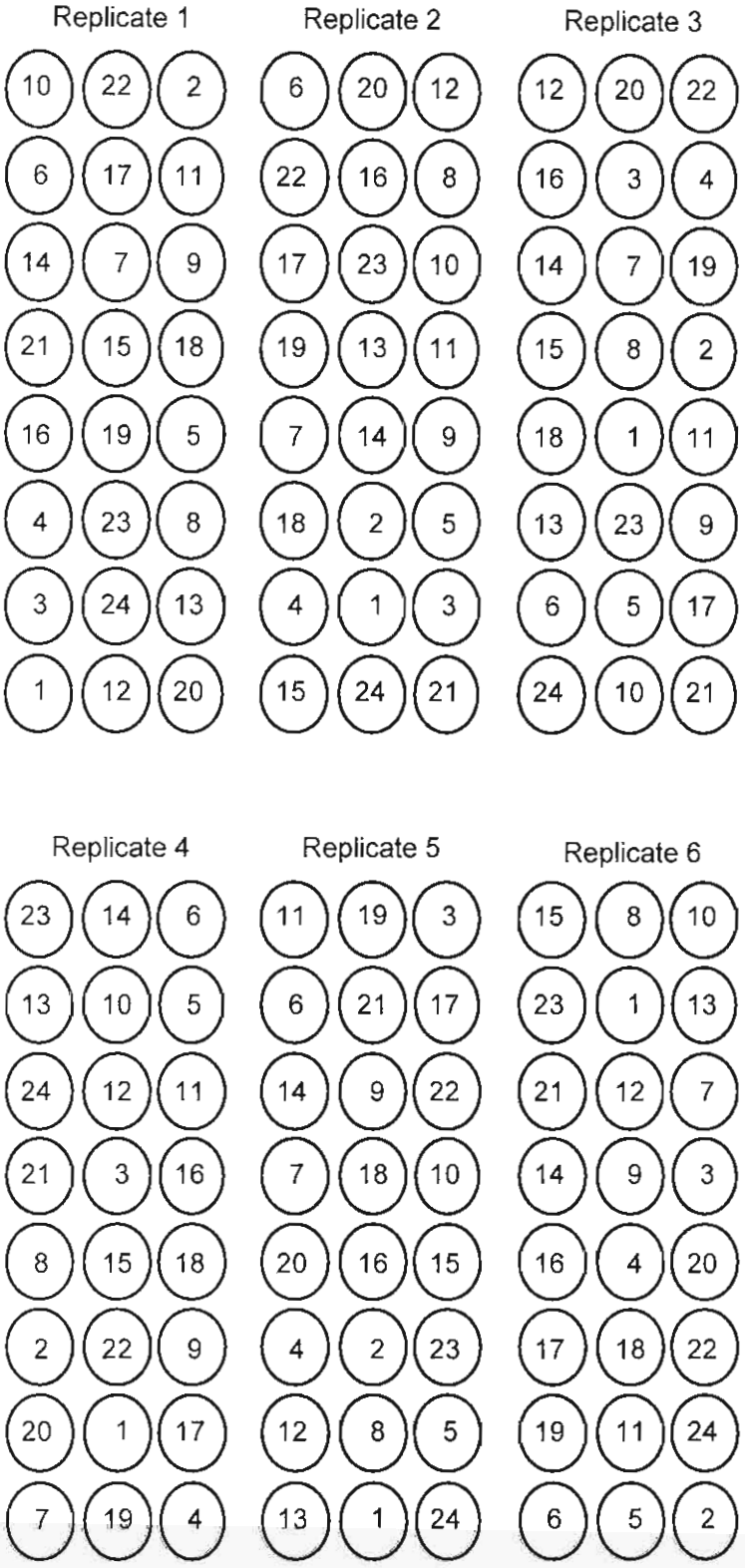
Figure 4. Experimental design and randomization used to evaluate thirteen local pumpkin genotypes for host suitability to *Meloidogyne incognita* (race 2) and *Meloidogyne javanica*, respectively, in two separate greenhouse trials.



Genotypes

1. Scallopini (squash)
2. Caserta (squash)
3. Rolet (squash)
4. Jack Be Little (traditional pumpkin)
5. Long Green Bush (marrow)
6. Early Jarrah (traditional pumpkin)
7. Jarradale (traditional pumpkin)
8. Queensland Blue (traditional pumpkin)
9. Green Hubbard (squash)
10. Star 7022 (traditional pumpkin)
11. Early Butternut (squash)
12. Waltham (butternut)
13. Star 8001 (squash)
14. Star 7020 (traditional pumpkin)
15. Flat White Boer Van Niekerk (traditional pumpkin)
16. Star 7001 (traditional pumpkin)
17. Green Hubbard Chigago Warted (squash)
18. Pagoda Gold (squash)
19. President (squash)

Figure 5. Experimental design and randomization used to evaluate nineteen local pumpkin genotypes for host suitability to *Meloidogyne incognita* (race 2) and *Meloidogyne javanica*, respectively, in two separate greenhouse trials.



Genotypes

1. Puma (cabbage)
2. Big Cropper (cabbage)
3. Cape Spitz/Sugar Loaf (cabbage)
4. Conquistador (cabbage)
5. Long Island (brussels sprouts)
6. Green Valiant (broccoli)
7. Wallaby (cauliflower)
8. F1 Intro (cabbage)
9. Copenhagen Market (cabbage)
10. Drumhead (cabbage)
11. Glory of Enkhuizen (cabbage)
12. Green Star (cabbage)
13. Green Coronet (cabbage)
14. Star 3301 (cabbage)
15. Star 3315 (cabbage)
16. Star 3311 (cabbage)
17. Hercules (cabbage)
18. Gloria P1 (cabbage)
19. Snowball (cauliflower)
20. Suntop (broccoli)
21. Malimba (cauliflower)
22. Adagia (brussels sprouts)
23. Graffiti (cauliflower)
24. Discovery (cabbage)

Figure 6. Experimental design and randomization used to evaluate twenty-four local *Brassica cultivars* for host suitability to *Meloidogyne incognita* (race 2) and *Meloidogyne javanica*, respectively, in two separate greenhouse trials.

3.3. Results

Substantial variation existed among the genotypes of each vegetable crop for all nematode parameters evaluated with regard to each respective root-knot nematode species or race (Tables 3, 4, 5, 6, 7, 8, 9 & 10). None of the crop genotypes were, however, immune to either *M. incognita* race 2 or *M. javanica*, since both species reproduced on all genotypes evaluated.

3.3.1.Tomato

Four tomato genotypes were identified having resistance to *M. incognita* race 2 according to RF-values, while no genotype exhibited resistance to *M. javanica* (Tables 3 & 4).

3.3.1.1. *Meloidogyne incognita* race 2

RF-values

Of the 21 tomato genotypes evaluated for host suitability to *M. incognita* race 2 Rhapsody, MFH 9324, FA 1454 and FA 593 had RF values ≤ 1 (Tables 1 & 3). These genotypes also exhibited ELF indices ≤ 2, indicating resistance to this population of *M. incognita* race 2 (Table 3). The latter genotypes also maintained significantly lower numbers of egg masses, eggs/root system and eggs/g of roots compared to the susceptible standard Moneymaker. Rhapsody maintained the lowest RF-value, number of egg masses as well as eggs/root system, while the susceptible standard Moneymaker had the highest RF value and number of eggs/root system. Though not the highest, Moneymaker had an ELF-index of 4.5 and the number of egg masses/root system and eggs/g of roots of this latter cultivar were also high.

Primepak, FA 1418, FA 1419, Roma, MRS 0457 and Floradade are good hosts to *M. incognita* race 2 with RF-values ranging between 1 and 5 (Tables 1 & 3). MFH 9318, Heinz, Star 9030, Star 9001, Fransesca, MFH 9343, FA 1453, Star 9006, Brilliante, FA 1410 and Moneymaker are excellent hosts with RF-values > 5 (Windham & Williams, 1988).

ELF-indices

Some genotypes, viz. Primepak, FA 1418, Roma and Floradade had low ELF-indices (≤ 2), but RF-values greater than 1, implying that relatively small numbers of females in the roots of these genotypes produced on average high numbers of eggs/egg mass (Tables 1 & 3).

No genotype could be classified as resistant to *M. incognita* race 2 with regard to this criterion (Table 2) since no one exhibited an ELF-index of zero (Murray *et al.*, 1986; Table 3). Rhapsody, MFH 9324, FA 1454, FA 593, Primepak, FA 1418, FA 1419, Roma, MRS 0457, Floradade and FA 1453, however, exhibited moderate resistance, while MFH 9318 is susceptible. Heinz, Star 9030, Star 9001, Fransesca, MFH 9343, Star 9006, Brilliante, FA 1410 and Moneymaker are, however, highly susceptible to this root-knot nematode race according to the index of Murray *et al.* (1986).

Egg masses

In terms of the number of egg masses per plant, no tomato genotype could be considered resistant (Table 2) to *M. incognita* race 2, since none had zero egg masses per plant (Murray *et al.*, 1986; Table 3). Rhapsody, MFH 9324, FA 1454, FA 593, Primepak, FA 1418, FA 1419 and Floradade could be classified as moderately resistant and Roma and MRS 0457 are susceptible to this root-knot nematode species and race. MFH 9318, Heinz, Star 9030, Star 9001, Fransesca, MFH 9343, FA 1453, Star 9006, Brilliante, FA 1410 and Moneymaker are, however, highly susceptible to this root-knot nematode according to the index of Murray *et al.* (1986).

Table 3. Reproduction of *Meloidogyne incognita* race 2 on local tomato genotypes measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage larvae (J2) in a greenhouse trial during the 2004 growing season.

Cultivar	RF-value	ELF-index	No. of egg masses/ root system	No. of eggs and J2/root system	No. of eggs and J2/g root
1. Rhapsody	0.3a	0.8ab	1a	1 674a	370ab
2. MFH 9324	0.6a	0.5a	2a	2 853a	165a
3. FA 1454	0.7a	1.8cd	4a	3 728a	1 442abcdef
4. FA 593[1]	0.7a	0.8ab	3a	3 728a	612abcd
5. Primepak	1.2a	1.6bc	3a	6 078a	869abcde
6. FA 1418	1.4a	1.5bc	5a	7 122a	274ab
7. FA 1419	2.4a	2.7def	13a	11 935a	496abc
8. Roma	2.9a	2.0cde	22abc	14 368a	846abcde
9. MRS 0457	2.1a	3.0fg	19ab	14 770a	594abcd
10. Floradade	4.4a	1.3abc	13a	21 975a	1 269abcdef
11. MFH 9318	6.5ab	3.7gh	37bc	32 258ab	1 855abcdef
12. Heinz	12.4bc	4.0hi	71d	61 833bc	2 637bcdef
13. Star 9030	12.6bc	4.2hi	82d	63 058bcd	2 816cdef
14. Star 9001	13.7bcd	4.0hi	72d	68 600bcde	3 009def
15. Fransesca	14.0bcd	4.0hi	71d	81 427cde	2 579abcdef
16. MFH 9343	15.7cd	4.2hi	88d	78 400cde	2 207abcdef
17. FA 1453	19.0cde	2.8efg	42c	94 967cdef	6 833g
18. Star 9006	20.1cde	4.5hi	91d	100 392def	3 648f
19. Brilliante	21.3de	4.2hi	76d	106 692ef	3 399ef
20. FA 1410	25.0e	4.8i	92d	124 950f	6 582g
21. Moneymaker[2]	33.0f	4.5hi	91d	164 792g	6 639g

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for signicance.

[1] resistant stardard

[2] susceptible standard

3.3.1.2. *Meloidogyne javanica*

RF-values

None of the 21 tomato genotypes were resistant to *M. javanica*, since none of them exhibited RF-values ≤ 1 when screened for host suitability against this root-knot nematode species (Tables 1 & 4). Although Rhapsody had the lowest values for all nematode parameters used during this study, i.e. RF-value of 1.8, an ELF index of 2, 11 egg masses/root system, 8 925 eggs/roots system and 566 eggs/g of root, it still is susceptible to this root-knot nematode species (Windham & Williams, 1988; Hussey & Boerma, 1981).

Rhapsody, Star 9030, FA 1410, FA 593 and FA 1453 are good hosts to *M. javanica* (Windham & Williams, 1988; Tables 1 & 4). Star 9006, FA 1419, Star 9001, FA 1418, MFH 9324, MFH 9318, Rodade, Primepak, Fransesca, MRS 0457, Brilliante, MFH 9343, Heinz, Moneymaker, Floradade and Roma are, however, excellent hosts according to the index of Windham and Williams (1988).

ELF-indices

Rhapsody, Star 9030, FA 1410, FA 593, FA 1453, Star 9006 and FA 1419 exhibited moderate resistance to *M. javanica*, since they all had ELF-indices ≤ 3.2 (Murray *et al.*, 1986; Tables 1 & 4). Star 9001, FA 1418, MFH 9324, MFH 9318, Rodade, Primepak, Fransesca, MRS 0457, Brilliante, MFH 9343, Heinz, Moneymaker, Floradade and Roma are highly susceptible to this root-knot nematode species.

Egg masses

Based on number of egg masses per plant no tomato genotype was resistant to *M. javanica* (Murray *et al.*, 1986; Tables 2 & 4). Rhapsody, Star 9030, FA 1410 and FA 1453 are moderately resistant according to this index while FA 593 and Star 9001 are classified as susceptible. Star 9006, FA 1419, FA 1418, MFH 9324, MFH 9318, Rodade, Primepak, Frensesca, MRS 0457, Brilliante, MFH 9343, Heinz, Moneymaker, Floradade and Roma are, however, classified as highly susceptible to this root-knot nematode species according to the index of Murray *et al.* (1986).

Table 4. Reproduction of *Meloidogyne javanica* on local tomato genotypes measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage larvae (J2) in a greenhouse trial during the 2004/05 growing season.

Cultivar	RF-value	ELF-index	No. of egg masses/root system	No. of eggs and J2/root system	No. of eggs and J2/g root
1. Rhapsody	1.8a	2.0a	11a	8 925a	566a
2. Star 9030	2.1a	2.2ab	12a	10 681a	887a
3. FA 1410	2.2a	2.7bc	14a	10 833a	1 185a
4. FA 593[1]	2.4a	2.7bc	16ab	11 830a	1 375a
5. FA 1453	2.6a	2.8c	15ab	13 195a	1 080a
6. Star 9006	24.3ab	3.0c	27b	133 989ab	6 536ab
7. FA 1419	55.3abc	3.2c	98d	276 617abc	25 624bcd
8. Star 9001	60.5abc	4.0d	18ab	302 598abc	14 295abc
9. FA 1418	64.8abc	4.7e	98d	323 867abc	21 332abcd
10. MFH 9324	68.4abc	4.8e	100d	342 008abc	20 198abcd
11. MFH 9318	95.3bcd	4.8e	100d	476 558bcd	20 790abcd
12.Rodade	111.1cde	4.8e	100d	555 742cde	30 811cde
13. Primepak	116.7cde	4.8e	100d	866 453def	39 113de
14. Fransesca	116.8cde	4.8e	98d	584 033cde	36 009cde
15. MRS 0457	119.2cde	4.8e	54c	596 225cde	41 358de
16. Brilliante	129.1cde	5.0e	100d	645 633cde	35 496cde
17. MFH 9343	159.7def	5.0e	100d	798 642def	32 981cde
19. Heinz	174.3ef	5.0e	100d	871 383ef	51 614ef
18. Moneymaker[2]	207.9f	5.0e	100d	1 039 910f	79 973g
20. Floradade	211.2f	5.0e	100d	1 055 780f	64 497fg
21. Roma	215.1f	5.0e	100d	1 075 610f	50 661ef

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for significance.
[1]= resistant standard.
2= susceptible standard

3.3.2. Green bean

None of the green bean genotype were resistant to either one of the root-knot nematode species used in this study, because none had an RF-value ≤ 1. All green bean entries could therefore be classified as susceptible to *M. incognita* race 2 and *M. javanica*, respectively.

3.3.2.1. *Meloidogyne incognita* race 2

RF-values

Although no green bean genotype could be regarded as resistant on account of any of the criteria used, the level of susceptibilty to *M. incognita* race 2 varied greatly (Tables 1 & 5). Witsa had the lowest RF value of 4.1 and genotype Contender had the

highest (40.3). Witsa is a good host to *M. incognita* race 2, while Tokai, Lazy Housewife, Espada, Top Crop, Winter Green, Provider, Star 2000, Nelson, Tongati, Newton, Quteniqua and Contender are excellent hosts to this root-knot species (Windham & Williams, 1988; Tables 1 & 5).

ELF indices

Only Witsa could be classified as moderately resistant to *M. incognita* race 2, while Lazy Housewife, Winter Green and Newton are susceptible (Murray *et al.*, 1986; Tables 2 & 5). Tokai, Espada, Top Crop, Provider, Star 2000, Nelson, Tongati, Quteniqua and Contender could be classified as highly susceptible according to the index of Murray *et al.* (1986).

Egg masses

With regard to the number of egg masses/root system, Witsa could be classified as susceptible to *M. incognita* race 2 (Murray *et al.*, 1986; Tables 2 & 5). Tokai, Lazy Housewife, Espada, Top Crop, Winter Green, Provider, Star 2000, Nelson, Tongati, Newton, Quteniqua and Contender are highly susceptible to this root-knot nematode species and race according to the index of Murray *et al.* (1986).

Table 5. Reproduction of *Meloidogyne incognita* race 2 on local green bean genotypes measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage juveniles (J2) in a greehouse trial during the 2005 growing season.

Cultivar	RF-value	ELF-index	No. of egg masses/root system	No. of eggs and J2/root system	No. of eggs and J2/g root
1. Witsa	4.1a	2.8a	17a	20 320a	2 251a
2. Tokai	10.4ab	3.8bcd	44b	52 063ab	5 626abc
3. Lazy Housewife	12.6ab	3.7bc	39ab	63 000ab	8 458abcd
4. Espada	13.7bc	4.2cd	73cd	68 688bc	5 525ab
5. Top Crop	14.1bc	4.0bcd	50bc	70 438bc	7 148abcd
6. Winter Green	14.3bcd	3.7bc	41ab	71 604bcd	15 665d
7. Provider	16.5bcde	4.3de	73cde	82 251bcde	11 325bcd
8. Star 2000	16.5bcde	4.2cd	64bcd	82 688bcde	5 815abc
9. Nelson	18.4bcde	4.3de	71cd	9 193bcde	14 552cd
10. Tongati	22.6cde	4.2cd	63bcd	113 167cde	11 949bcd
11. Newton	23.2de	3.5b	41ab	116 231de	11 917bcd
12. Quteniqua	23.6e	4.2cd	78de	117 833e	14 157bcd
13. Contender	40.3f	4.8e	99e	201 542f	3 215e

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for significance.

3.3.2.2. *Meloidogyne javanica*

RF-values

All green bean genotypes were susceptible to *M. javanica*, since none of them had RF-values ≤ 1 (Table 6). In fact, RF-values were very high. Tokai exhibited the lowest RF-value of 39.8. As expected all genotypes also maintained high ELF-indices, egg masses/root system, eggs and J2/root system and eggs and J2/g of root.

Tokai, Witsa, Tongati, Lazy Housewife, Star 2000, Top Crop, Nelson, Espada, Contender, Provider, Newton, Winter Green and Quteniqua are excellent hosts to this root-knot nematode species since they all had RF-values > 5 (Windham & Williams, 1988; Tables 1 & 6).

ELF-indices

All green bean genotypes evaluated were very susceptible to *M. javanica* (Murray *et al.*, 1986; Tables 2 & 6) with ELF-values ranging between 4.3 (Newton and Winter Green) and 5 (Witsa, Star 2000, Nelson, Espada, Provider and Quteniqua).

Egg masses

Egg mass numbers/root system ranged between 79 (Newton and Winter Green) and 100 (Witsa, Star 2000, Nelson, Espada, Provider, Quteniqua) (Table6). Therefore no green bean genotype could be classified as resistant to *M. javanica* according to the index of Murray *et al.*, 1986)

Table 6. Reproduction of *Meloidogyne javanica* on local green bean genotypes measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage juveniles (J2) in a greenhouse experiment during the 2005 growing season.

Cultivar	RF-value	ELF-index	No. of egg masses/root system	No. of eggs and J2/root system	No. of eggs and J2/ g root
1. Tokai	39.8a	4.5ab	90a	198 975a	13 441a
2. Witsa	60.7ab	5.0b	100a	303 725ab	19 501ab
3. Tongati	79.9ab	4.5ab	82a	399 262ab	20 052ab
4. Lazy Housewife	84.0ab	4.5ab	92a	420 088ab	19 346ab
5. Star 2000	85.1ab	5.0b	100a	425 687ab	15 721a
6. Top Crop	91.0ab	4.8ab	97a	454 913ab	29 725abc
7. Nelson	94.3abc	5.0b	100a	471 625abc	16 175a
8. Espada	111.4abc	5.0b	100a	556 850abc	20 824ab
9. Contender	114.3abc	4.8ab	91a	571 463abc	36 780abc
10. Provider	117.0bc	5.0b	100a	585 113bc	30 639abc
11. Newton	129.3bc	4.3a	79a	646 713bc	51 853c
12. Winter Green	133.8bc	4.3a	79a	668 763bc	35 705abc
13. Quteniqua	168.5c	5.0b	100a	842 650c	45 127bc

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for significance.

3.3.3. Pumpkin

All pumpkin genotypes were susceptible to *M. incognita* race 2 and *M. javanica*, respectively (Tables 7 & 8), since they all had RF-values > 1.

3.3.3.1. *Meloidogyne incognita* race 2

RF-values

All pumpkin genotypes screened against this species and race had RF-values > 1 (Table 7) and maintained high ELF-indices, number of egg masses/root system, number of eggs and J2/root system and number of eggs/g of roots. All these

genotypes could be classified as excellent hosts to *M. incognita* race 2 (Windham & Williams, 1988; Tables 1 & 7).

ELF-indices

All pumpkin genotypes exhibited ELF-values > 3.8 and were classified as very susceptible to *M. incognita* race 2 (Murray *et al.*, 1986; Tables 2 & 7).

Egg masses

No genotype was classified as resistant to *M. incognita* race 2 (Murray *et al.*, 1986), since no genotype had zero egg masses/root system (Table 2 & 7).

Table 7. Reproduction of *Meloidogyne incognita* race 2 on local pumpkin genotypes measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage larvae (J2) in a greenhouse trial during 2005 growing season.

Cultivar	**RF-value**	**ELF-index**	**No. of egg masses/root system**	**No. of eggs and J2/root system**	**No. of eggs and J2/g root**
1. Caserta	11.2a	4.2a	64a	56 233a	8 323a
2. Flat White Boer Van Niekerk	18.8ab	5.0b	100b	117 658abcd	8 512a
3. Rolet	20.4ab	4.5ab	85b	121 975abcd	10 131a
4. Long Green Bush	23.5abc	4.6ab	85b	116 202abcd	10 335a
5. Star 7001	24.4abc	5.0b	100b	102 142ab	11 337a
6. President	24.9abcd	4.8ab	93b	174 008abcdef	11 751a
7. Jack Be Little	25.9abcde	4.8ab	95b	157 208abcdef	13 791a
8. Green Hubbard	27.5abcde	4.8ab	99b	137 667abcde	17 900a
9. Queensland Blue	31.4abcdef	4.8ab	93b	286 842f	18 288a
10. Star 7020	34.8abcdef	4.4ab	93b	144 891abcdef	19 038a
11. Early Butternut	40.4abcdef	5.0b	100b	217 350bcdef	29 103ab
12. Green Hubbard Chigago Warted	43.5bcdef	4.5ab	93b	262 092cdef	29 505ab
13. Pagoda Gold	47.6bcdef	4.7ab	90b	249 579cdef	31 640ab
14. Star 8001	49.9cdef	4.7ab	93b	201 780abcdef	33 899ab
15. Scallopini	**52.4cdef**	4.8ab	**95b**	129 442abcde	34 297ab
16. Star 7022	**53.9def**	4.7ab	98b	**275 392ef**	**36 388ab**
17. Waltham	**55.1ef**	5.0b	100b	**237 883bcdef**	**38 360ab**
18. Jarradale	57.4f	4.2a	83b	269 967def	58 824b

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for significance.

3.3.3.2. *Meloidogyne javanica*

RF-values

All 19 pumpkin genotypes evaluated against *M. javanica* had RF-values > 1, indicating susceptibility (Table 8). All genoytypes also maintained high ELF-indices, egg masses/root system, number of eggs and J2/root system and number of eggs and J2/g of roots. Genotype Star 8001 exhibited the lowest RF-value of 43.3.

ELF-indices

All pumpkin genotypes used in this study are classified as excellent hosts to this parasite since they all had ELF-indices ranging between 4.8 and 5.0 (Murray *et al.*, 1986).

Egg masses

Concerning the number of egg masses/root system, all genotypes are classified as very susceptible to this species (Murray *et al.*, 1986).

Table 8. Reproduction of *Meloidogyne javanica* on local pumpkin genotypes measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage juveniles (J2) in a greenhouse trial during 2005 growing season.

Cultivar	RF-value	ELF-index	No. of egg masses/root system	No. of eggs and J2/root system	No. of eggs and J2/g root
1. Star 8001	43.3a	4.8a	99ab	216 358a	63 182ab
2. Caserta	61.8ab	5.0a	100b	308 992ab	46 197a
3. Queensland Blue	72.6abc	5.0a	100b	363 067abc	64 169ab
4. Rolet	79.6abc	5.0a	100b	397 833abc	95 387abcde
5. Long Green Bush	87.0abcd	5.0a	100b	435 167abcd	61 196ab
6. Flat White Boer Van Niekerk	87.4abcd	5.0a	100b	437 092abcd	65 044ab
7. President	111.5abcde	5.0a	100b	557 492abcde	99 249bcde
8. Pagoda Gold	111.6abcdef	5.0a	100b	558 133abcde	117 718cdefg
9. Green Hubbard	117.2bcdefg	5.0a	100b	585 842bcdef	78 356abcd
10. Green Hubbard Chigago Warted	120.8bcdefg	4.8a	100b	603 983bcdef	119 077defg
11. Jarradale	134.4cdefg	5.0a	100b	679 408cdef	10 7415bcdef
12. Waltham	135.9cdefg	5.0a	100b	688 842cdef	69 722abcd
13. Star 7001	151.2defgh	5.0a	100b	755 942defg	68 294abc
14. Scallopini	161.1efgh	5.0a	100b	805 642efg	160 620g
15. Jack Be Little	161.1efgh	5.0a	100b	822 308efg	140 739efg
16. Early Butternut	166.9efgh	5.0a	100b	834 283efg	157 037fg
17. Star 7020	180.3fgh	5.0a	100b	901 483efg	78 545abcd
18. Early Jarrah	182.8gh	5.0a	100b	913 908fg	93 341abcde
19. Star 7022	215.6h	4.8a	98a	1 077 940g	111 232bcdefg

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for significance.

3.3.4. Brassica

Fourteen Brassica cultivars exhibited resistance to *M. incognita* race 2, while all genotypes showed resistance to *M. javanica*, since they exhibited RF-values ≤ 1 when evaluated against this root-knot nematode species.

3.3.4.1. *Meloidogyne incognita* race 2

RF-values

Fourteen of the 24 Brassica cultivars, namely, Cape Spitz/Sugar Loaf, Green Valiant, Suntop, Graffiti, Wallaby, Star 3315, Snowball, Conquistador, Malimba, Puma, Green Coronet, Star 3311, Hercules and Big Cropper exhibited RF values ≤ 1 (Windham & Williams, 1988; Tables 1 & 9) and ELF-indices ≤ 2 (Murray *et al.*, 1986; Tables 2 & 9), indicating resistance to *M. incognita* race 2. The other ten genotypes namely,

Adagia, F1 Intro, Long Island, Copenhagen Market, Glory of Enkhuizen, Gloria P1, Star 3301, Discovery, Green Star and Drumhead had RF-values > 1 when screened against this root-knot nematode species and race and are classified as excellent hosts to this root-knot nematode species. Cape Spitz/Sugar Loaf exhibited the lowest RF-value, while Drumhead exhibited the highest RF-value.

ELF-indices

Cape Spitz/Sugar Loaf, Green Valiant, Suntop, Wallaby and Star 3315 exhibited resistance to *M. incognita* race 2 because they all had ELF-indices ranging between zero and one (Murray *et al.*, 1986; Tables 2 & 9). Graffiti, Snowball, Conquistador, Malimba, Puma, Green Coronet, Star 3311, Hercules Big cropper, Adagia, F1 Intro, Long Island, Copenhagen Market, Glory of Enkhuizen and Star 3301 are moderately resistant to *M. incognita* race 2 with ELF-indices ranging between one and 3.2, while Gloria P1, Discovery, Green Star and Drumhead were classified as very susceptible to this root-knot species with ELF-indices ranging between 3.8 and 5.

Egg masses

With regard to the number of egg masses/root system, only Green Valiant could be classified as resistant to *M. incognita* race 2 (Murray *et al.*, 1986), since this genotype maintained zero egg masses/root system (Tables 2 & 9). Suntop also maintained a mean of less than one egg mass/root system. Cape Spitz/Sugar loaf, Graffti, Wallaby, Star 3315, Snowball, Conquistador, Malimba, Puma, Green Coronet, Star 3311, Hercules, Big Cropper, Adagia and F1 Intro could be classified as moderately resistant. On the other hand, Long Island, Copenhagen Market and Glory of Enkhuizen could be classified susceptible while Gloria P1, Star 3301, Discovery, Green Star and Drumhead could be classified as highly susceptible to this root-knot nematode species according to the index of Murray *et al.* (1986).

Table 9. Reproduction of *Meloidogyne incognita* race 2 on local Brassica cultivars measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage juveniles (J2) in a greenhouse trial during the 2005 growing season.

Cultivar	RF-value	ELF-index	No. of egg masses/root system	No. of eggs and J2/root system	No. of eggs and J2/g root
1. Cape Spitz/Sugar Loaf	0.01a	0.5abc	1ab	47a	6a
2. Green Valiant	0.02a	0a	0a	93a	8a
3. Suntop	0.04a	0.2ab	0.3a	181a	33a
4. Graffiti	0.08a	1.0bcde	2ab	385a	170a
5. Wallaby	0.1ab	0.5abc	1ab	508ab	117a
6. Star 3315	0.2ab	0.6abcd	1ab	811ab	87a
7. Snowball	0.2ab	1.7efgh	5abcd	1 097ab	386a
8. Conquistador	0.3ab	1.0bcde	2ab	1 482ab	240a
9. Malimba	0.3ab	1.3cdef	3abc	1 587ab	434a
10. Puma	0.3ab	1.8efgh	4abcd	1 616ab	243a
11. Green Coronet	0.4ab	1.5defg	4abcd	2 001ab	184a
12. Star 3311	0.4ab	1.0bcde	3abc	2 164ab	272a
13. Hercules	0.6ab	1.5defg	4abc	3 179ab	225a
14. Big Cropper	0.8ab	1.7efgh	7abcd	3 897ab	479a
15. Adagia	1.1ab	2.0fghi	5abcd	5 349ab	687a
16. F1 Intro	1.2abc	2.3ghij	9abcd	5 997abc	969a
17. Long Island	1.5abc	2.5hij	16bcd	7 642abc	695a
18. Copenhagen Market	2.4bcd	2.5hij	18cde	11 954bcd	1 554ab
19. Glory of Enkhuizen	3.4cde	2.8ij	19de	17 185cde	1 767ab
20. Gloria P1	4.0def	3.8k	42fg	19 868def	2 887bc
21. Star 3301	4.2def	3.2k	33ef	21 123def	3 985cd
22. Discovery	5.4efg	3.8k	52gh	26 886efg	3 713cd
23. Green Star	6.2fg	3.8k	65hi	31 168fg	5 133d
24. Drumhead	6.6g	3.8k	77i	32 900g	3 948cd

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for significance.

3.3.4.2. *Meloidogyne javanica*

RF-values

All Brassica cultivars had RF-values ≤ 1 and exhibited ELF-indices < 2 when screened against *M. javanica* in this study (Murray *et al.*, 1986; Windham & Williams, 1988; Tables 1, 2 & 10). The genotypes also maintained very low numbers of eggs/root system and eggs/g of root. Long Island had the lowest RF-value, with Discovery having the highest.

ELF-indices

All Brassica cultivars exhibited resistance to *M. javanica* since they all had ELF-indices between zero and one (Murray *et al.*, 1986; Tables 2 & 10).

Egg masses

Concerning the number of egg masses per plant, all the genotypes are classified as resistant to *M. javanica* according to the ELF-index of Murray *et al.* (1986) since all genotypes had zero egg masses/root system (Tables 2 & 10). Although none of the genotypes maintained any visible egg masses, very low numbers of eggs and J2 were extracted from root systems of these genotypes.

Table 10. Reproduction of *Meloidogyne javanica* on local Brassica cultivars measured 56 days after inoculation (DAI) with 5 000 eggs and second-stage juveniles (J2) in a greenhouse trial during 2005/06 growing season.

Cultivar	**RF-value**	**ELF-index**	**No. of egg masses/root system**	**No. of eggs/root system**	**No. of eggs/g roots**
1. Long Island	0.00003a	0.0a	0.0a	0.2a	0.01a
2. Big Cropper	0.00006a	0.0a	0.0a	0.3a	0.02a
3. Suntop	0.0001a	0.0a	0.0a	1a	0.1a
4. Green Valiant	0.0001a	0.0a	0.0a	1a	0.1a
5. Star 3315	0.0001a	0.0a	0.0a	1a	0.04a
6. Puma	0.0002a	0.0a	0.0a	1a	0.1a
7. Adagia	0.0002a	0.0a	0.0a	1a	0.1a
8. Wallaby	0.0002a	0.0a	0.0a	1a	0.2a
9. Hercules	0.0002a	0.0a	0.0a	1a	0.1a
10. Copenhagen Market	0.0002a	0.0a	0.0a	1a	0.1a
11. Malimba	0.0002a	0.0a	0.0a	1.2a	0.2a
12. Green Star	0.0002a	0.0a	0.0a	1.2a	0.1a
13. Glory of Enkhuizen	0.0002a	0.0a	0.0a	1.2a	0.1a
14. Star 3301	0.0003a	0.0a	0.0a	2a	0.2a
15. F1 Intro	0.0003a	0.0a	0.0a	2a	0.2a
16. Star 3311	0.0004a	0.0a	0.0a	2a	0.1a
17. Green Coronet	0.0005a	0.0a	0.0a	2a	0.2a
18. Drumhead	0.0005a	0.0a	0.0a	2a	0.2a
19. Snowball	0.0005a	0.0a	0.001a	4a	0.3a
20. Gloria P1	0.0007a	0.2b	0.2b	4a	0.3a
21. Cape Spitz/Sugar Loaf	0.0009a	0.0a	0.0a	5a	1a
22. Conquistador	0.0012a	0.0a	0.0a	6a	1a
23. Graffiti	0.0023a	0.0a	0.0a	11a	2a
24. Discovery	0.02b	0.0a	0.0a	89b	9b

Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test for significance.

Table 11. Correlation coefficients indicating the relationship between the number of egg masses and egg-laying females (ELF), respectively, and RF-values for the various vegetable crops during evaluations for host suitability to root-knot nematodes.

Vegetable crop	Root-knot nematode species (race)	Nematode parameters	Correlation coefficient (r)
Tomato	*M. incognita race 2*	Egg mass	0.81
Tomato	*M. incognita race 2*	ELF	0.72
Tomato	*M. javanica*	Egg mass	0.63
Tomato	*M. javanica*	ELF	0.59
Brassica	*M. incognita race 2*	Egg mass	0.86
Brassica	*M. incognita race 2*	ELF	0.70
Brassica	*M. javanica*	Egg mass	-0.014
Brassica	*M. javanica*	ELF	-0.014
Grean bean	*M. incognita race 2*	Egg mass	0.60
Grean bean	*M. incognita race 2*	ELF	0.60
Grean bean	*M. javanica*	Egg mass	0.31
Grean bean	*M. javanica*	ELF	0.28
Pumpkin	*M. incognita race 2*	Egg mass	0.27
Pumpkin	*M. incognita race 2*	ELF	0.21
Pumpkin	*M. javanica*	Egg mass	0.21
Pumpkin	*M. javanica*	ELF	0.26

Significant, positive relationships with correlation coefficients of 0.81 and 0.72 were obtained between the number of egg masses and ELF, respectively, when plotted against RF-values for tomato genotypes evaluated against *M. incognita* race *2* (Table 11). On the other hand correlation coefficients of 0.63 and 0.59 were obtained when the number of egg masses and ELF, respectively, were plotted against RF-values for tomato genotypes evaluated against *M. javanica.*

Significant, correlation coefficients of 0.86 and 0.70 were obtained when the number of egg masses and ELF, respectively, were plotted against RF-values for Brassica cultivars evaluated against *M. incognita* race 2 (Table 11), indicating positive, strong relationships. Weak, negative relationships (r = -0.014) were however, obtained when the number of egg masses and ELF, respectively, were plotted against RF-values for Brassica cultivars evaluated against *M. javanica.*

With regard to the green bean genotypes evaluated for host suitability against *M. incognita* race 2, moderately strong, positive relationships existed when the number of egg masses and ELF, respectively, were plotted against RF-values with correlation coefficients of 0.60 and 0.60 (Table 11). On the other hand, non-significant, positive relationships (r = 0.31 and r = 0.28) existed when the number of egg masses and ELF, respectively, and RF-values were plotted against each other.

Non-significant, positive relationships existed for pumpkin genotypes when screened against *M. incognita* race 2 when the number of egg masses and ELF, respectively, were plotted against RF-values with correlations coefficients of 0.27 and 0.21 (Table 11). The same trend was observed when pumpkin genotypes when screened against *M. javanica* when the number of egg masses and ELF, respectively, were plotted against the number of eggs and J2 with correlations coefficients of 0.21 and 0.26.

3.4. Discussion

None of the vegetable crop genotypes evaluated in this study were immune to either *M. incognita* race 2 or *M. javanica*, since these parasites reproduced in roots of all these genotypes.

Although a variety of nematode parameters were used to classify resistance in this study, RF-values were used as the main criterium to select for resistance in tomato, green bean, pumpkin, and Brassica cultivars. This criterium was also used by Swanson and Van Gundy (1984), Luzzi *et al.* (1987) and Fourie (2005) when soybean cultivars were screened for resistance to root-knot nematodes. Shepherd (1979) as well as Bernard and Keyserling (1985) also found egg counts to be more appropriate than egg mass counts when screening sunflower for host suitability to *Meloidogyne* spp. According to Williams and Williams (1987), RF-values are the most reliable parameter to identify root-knot nematode resistance as it provides a basic measurement for the usefulness of the resistance present.

Based on RF-values (Windham & Williams, 1988) four tomato (Rhapsody, MFH 9324, FA 1454 and FA 593) and a range of Brassica cultivars were identified with resistance against *M. incognita* race 2 and *M. javanica*, respectively, during this study. These four tomato genotypes all had RF-values ≤ 1, ELF-indices ≤ 2 and a low number of root-knot nematode eggs and J2 as well as egg masses/root system, which differed significantly from the susceptible standard cv. Moneymaker as well as from a range of other susceptible genotypes. Although it is known that root-knot nematode resistance in tomato is conferred by the *Mi* gene (Gilbert & Mc Guire, 1956; Roberts, 1992), the root-knot nematode resistance source present in local tomato genotypes is not reported (Anon, 2005). Furthermore, root-knot nematode resistance present in

currently available tomato genotypes world-wide is, however, not derived from breeding lines that posses the *Mi* gene only (Ammanti *et al.*, 1985, Cap *et al.*, 1993; Roberts *et al.*, 1990). New resistant sources that are not allelic to the *Mi* gene have been identified in available tomato cultivars (Ammanti *et al.*, 1985, Cap *et al.*, 1993; Roberts *et al.*, 1990).

The *Mi* gene that confers resistance to *Meloidogyne arenaria*, *M. incognita* and *M. javanica,* consists of a single, dominant gene (Gilbert & Mc Guire, 1956) and is effective against a wide range of populations of these species (Roberts, 1992). Root-knot nematode resistance conferred by the *Mi* gene is, however, not sustainable under high temperatures when soil temperatures exceed 28°C (Dropkin, 1969). Natural, virulent populations of *M. incognita*, *M. javanica* and *M. arenaria* have also been reported that develop and reproduce on tomato genotypes exhibiting the *Mi* gene (Castagnone-Sereno *et al.*, 1993; Castagnone-Sereno *et al.*, 1994; Roberts *et al.*, 1990). Examples of resistance confered by the *Mi* gene include several breeding lines and cultivars, i.e. cvs. Celebrity, Carmelo, Luxor, Mont, Small Fry, etc. and lines ARP 365-2, ARP 367-1, ARP 367-2, PSR8991994, F8A, F8B, etc. (Castagnone-Sereno *et al.*, 1994; Mani & Zidgali, 1995; Tzortzakakis *et al.*, 1998; Ornat *et al.*, 2001; Nono-Womdin *et al.*, 2002).

No local tomato genotype, however, had an RF-value ≤ 1 when screened against *Meloidogyne javanica* during this study and is therefore susceptible to this population according to our interpretation. Although resistance to *M. incognita* race 2 is present in local genotypes Rhapsody, MFH 9324, FA 1454 and FA 593 it does not protect these genotypes against the local *M. javanica* population used in this study. This is in accordance with reports by Netscher and Sikora, (1990), namely that a cultivar resistant to one root-knot nematode species does not necessarily exhibit resistance to another species of the same genus. Although the local genotype MFH 9343 was reported resistant to root-knot nematodes (Anon, 2005), results from this study indicate the contrary, namely that this genotype is susceptible to local populations of *M. incognita* race 2 and *M. javanica*.

In terms of Brassica, 13 and 24 of the local genotypes evaluated, respectively, exhibited potential resistance to the local *Meloidogyne incognita* race 2 and *M.*

javanica populations used in this study, since they had RF-values ≤ 1. Since reproduction of both root-knot nematode species were generally low on the majority of local Brassica cultivars used during this study, it confirms reports that crucifers are generally poor hosts to *Meloidogyne* species (Liebanas & Castillo, 2004). It can, however, not be concluded that these local Brassica cultivars are resistant to particularly the local *M. javanica* population used, since all genotypes had RF-values ≤ 1 in this trial. The trial should therefore be repeated with inclusion of an exotic *M. javanica*-resistant genotype or at least a *M. javanica* susceptible crop, i.e. tomato (susceptible cv. Moneymaker). Thus resistance to this root-knot nematode species in these Brassica cultivars can be verified.

Brassica cultivars (cabbage in this case) with resistance to an Australian population of *M. javanica* have, however, been reported by Stirling and Stirling (2003), i.e. in mustard (cv. Nemafix), in canola (cv. Dunkeld) and in rape (cvs. Rangi, Nemcon and Striker). These genotypes could, however, not be obtained in time to include in these local screening trials.

Isothiocyanates (ITCs) are the most active biocides present in roots and leaves of Brassica crops and are responsible for the reduction in plant-parasitic nematode populations (Brown & Moora, 1997; Rosa *et al.*, 1997). According to Mc Leod *et al.* (2001), poor host suitability of Brassica crops to *M. javanica* is the result of delayed or retarded (slower) development of individuals from this species compared to that in good hosts, i.e. tomato cv. Grosse and pea cv. Dun. However, in contrast to the latter authors, a study by Liebanas and Castillo (2004) indicated that *M. incognita*, *M. javanica* and *M. arenaria* reproduced substantially on crucifers although nematode production was lower than on susceptible tomato genotypes included in the same trial. Several factors, i.e. Brassica cultivars (Stirling & Stirling, 2001), experimental and environmental conditions and virulent *Meloidogyne* populations (Liebanas & Castillo, 2004) are, however, considered to be responsible for higher root-knot nematode reproduction in genotypes of some Brassica cultivars. It is therefore imperative that only Brassica cultivars that are resistant to root-knot nematodes are planted in warm climates in rotation/intercropping systems (Stirling & Stirling, 2003). Since Brassica crops are adapted to cool climates, root-knot nematode susceptible Brassica cultivars

can also be planted during winter months when nematodes multiply slower at low temperatures (Stirling & Stirling, 2003).

No root-knot nematode resistance was identified in local green bean (*Phaseolus vulgaris*) and pumpkin (*Cucurbit* spp.) genotypes used in this study. Resistance to *M. incognita* race 2 has, however, been reported in the exotic green bean genotype Nemasnap (Sikora *et al.*, 2005) while resistance to *M. javanica* and *M. incognita* has been recorded in several dry bean genotypes (*Phaseolus vulgaris*) (Ngundo, 1977; Omwega *et al.*, 1989; Sikora *et al.*, 2005). Since seed of the *M. incognita*-resistant cultivar Nemasnap ordered from the Centre Internacional de Agricultura Tropical (CIAT) in Colombia, South America only arrived after commencement of the respective green bean trials, this resistant standard could not be included in this study. It is, however, important that cv. Nemasnap should be evaluated against local populations of *M. incognita* to establish whether the reported resistance will be sustainable under these populations.

In terms of the pumpkin genotypes evaluated for resistance to *M. incognita* race 2 and *M. javanica*, respectively, during this study, all these genotypes were susceptible to these respective species and race. These results confirm an earlier report by Brust *et al.* (2003) that little research has been done in developing pumpkin varieties resistant to root-knot nematodes on a world-wide scale.

The reason that RF-values were used in this study is that the primary objective in a crop rotation system is to apply the management strategy that will result in the lowest number of plant-parasitic nematodes maintained by the current crop. However, root galling and ELF-indices are also used widely as criteria to determine root-knot nematode resistance, especially under field conditions where laboratory infrastructure are not available to extract eggs and J2 (Hussey & Boerma, 1981; Griffin & Rumbaugh, 1996). These indices are, however, not as accurate as RF-values (1987).

To illustrate why egg-mass and ELF-indices were not used as the main criteria in this study, some tomato and *Brassica cultivars* had ELF-indices < 1, indicating moderate resistance (Murray *et al.*, 1986) to the relevant root-knot nematode species and/or race. In such cases these higher ELF-indices and low RF-values (≤ 1) imply that root-

knot nematode females produced many eggs, but these egg masses contained little or no eggs (Fourie, 2005). Examples include the tomato genotype FA 1454 and *Brassica cultivars* Graffiti, Snowball, Conquistador, Malimba, Puma, Green Coronet, Star 3311, Hercules and Big Cropper. Conversely, tomato genotypes Primepak, Floradade, FA 1418 and Roma had ELF-indices ≤ 2, but RF-values ≥ 1 when evaluated against *M. incognita* (race 2). In this case the low number of root-knot nematode egg masses contained relatively high number of eggs per egg mass. This was observed also during this study in genotype Rhapsody when evaluated for host suitability for *M. javanica*. Hussey and Boerma (1981), Niblack *et al.* (1986) and Fourie (2005) also reported the latter phenomenon when evaluating soybean for resistance to root-knot nematodes. Although some of these authors reported a correlation between gall ratings and egg mass indices when plotted against total nematode numbers on soybean roots, a few exceptions occurred where some soybean cultivars exhibited low gall ratings but had high egg mass indices as well as high total numbers of root-knot nematode eggs per root system. This was also true for the vegetable crops used in this study when screened against the appropriate root-knot nematode species.

Valuable information with regard to the identification of resistant sources in tomato and Brassica cultivars against *M. incognita* race 2 as well as against *M. incognita* race 2 and *M. javanica*, respectively, has finally been obtained as a result of this study. Although no resistant green bean or pumpkin genotype has been identified in this study, genotypes with the lowest RF-values such as green bean genotypes Witsa (for *M. incognita* race 2) and Tokai (for *M. javanica*) as well as pumpkin genotypes Caserta (for *M. incognita* race 2) and Star 8001 (for *M. javanica*) can, however, be recommended for planting by farmers rather than the highly susceptible varieties of these crops. The need, however, exists to identify and develop root-knot nematode resistant genotypes from a wider range of vegetable crops to ensure household food security where root-knot nematodes pose a serous threat to sustainable crop production.

Chapter 4

Verification of *Meloidogyne incognita* race 2-resistance in a microplot study using a range of initial inoculation densities (Pi).

4.1. Introduction

Tomato hosts a wide variety of plant-parasitic nematodes, including root-knot nematodes (Overman, 1995; Sikora & Fernandez, 2005). *Meloidogyne incognita* is the predominant root-knot nematode species in tomato world-wide and rank second to *M. javanica* in tropical and subtropical regions (Nono-Womdim *et al.*, 2002). According to Abawi *et al.* (1994) root-knot nematodes cause major reduction in tomato yield if proper management strategies are not applied. Estimated production losses exceeding 50 % were reported in tomato due to infection by root-knot nematodes (Nono-Womdim *et al.*, 2002).

Development and availability of root-knot nematode resistant tomato cultivars are crucial, particularly to small-scale farmers. These parasites are widely distributed and attack the tomato crop wherever it is grown. A number of root-knot nematode resistant tomato cultivars are available (Bundoora, 1996; Roberts, 1992; Sikora *et al.*, 2000). Although there are reports of tomato genotypes resistant to root-knot nematodes under greenhouse conditions (Sharma *et al.*, 2005) the resistance of those materials needs to be verified under general production conditions. Those include field or microplot trials to determine the levels of resistance under uncontrolled or semi-controlled circumstances. The objectives of the present study were therefore to verify resistance identified in tomato to *M. incognita* race 2 in a greenhouse (see Chapter 3 paragraph 3.2.1.1.) under natural environmental conditions in a microplot trial using a range of initial inoculation densities (Pi).

4.2. Materials and methods

To achieve the objective of the present study, a microplot trial was conducted during the 2005/2006 growing season on the premises of the Agricultural Research Council-Grain Crops Institute (ARC-GCI) in Potchefstroom (North West Province).

4.2.1. Tomato germplasm

Tomato seedlings were made from a *M. incognita* race 2-susceptible and -resistant cultivar Moneymaker (Hadisoeganda & Sasser, 1982; Anwar *et al.*, 1994) and Rhapsody, respectively. The latter cultivar was identified as resistant to this species and race in a host-suitability study described in Chapter 3. Seeds were planted in seedling trays filled with potting soil and the trays were watered regurlarly. Six weeks after germination the seedlings were transplanted into pre-prepared microplots (described in paragraph 4.2.2.).

4.2.2. Microplot trial

Microplots used in the present study consisted of circular concrete tubes 1m in diameter and 1.25m high that were partially buried vertically in the soil in a field. The average daily temperature recorded for the duration of the microplot experiment was ± 22.5 °C, while the minimum and maximum temperatures were 14.0 and 32.9 °C, respectively for this period (86 days). Total rainfall was 152.4 mm from planting to harvesting. Temperature and rainfall data were obtained from the Weather Department of the ARC-GCI, Institute for Soil, Climate and Water (ISCW), Potchefstroom.

Microplots were filled with methyl bromide-fumigated soil (1,162g a.i/$2m^3$) consisting of 3.9 % clay, 1.9 % silt, 93.6 % sand and 0.6 % organic material, with a pH (H_20) of 6.25. Plant nutrients were added according to a soil nutrient analysis. They constituted of 3.12g potassium chloride (KCl) (1.56g per microplot at planting and 1.56g per microplot at flowering) and 40g super phosphate (10,5 % phosphorus) per microplot at planting.

Four tomato seedlings were planted in each microplot. Plants were irrigated three times a week with sprayers fitted in each microplot, delivering 25 ± 4mm water in 15 minutes. When it rained, irrigation schedules were adapted to prevent water logging. Nematode sampling was conducted 86 days after planting and inoculation. Tomato

roots were rinsed free of adhering soil and debris and the eggs and second-stage juveniles (J2) extracted from each tomato root system.

4.2.3. Nematode inoculation

Eggs and J2 from the same *M. incognita* race 2 population used in the host-suitability trial (see Chapter 3, paragraph 3.2.3.) were used for inoculation purposes in the microplot trial. This root-knot nematode species was identified (see Chapter 2) and reared *in vivo* as described in Chapter 3, paragraph 3.2.2. Initial nematode inoculum levels (Pi) consisted of approximately 0, 100, 500, 1 000, 5 000, 10 000 and 20 000 *M. incognita* race 2 eggs and J2 per seedling. The inoculum was prepared in tap water and inoculated as described in Chapter 3, paragraph 3.2.3.

4.2.4. Experimental design and data analysis

The experimental layout was a randomised complete block design including two tomato cultivars (Moneymaker and Rhapsody), six replicates and seven treatments (Figure 7). Untreated control treatments for each of the tomato genotype were included as nematode-free (Pi = 0), EDB-fumigated treatments. Root systems of each tomato plant from each treatment were treated as one replicate. For yield analysis fruits from the four plants were pooled together. Final nematode population densities (Pf) in the soil and roots as well as nematode reproduction factors [RF=final egg and J2 numbers (Pf) / initial egg and J2 numbers (Pi)] were analysed by means of factorial analysis of variance (Statgraphics Plus 5 for Windows) as well as non-linear regression analysis (Genstat for Windows). Means were separated by Tukey's test ($P \leq 0.05$), while degrees of freedom (error) > 18 (Van Ark, 1981) were always persued.

4.2.5. Nematode reproduction assessment

Tomato root systems of both genotypes were removed 86 days after planting, rinsed free of adhering soil and debris with running tap water, blotted on towel paper and weighed. The following nematode variables were determined: i) final population density (Pf) in the soil and in roots and ii) nematode reproduction factors (RF). Nematode eggs and J2 were extracted from tomato root systems using Riekert's

(1995) modified NaOCl-method (described in Chapter 3, praragraph 3.2.5) and counted under a stereo microscope. Nematodes were extracted from 200 ml soil samples using the decanting and sieving method (Cobb, 1918, paragraph 4.2.6), followed by the sugar flotation method (Caveness & Jensen, 1955, paragraph 4.2.7). The reproductive potential of *M. incognita* race 2 was determined using Oostenbrink's reproduction factor (RF) (Windham and Williams, 1987) described in Chapter 3, paragraph 3.2.4.

Nematode data, namely Pf in soil and roots and reproduction factors (dependent variables) were non-linearly regressed on the range of Pi levels (independent variables) using the rational, linear-divided-by-linear (ldl) model, Y= A+B/(1+D*X), the quadratic-divided-by-quadratic model (qdq) Y= A+(B+C*X)/(1+D*+E^X), the exponential model Y= A+B*(R^X) as well as the quadratic model Y = A + B*(R^X) (Genstat for Windows). The Pf in roots and soil were log(x+1) tranformed for data analysis purposes. Correlation coefficients were calculated to obtain the relationship between RF- and Pi using a Multiple-Variable Analysis (Statgraphics 5 Plus for Windows). With regard to yield data, the percentage yield loss [(x yield of untreated control/x for each respective Pi)/100-100] was calculated for each cultivar and treatment (Table 11).

4.2.6. Decanting and sieving method (Cobb, 1918)

After soaking the soil sample in water the soil particles having a diameter of more than 1 mm are removed by passing the soil sample through a 1 mm aperture sieve in a 4,5 L bucket. The residue on the sieve is washed with a fine spray and discarded. The bucket is then filled up to a volume of 4,5 L, the soil sample is then throughly mixed with water and the mixture allowed to settle for about 30 seconds. It is then decanted through 53-µm aperture sieve and a 38-µm aperture sieve, leving behind the sediment that has settled to the bottom of the bucket. The nematodes and fine soil particles retained in the sieves are washed (using a gentle stream of water applied to the underside of the inclined sieve) into a beaker.

4.2.7. Sugar centrifugal-flotation method (Caveness and Jensen, 1955)

(a) Water centrifugation

The soil is washed with watern in 50 ml or 100 ml centriguge tubes and centrifuged for 5 minutes with a Relative Centrifugal Force (RCF) of 1800 g. After the centrifugation the supernatant is poured off carefully.

(b) Sucrose centrifugation

A sucrose soultion with a density of 1,15 is now added to the centrifuge tubes. Then the sucrose solution and the sediment in the centrifuge tubes are thoroughly mixed, i.e. with a vibromixer, and immediately centrifuged during a 1 minute with RCF of 1800 g. After centrifugation has ended, the supernatant liquid (with the nematodes) is poured off on a 38-µm aperture sieve and gently rinsed with water in order to remove the sucrose. Was into a beaker or botlle with a little water. The nematodes are then examined.

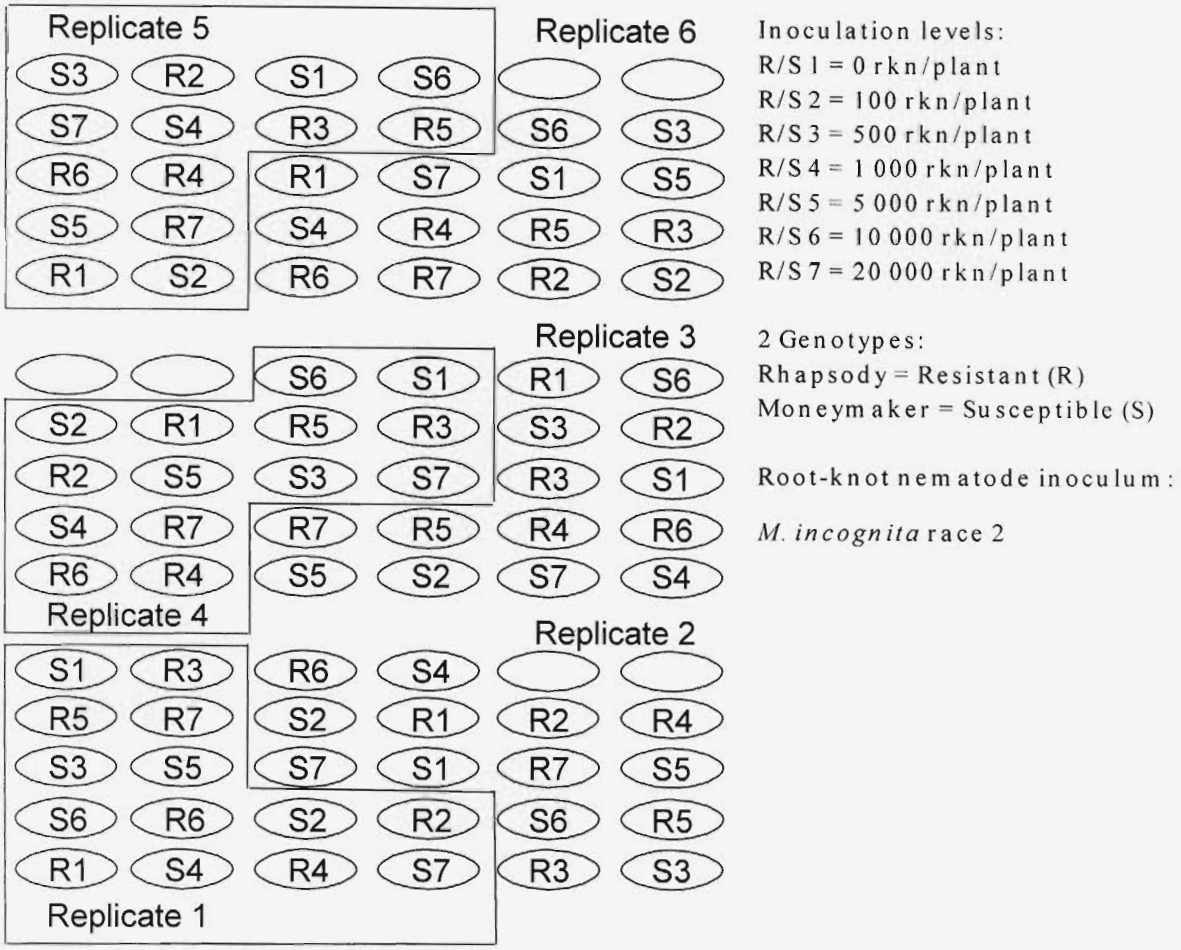


Figure 7. Trial layout for the verification of *Meloidogyne incognita* race 2-resistance in tomato cultivar Rhapsody using a range of initial inoculation densities (Pi) in a microplot trial at Potchefstroom during the 2005/06 growing season together with the susceptible cultivar Moneymaker.

4.3. Results

The relationship between initial (Pi) and final (Pf) *M. incognita* race 2 populations in both roots and soil as well as between Pi and RF-values were best described by non-linear equations (Figure 8 A, B & C).

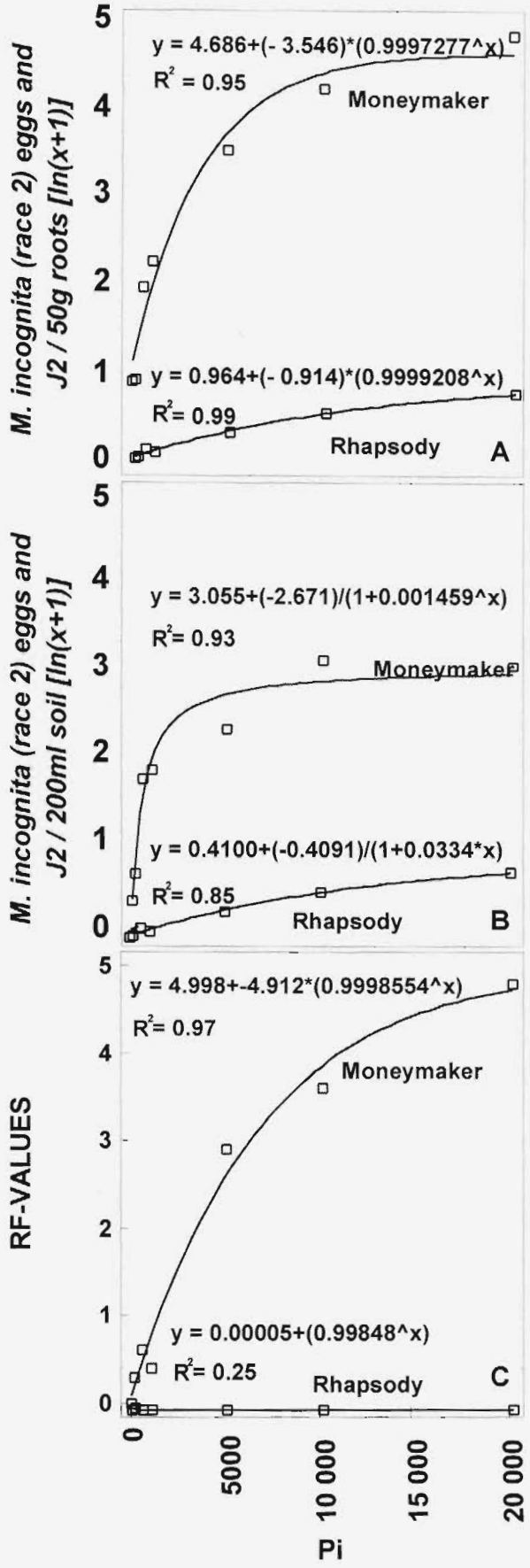


Figure 8. Relationships between initial (Pi) and final *Meloidogyne incognita* race 2 populations (Pf) in 50g tomato roots (A), 200ml soil (B) as well as for RF-values (C) at 86 days after inoculation (DAI) for a susceptible (Moneymaker) and a resistant (Rhapsody) tomato cultivar in a microplot trial at Potchefstroom.

4.3.1. Pf in roots

Reproduction of *M. incognita* race 2 was significantly lower in roots of Rhapsody compared to those in roots of Moneymaker in the microplot trial (Figure 8 A; Table 12). Significantly higher numbers of eggs and J2 were present in roots of Moneymaker compared to Rhapsody at Pi = 100, 500, 5 000, 10 000 and 20 000. Pf for Rhapsody was very low and increased from 0 (Pi = 0) to only 11 eggs and J2 at Pi = 20 000. The Pf for Moneymaker was nine-fold more, namely a mean of 96 532 eggs and J2 at Pi = 20 000.

Strong relationships between Pi and Pf in roots were best described by non-linear models for both the cultivars, explaining a significant 99 % and 95 % of the variation for Rhapsody and Moneymaker, respectively (Fig. 8 B). The regression line for Moneymaker generally leveled off between Pi = 5 000 and Pi = 10 000, showing that Pf in roots did not increase substantially when more than 5 000 – 10 000 eggs and J2 were inoculated per plant. On the other hand, the regression line for Rhapsody seemed to level of after a slight increase in eggs and J2 at the highest Pi = 20 000, explaining the difference in slopes of the regression lines for the two cultivars. It is however important to bear in mind that a low, final Pf of 11 eggs and J2 was recorded at the highest Pi = 20 000 for the latter cultivar.

4.3.2. Pf in soil

Pf-values in soil for both tomato cultivars were best described by non-linear regression equations (Fig. 8 B). Significant R-square (R^2) values of 0.85 and 0.93, respectively, were recorded for Rhapsody and Moneymaker, explaining 85 % and 93 % of the variation. The regression line for Moneymaker generally leveled off between Pi = 1 000 and Pi = 5 000, while the regression line for Rhapsody showed a constant increase in *M. incognita* race 2 J2 towards the final Pi = 20 000. It is, however, important to bear in mind that a low mean of only two J2 were present in soil samples from the rhisophere of Rhapsody at Pi = 20 000. Substantially more J2 were present in soil samples from the rhizosphere of cultivar Moneymaker compared to those in the rhizosphere of genotype Rhapsody (Fig. 8 B; Table 12). At the highest inoculum level

of Pi = 20 000 Rhapsody and Moneymakey maintained an average of 2 and 2 648 J2 per 200ml soil, respectively.

4.3.3. RF-values

No significant relationship (R^2 = 0.25) was obtained between Pi and RF-values for cultivar Rhapsody using a quadratic linear model as well as range of other models (Fig. 8 C). A strong relationship was, however, obtained between these parameters for Moneymaker and was best described by a non-linear model with a significant R^2-value of 0.97.

RF-values were inversely related to Pi for Rhapsody (r = -0.3), decreasing from 0.002 at Pi = 100 to 0.0005 at the highest Pi = 20 000 (Table 9). On the other hand, RF-values for Moneymaker gradually increased from 0.3 at Pi = 100 to 4.8 at Pi = 20 000 (r = 0.94).

4.3.4. Percentage yield loss

Yield data were adversely affected by excessive rains throughout the growing season. Percentage yield loss was, however, generally substantially higher for cultivar Moneymaker compared to Rhapsody (Table 12). At the highest Pi = 20 000 yield losses of up 18.9 % were recorded for Moneymaker in contrast to 0.2 % for Rhapsody. High yield losses of 12.8 % and 9.2 % were, however, recorded for genotype Rhapsody at Pi = 500 and Pi = 1 000.

Table 12. *Meloidogyne incognita* race 2 data on final nematode population density (Pf) in roots and soil, reproduction factor (RF) and yield loss (%) at 86 days after inoculation (DAI) for a susceptible (Moneymaker) and a resistant (Rhapsody) tomato cultivar in a microplot trial at Potchefstroom.

Pi	Tomato cultivar	Pf (roots)	Pf (soil)	RF	% Yield loss
0	Rhapsody	0.1 (0.07)a	0 (0)a	0a	0
	Moneymaker	13 (2.1) a	4 (0.8)a	0a	0
100	Rhapsody	0.2 (0.1) a	2 (0.8)b	0.002g	0.06
	Rhapsody	25 (2.1) a	9 (1.6)b	0.3b	7.8
500	Rhapsody	0.5 (0.3) a	2 (0.8)b	0.001f	12.8
	Moneymaker	304 (4.6) b	122 (4.0)b	0.6d	5.6
1000	Rhapsody	0.4 (0.3) a	2 (0.7)b	0.0005d	9.2
	Moneymaker	367 (5.2) b	106 (4.2)b	0.4c	8.7
1500	Rhapsody	1.6 (0.8) a	2 (0.8)b	0.00032b	4.9
	Moneymaker	14 318 (8.2) c	530 (5.4)c	2.9e	5.6
10000	Rhapsody	4.15 (1.3) b	3 (1.1)b	0.00042c	0.3
	Moneymaker	36 012 (9.9) d	1 939 (7.2)d	3.6f	20.6
20000	Rhapsody	10.55 (1.8) b	2 (0.9)b	0.0005e	0.2
	Moneymaker	96 532 (11.2) e	2 648 (7.1)d	4.8g	18.9

* Means in the same column followed by the same letter do not differ significantly ($P \leq 0.05$) according to the Tukey test.
log(x+1)-transformed data in parenthesis.

4.4. Discussion

Data obtained on the quantitative relationships between initial population densities and final population densities of *M. incognita* race 2 in tomato root and soil samples indicated that the resistant Rhapsody suppressed nematode reproduction in its roots significantly compared to the susceptible Moneymaker. The latter cultivar maintained significantly higher populations of this nematode species. Data furthermore showed that Rhapsody has a higher damage threshold level of at least four times that of Moneymaker, since the regression line for Moneymaker leveled off from Pi = 5 000 and for Rhapsody from Pi = 20 000 eggs and J2 per root system. This information could play an important role in tomato production, particularly for small-scale producers, since it is crucial for decision-making with regard to the economic management of these parasites.

Significantly lower RF-values exhibited by Rhapsody in this study are a further indication of the high level of resistance present in this genotype compared to

Moneymaker. Decreasing RF values obtained for Rhapsody in this study with regard to *M. incognita* race 2 also corresponds with reports by other authors (Di Vito *et al.*, 1991; Charchar *et al.*, 2003) in terms of *M. incognita* race 1 and *M. javanica* on tomato.

Furthermore, results from this study indicate that the *M. incognita* race 2-resistant local tomato cultivar Rhapsody maintained lower root-knot nematode populations and generally sustained lower yield losses than the susceptible Moneymaker, showed similar trends to that of a resistant cultivar DISA N in the Mediterranean when a much lower range of Pi-levels (0.031 to 128 eggs and J2) for *M. incognita* race 1 were used (Di Vito *et al.*, 1991). According to the latter author DISA N sustained less yield loss and a higher marketable yield than the susceptible cultivar Ventura. Furthermore, DISA N also maintained significantly lower numbers of *M. incognita* race 1 in roots at various Pi-levels than susceptible cultivar Ventura.

Low numbers of J2 (J2/200ml soil) detected in soil samples from the rhizosphere of Rhapsody at 86 days after inoculation in this study is also in agreement with findings from Di Vito *et al.* (1991) for cultivar DISA N. The latter author reported low populations of *M. incognita* race 1 (0.4 J/1cm^3 soil) in the rhizosphere of the latter cultivar 70 days after inoculation in a microplot trial. According to Di Vito *et al.* (1991) this phenomenon is due to the fact that most J2 that penetrated the roots of DISA N were unable to complete their development within the roots, resulting in a decline in nematode numbers in the soil at harvest. The presence of low root-knot nematode populations in soil before planting, resulting from a resistant tomato cultivar preceeding a follow-up crop will be beneficial to producers contrary to a preceeding susceptible cultivar, e.g. Moneymaker. However, when a highly susceptible follow-up crop e.g. potato is planted after Rhapsody, for example, the possibility also exists that severe damage could still occur. Therefore, the level of resistance present in a tomato genotype is very important since it will indicate which additional nematode control strategy would be required in a crop rotation system.

Although significant differences in nematode population densities between the two cultivars coincided with a significant difference in yield loss percentage, excessive rains during critical growth stages of the crop may have had an adverse effect on yield

data obtained for both cultivars. This was evident when Rhapsody showed high yield losses with low Pi. This genotype, however, generally showed yield reduction than the susceptible Moneymaker. Higher yield losses compared to the maximum yield loss of 20,6 % in the susceptible cultivar Moneymaker in this study have, however, been reported on susceptible tomato in tropical regions due to *M. incognita* infection (Subramaniyan *et al.*, 1990; Charchar *et al.*, 2003). Levels of yield suppression for the respective cultivars recorded in this study showed the same trend, i.e. higher yield losses for a susceptible as opposed to a resistant cultivar, as those reported by Di Vito *et al.* (1991) in the Mediteranean.

Data obtained in this study with regard to the susceptibility of Moneymaker to *M. incognita* race 2 is also in agreement with reports by Anwar *et al.* (1994) and Hadisoeganda and Sasser (1982). According to these authors Moneymaker is highly susceptible to the *M. incognita* populations used in their respective studies. This cultivar allowed rapid development of J2 into adult females with a subsequent high fecundity rate, resulting in high numbers of eggs produced at all Pi's used in their studies. The same trend was observed with regard to the reproduction rate of *M. incognita* race 1 in the susceptible tomato cultivar Ventura planted in microplots (Di vito *et al.*, 1991)

Identification and verification of tomato genotypes with resistance to *M. incognita* race 2 is important for commercial, but particularly for small-scale farmers who have limited resources. Use of the highly resistant Rhapsody remains at present one of the best strategies to keep *M. incognita* race 2 numbers beyond damage threshold levels when included in crop rotation systems with other resistant vegetable crops (i.e. *Brassica* spp.). It is however, important to bear in mind that factors such as environmental conditions, crop history, cultivar planted, nematode species or race present, nematode distribution patterns and initial inoculation densities are amongst the numerous factors that influence the severity of eventual crop damage and yield reduction (Baldwin *et al.*, 1979; Brown, 1987). Therefore, it is important to repeat this study during the following season.

Chapter 5

5.1. Conclusions and recommendations

The need exists to maximise yield of vegetable crops whilst maintaining root-knot nematodes below damage-threshold levels without increasing production costs. Therefore, the present study entailed the identification and verification of resistance to root-knot nematodes species in locally available vegetable crops. The nematodes were identified by means of molecular techniques. This study was thus conducted to make a practical and applicable contribution to sustainable production systems, particularly to the subsistence-farming sector.

The DNA-based SCAR-PCR method was successfully used to identify *M. incognita* race 2 and *M. javanica* as monospecific populations during this study. These respective root-knot nematodes populations could, therefore, be used as inoculum for the greenhouse and microplot trials conducted during this study.

Reproduction factor was used as the main criteria to identify resistance in local tomato, green bean, pumpkin and Brassica cultivars evaluated against *M. incognita* race 2 and *M. javanica*, respectively, in greenhouse trials. Resistance to *M. incognita* race 2 was identified in four tomato genotypes, viz. Rhapsody, MFH 9324, FA 1454 and FA 593, since they had RF-values ≤ 1. Resistance to *M. incognita* race 2 and *M. javanica*, respectively, was also identified in a range of Brassica cultivars. No resistant pumpkin or green bean genotype was, however, identified. Important to bear in mind is that none of the vegetable crop genotypes evaluated against the respective root-knot nematode species and races in this study were immune to these parasites, since the nematodes reproduced on all genotypes.

During verification of *M. incognita* race 2 resistance in the tomato genotype Rhapsody in a microplot trial, using a range of Pi the latter genotype consistently maintained significantly lower numbers of eggs and J2 as well as RF-values compared to the susceptible standard Moneymaker. Thus superior resistance in Rhapsody was confirmed. Furthermore, relationships among all nematode parameters studied were

best described by non-linear equations. Rhapsody had a damage threshold value of at least four times higher than that of Moneymaker and generally sustained lower yield loss than the latter cultivar. A further advantage of using Rhapsody is the low numbers of J2 detected in soil samples compared to significantly higher numbers of J2 in the rhizosphere of Moneymaker at 86 days after planting. This emphasizes the usefulness of this resistant source, particularly for small-scale tomato producers. Since Rhapsody does not allow build-up of high *M. incognita* race 2 numbers in the soil, it minimizes yield losses, particularly in systems where susceptible crops are used for intercropping or crop rotation. Information gained on the verification of *M. incognita* race 2 as well as on damage threshold values for Rhapsody is invaluable for small-scale farmers since it simplifies the decision-making process for managing these parasites economically.

Future prospects include recommendation of the root-knot nematode-resistant tomato and Brassica cultivars identified in this study to be used by subsistence farmers, rather than the susceptible varieties of these crops. Thus it will prevent build-up of high root-knot nematode populations, which is difficult to manage. The need, however, exists to identify and develop root-knot nematode-resistant genotypes from a wider range of vegetable crops to ensure food security where root-knot nematodes pose a threat to sustainable crop production. The use of host plant resistance identified in this study and other alternative, cost-effective and environmentally-friendly nematode control strategies will be further investigated for inclusion in Integrated Pest Management (IPM) systems. This forms part of the internationally funded project "Mobilising participatory ICM (Integrated Crop Management) for sustainable nematode management in household and community gardens of resources –poor farmers in South Africa" by the Flemish Inter University Council (VL.I.R.). Thus household food security will be supported in subsistence farming systems in South Africa.

References

ABAWI, G.S. & CHEN, J. 1998. Concomitant pathogen and pest interaction. *In*: Plant and nematode interactions. (eds: K.R. Barker, G.A. Pederson, G.L. Windham). Madison, Wisconson, USA. pp 135-157.

ABAWI, G.S., MULLIN, B.A. & MAI, W.F. 1991. Diseases caused by Nematodes. *In*: Compendium of Bean Diseases. (ed: Hall, R). APS Press. USA. pp 32-36.

ABUBAKUR, U., ADAMU, T. & MANGA, S.B. 2004. Control of *Meloidogyne incognita* (Kofoid & White) Chitwood (root-knot nematode) of *Lycopersicon esculentum* (tomato) using cow dung and urine. *African Journal of Biotechnology* 3(8): 379-381.

AGRIOS, G.N. 1997. Plant Pathology, 4th edition. Academic Press, London, UK. pp 565-597.

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

ANONYMOUS, 2003f. France tomato production http://www.google.co.za/search?hl=en&q=tomato+production+in+France&btnG=Google [Access date: 21/11/06]

ANONYMOUS. 2005e. Mayford-groentebylae. Mayford het tamatise vir elke tyd en plek. pp 1-7.

ANONYMOUS. 2006a. Wikipedia Foundation. Inc. [Available on internet:] En.wikipedia.org/wiki/vegetable [Access date: 22/05/06]

ANONYMOUS, 2006b. South Africas Farming Sector. [Available on internet:] http://www.southafrica.info/doing-business/economy/key-sectors/542547.htm [Access date: 15/09/06]

ANONYMOUS. 2006c. Cabbage. [Available on internet:] http://www.answers.com/topic/cabbage [Access date: 27/03/06]

ANONYMOUS. 2006d. Pumpkin and squashes. [Available on internet:] http://www.answers.com/topic/cabbage [Access date: 27/03/06]

ANONYMOUS, 2006g. Tomato in Portugal http://www.google.co.za/search?hl=en&q=tomato+production+in+Portugal&btnG [Access date: 21/11/06]

ANWAR, S.A., TRUDGILL, D.L. & PHILLIPS, M.S. 1994. The contribution of variation in invasion and development rates of *Meloidogyne incognita* to host status differences. *Nematologica* 40: 579-586.

BALDWIN, J.G., BARKER, K.R. & NELSON, L.A. 1979. Effects of *Meloidogyne incognita* on nitrogen fixation in soybean. *Journal of Nematology* 11: 156-161.

BARKER, K.R., SHOEMAKER, P.B. & NELSON, L.A. 1976. Relationships of initial population densities of *Meloidogyne incognita* and *M. hapla* to yield of tomato. *Journal of Nematology* 8: 232-239.

BERNARD, E.C. & KEYSERLING, M.L. 1985. Reproduction of root-knot, lesion, spiral and cyst nematodes on sunflower. *Plant Disease* 69 (2): 103-105.

BLOK, V.C., PHILLIPS, M.S., McNICOL, J.W. & FARGETTE, M. 1997. Genetic variation in tropical *Meloidogyne* species as shown by RAPDs. *Fundamental and Applied Nematology* 20: 127-133.

BOERMA, H.R. & HUSSEY, R.S. 1992. Breeding plants for resistance to nematodes. *Journal of Nematology* 24 (2): 242-252.

BOS, L. & PARLEVLIET, J.E. 1995. Concepts and Terminology on Plant Pest Relationship: Toward Consensus in Plant Pathology and Crop Protection. *In*: (eds:

R.K. Webster, G.A., Zentmyer, G., Shaner). *Annual Review of Phytopathology* 33. pp 69-102.

BRIDGE, J. 1983. Nematodes. *In*: Pest Control in Tropical Tomatoes. Centre for Overseas Pest Research, Overseas Development Administration, London. pp 69-84.

BRIDGE, J. 1996a. Nematode management in sustainable and subsistence agriculture. *Annual Review of Phytopathology* 34: 201-225.

BRIDGE, J. 1996b. Introduced and indigenous plant nematodes of different crops and cropping systems in Africa: a personal perspective. *African Plant Protection* 2 (1): 67-75.

BRIDGE, J. 1987. Control strategies in subsistence agriculture. *In*: Principles and Practice of Nematode Control in Crops. (eds: R.H. Brown, B.R. and Kerry). Academic Press, New York. pp 389-420.

BROWN, R.H. 1987. Control strategies in low-value crops. *In*: Principles and practice of nematode control in crops. (eds: R.H. Brown & B.R. Kerry). Academic Press, London, UK. pp 351-382.

BROWN. D. & MOORA, M.J. 1997. Control of soil-borne plant pests using glucosinate-containing plants. *Advances in Agronomy* 61: 167-231.

BRUST, G.E., SCOTT, W.D. & FERRIS, J.M. 2003. Root-knot nematode control in melons. [Available on Internet:] http://www.ces.purdue.edu/extmedia> [Access date: 27/03/06]

BUNDOORA, J.H. 1996. Root-knot nematode on vegetables. Department of Primary Industries.

CAP, G.B., ROBERTS, P.A, THOMASON, I.J. 1993. Inheritance of heat-stable resistance to *Meloidogyne incognita* in *Lycopersicon peruvianum* and its relationship to the *Mi* gene. *Theoretical and Applied Genetics* 85: 777-783.

CASTAGNONE, P., PIOTTE, C., ABAD, P., BONGIOVANNI, M. & DALMASSO, A. 1991. Isolation of repeated DNA probe showing polymorphism among *Meloidogyne incognita* populations. *Journal of Nematology* 23: 316-320.

CATAGNONE-SERENO, P., PIOTTE, P., UIJTHOFF, J., ABAD, P., WAJNBERG, E., VANLERBERGHE-MASUTTI, M., BONGIOVANNI, M. & DALMASSO, A. 1993. Phylogenetic relationships between amphimictic and parthenogenetic nematode of the genus *Meloidogyne* inferred from repetitive DNA analysis. *Heredity* 70: 195-204.

CATAGNONE-SERENO, P., BONGIOVANNI, M. & DALMASSO, A. 1994. Reproduction of virulent isolates of *Meloidogyne incognita* on susceptible and *Mi*-resistant tomato. *Journal of Nematology* 26 : 324-328.

CAVENESS, F.E. & JENSEN, H.J. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. *Proceedings of the Helminthological Society of Washington* 22: 87-89.

CENIS, J.L. 1993. Identification of four major *Meloidogyne* spp. by random amplified polymorphic DNA (RAPD-PCR). *Phytopathology* 83: 76-78.

CERKAUSKAS, R. 2004. Root-knot nematodes. AVRDC-The World Vegetable Center. [Available on internet:] www.avrdc.org [Access date: 19/06/06]

CHARCHAR, J.M., GONZAGA, V., GIORDANO, L.D., BOITEUX, L.S.,DOS REIS, N.V.B. & ARAG□O, F.A.D. 2003. Rea□o de Cultivares de Tomateiro

Infec □o por Popula□o Mista de *Meloidogyne incognita* ra□a 1 e *M. javanica* em Estufa Pl□stica e Campo. *Nematologia Brasileira* 27 (1): 49-54.

CHIKWAMBA, R. 2002. Comments on Arntzen and Mahoney's paper. http://listproc.vcdavis.edu/archives/vaccine/log0210/000/htm [Access date: 19/06/06]

COBB, N. A. 1918. Estimating the mean population of the soil. Agricultural Technical Circular Bureau of Plant Industries. United States Department of Agriculture. 1: 48.

COOK, R. & EVANS, K. 1987. Resistance and tolerance. *In*: Principles and Practice of Nematode Control in Crops. (eds: R.H. Brown & B.R. Kerry). Academic Press, London. pp 179-231.

COOK, R. & STARR, J.L., 2006. Resistant cultivars. *In*: Plant Nematology. (eds: R.N. Percy & M. Moens). CAB International, London. pp 370-389.

DAIBER, K.C. 1985. Tomato pests in South Africa, their potential damage and their control. *Horticultural Science* 2: 1-5.

DECRAEMER, W. & HUNT, D.J. 2006. Structure and classification. *In*: Plant Nematology. (eds. R.N. Perry & M. Moens). CAB International, UK. pp 4-32.

DeGUIRAN, G. & RITTER, M. 1979. Life cycle of *Meloidogyne* species and factors influencing their development. *In*: Root-knot nematodes (*Meloidogyne* species). Systematics, biology and control. (eds: F. Lamberti & C.E. Taylor). Academic press, London. pp. 173-191.

DILLARD, H.R., BELLINDER, R.R. & SHAH, D.A. 2004. Integrated Management of weeds and diseases in a cabbage cropping system. *Crop Protection* 23(2): 163-168.

DI VITO, M., CIANCIOTTA, V. & ZACCHEO, G. 1991. The effect of population densities of *Meloidogyne incognita* on yield of susceptible and resistant tomato. *Nematologia Mediterranea* 19: 265-268.

DROPKIN, V.H. 1980. Introduction to Plant Nematology. A Wiley-Interscience Publication, USA.

DROPKIN, V. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: reversal by temperature. *Phytopathology* 59: 1632-1637.

DUKE, J.A. 1983. *Phaseolus vulgaris* L. Handbbok of Energy Crops. Unpublished. www.hort.perdue.edu/newcrop/duke_energy/Phaseolus_vulgaris.html [Access date: 19/06/06]

EISENBACK, J.D. 1985. Detailed morphology and anatomy of second-stage juveniles, males and females of the genus *Meloidogyne* (root-knot nematodes). In: Advanced treatise on *Meloidogyne*. Volume 1. Biology and Control. (eds: J.N. Sasser & C.C. Carter.). North Caroline State University Graphics, North Carolina, USA. pp 47-78.

EL-SHERIF, A.G. 1991. Interaction between *Meloidogyne incognita* and *Agrobacterium tumefaciens*, and *Fusarium oxysporum f.sp. lycopersici* on tomato. *Journal of Nematology* 23: 239-242.

EPPS, J.M., YOUNG, L.D. & HARTWIG, E.E. 1981. Evaluation of nematicides and resistant cultivars for control of soybean cyst nematode. *Plant Disease* 65: 665-666.

FAO. 2004. www.fao.org [Access date: 19/06/06]

FARGETTE, M., PHILLIPS, M.S., BLOK, V.C., WAUGH, R. & TRUDGILL, D.L. 1996. An RFLP study of relationships between species, populations and resistance-breaking lines of tropical species of *Meloidogyne*. *Fundamental and Applied Nematology* 19 (2): 193-200.

FASSULIOTIS, G., DEAKIN, J.R. & HOFFMAN, J.C. 1970. Root-knot nematodes resistance in snap beans: breeding and nature of resistance. *Journal of American society of Horticultural Science* 95 (5): 640-645.

FERRIS, H. 1978a. Development of nematode damage functions and economic thresholds using *Meloidogyne incognita* on tomatoes and sweet potatoes. *Journal of Nematology* 10: 286-287.

FERRIS, H. 1978b. Nematode economic threshold: Derivation, requirements and theoretical considerations. *Journal of Nematology* 10: 341-349.

FERRAZ, L.C.C.B. & BROWN, D.J.F. 2002. An Introduction to nematodes. Plant Nematology. A student's textbook. Pensoft Publishers, Sofia, Bulgaria. pp 221.

FOURIE, H. 2005. *In vivo* evaluation of resistenc to *Meloidogyne incognita* race 2 (Nematoda: Tylenchida) and identification of genetic markers for this trait in soybean (*Glycine max*). Dissertationes de Agricultura. Katholieke Universiteit Leuven.

FOURIE, H. & Mc DONALD, A. 2000. Nematodes. Crop Protection Series No. 18. ARC-Grain Crops Institute. Potchefstroom, South Africa.

FOURIE, H., ZIJLSTRA, C. & Mc DONALD, A.H. 2001. Identification of root-knot nematode species occuring in South Africa using the SCAR-PCR technique. *Nematology* 3 (7): 675-680.

FRANCE, R.A. & ABAWI, G.S. 1995. Interaction between *Meloidogyne incognita* and *Fusarium oxysporum f.sp. phaseoli* on selected bean genotypes. *Journal of Nematology* 26: 464-474.

FRANKLIN, M. 1979. Economic importance of *Meloidogyne* spp. in temperate climates. *In*: Systematics, Biology and Control. (eds: F. Lamberti, C.E. Taylor). Academic Press, London, UK. pp 331-339.

GILBERT, J.C. & McGUIRE, D.C. 1956. Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. *American Society of Horticulture Science Proceedings* 68: 437- 442.

GRIFFIN, G.D. & RUMBAUGH, M.D. 1996. Host suitability of twelve leguminosae species to populations of *Meloidogyne hapla* and *M. chitwoodi*. *Journal of Nematology* 28 (3): 400-405.

GUIMARÃES, L.M.P., De MOURA, R.M. & PEDROSA, E.M.R. 2001. Efeito de Alta Temperatura do Solo na Interação Nematóide-Planta em Cultivares de Tomateiro Resistentes à *Meloidoginose*. *Nematologia Brasileira* 25 (2): 185-189.

HADISOEGANDA, W.W. & SASSER, J.N. 1982. Resistance of tomato, bean, Southern pea and garden pea cultivars to root-knot nematodes based on host suitability. *Plant Disease* 66: 145-150.

HALL, R. 1991. Bean Diseases. In: Compendium of Bean Diseases. (ed: R. Hall). APS Press. pp 4-5.

HANAN, J.J.H. 1998. Greenhouses: Advanced technology for protected horticulture. CRC Press, Boca, Raton, Florida.

HARTMAN, K.M. & SASSER, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential host treatise and perineal-pattern morphology. *In*: Advanced Treatise on *Meloidogyne*. Volume 11 Methodology. (eds: K.R. Barker, C.C. Carter, J.N., Sasser). North Carolina University Graphics, Raleigh, North Carolina: 69-78.

HEYNS, J. 1971. A guide to the plant and soil nematodes of South Africa. Pretoria. South Africa. pp 91-92.

HORBER, E. 1980. Types and classification of resistance. *In*: Breeding plant resistance to insects. (eds: F.C. Maxwell & P.R. Jennings). Wiley. pp 683.

HOOKS, C.R.R. & JOHNSON, M.W. 2003. Impact of agricultural diversification of the insect community of cruciferous crop. *Crop Protection* 22 (2): 223-238.

HUNT, W. 2000. A head of cabbage and the Irish potato. PersonalMD.com. [Available on internet:] http://www.personalmd.com/news/cabbage_potato_031700.shtml [Access date: 27/03/2006]

HUSSEY, R.S. & BOERMA, H.R. 1981. A greenhouse screening procedure for root-knot nematode resistance in soybeans. *Crop Science* 21: 794-796.

HUSSEY, R.S. & JANSSEN, G.J.W. 2002. Root-knot nematodes: *Meloidogyne* species. *In*: Plant Resistance to Parasitic Nematodes. (eds: J.L. Starr, R., Cook, J., Bridge). CAB International, Wallingford, UK. pp 43-70.

HUSSEY, R.S. & McGUIRE, J.M. 1987. Interaction with other organisms. *In*: Principles and practice of nematode control in crops. (eds: R.H. Brown & B.R. Kerry). Academic Press Inc, London. pp 293-328.

HUSSEY, R.S. & WILLIAMSON, V.M. 1998. Physiological and molecular aspects of nematode parasitism. *In*: Plant and nematode interactions. (eds: K.R. Barker, G.A. Pederson & G.L. Windham). Maison, Wisconson, USA. pp 87-108.

IBRAHIM, A.A.M. & IBRAHIM, I.K.A. 2000. Evaluation of non-chemical treatments in the control of *Meloidogyne incognita* on common bean. *Pakistan Journal of Nematology* 18 (1 & 2): 51-57.

JACQUET, M., BONGIOVANNI, M., MARTINEZ, M., VERSCHAVE, P., WAJNBERG, E. & CASTAGNONE-SERENO, P. 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. *Plant Pathology* 54: 93-99.

JENSEN, H.J. 1972. Nematode pests of vegetable and related crops. *In*: Economic Nematology. (ed: J.M. Webster). Academic Press Inc. London. pp 377-408.

JEPSON, S.B. 1987. Identification of root-knot nematodes. Wallingford, UK, CAB International. pp 256.

JOHNSON, A.W. & FASSULIOTIS, G. 1984. Nematode parasites of vegetable crops. *In*: Plant and Insect Nematodes. (ed: W.R. Nickle). Marcel Dekker Inc. New York. pp 323-372.

KAPLAN, M. & NOE, J.P. 1993. Effects of chicken-excrement amendments of *Meloidogyne arenaria*. *Journal of Nematology* 25: 71-77.

KEETCH, D.P. & BUCKLEY, N.H. 1984. A check-list of the plant-parasitic nematodes of Southern Africa. Technical Communication No. 195, Department of Agricultural Development, South Africa. pp 12-71.

KEETCH, D.P. & MILNE, D.L. 1982. The control of plant-parasitic nematodes. *In*: Nematology in Southern Africa. (eds: D.P. Keetch & J. Heyns).Science Bulletin No. 400. pp 113-129.

KLEYHANS, K.P.N. 1991. The root-knot nematodes of South Africa. Technical Communication No. 231, Department of Agricultural Development, South Africa.

KLEYNHANS, K.P.N., VAN DEN BERG, E., SWART, A., MARAIS, M. & BUCKLEY, N.H. 1996. Plant nematodes in South Africa. Plant Protection Research Institute Handbook. No. 8. Business Print. SA. pp 165.

LAMBERTI, F. 1979. Economic importance of *Meloidogyne* spp. in subtropical and Mediterranean climates. *In*: Systematics, Biology and Control. (eds: F. Lamberti, C.E. Taylor). Academic Press, London, UK. 341-357.

LAURIE, S. 1996. History and Importance of Tomato. Vegetable and Ornamental Plant Institute. RSA. Pretoria. pp 414-415.

LIEBANAS, G. & CASTILLO, P. 2004. Host suitability of some crucifers for root-knot nematodes in Southern Spain. *Nematology* 6 (1): 125-128.

LOUW, A., MADEVU, H., JORDAAN, D. & VERMEULEN, H. 2004. Southern Africa–local country studies. The South African Tomato Industry. http://www.regoverningmarkets.org/en/producers [Access date: 27/03/2006]

LUC, M., SIKORA, R.A. & BRIDGE, J. 1993. Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Aberystwyth, UK. pp 629.

LUC, M., BRIDGE, J. & SIKORA, R.A. 2005. Reflections on Nematology in Subtropical and Tropical Agriculture. In: Plant-parasitic nematodes in subtropical and tropical agriculture. 2nd ed. (eds: M. LUC, R.A. SIKORA, J., BRIDGE). CAB International. UK. pp 1-10.

LUZZI, B.M., BOERMA, H.R. & HUSSEY, R.S. 1987. Resistance to three species of root-knot nematode in soybean. *Crop Science* 27: 258-262.

MADULU, J.D.,TRUDGILL, D.L. & PHILLIPS, M.L. 1994. Rotational management of *Meloidogyne javanica* and effects on *Pasteuria penetrans* and tomato and tobacco yields. *Nematologica* 40 (3): 438-455.

MAGGENTI, A. 1981. General morphology. Springer-Verlag, New York. USA. pp 9-156.

MANI, A. & ZIDGALI, T.A. 1995. Screening of tomato cultivars for resistance against *Meloidogyne incognita*. *Nematologia Mediterranea* 23: 269.

MEDINA-FILHO, H.P. & TANKSLEY, S.D. 1983. Breeding for nematode resistance. *In*: Handbook of plant cell culture. (eds: D.A. Evans, W.R. Ammirato & P.V. Yamaday). Macmillian, New York. 1: 904-923.

McCREIGHT, J.D. 1996. Botany and culture. *In*: Compendium of cucurbits diseases. (eds: T.A. Zitter, D.L. Hopkins, & C.E. Thomas). APS Press. USA.

McLEOD, R.W., KIRKEGAARD, J.A. & STEEL, C.C. 2001. Invasion, development, growth and egg laying by *Meloidogyne javanica* in Brassicaceae crops. *Nematology* 3 (5): 463-472.

McSORLEY, R. & DICKSON, D.W. 1995. Effect of tropical rotation crops on *Meloidogyne incognita* and other plant-parasitic nematodes. *Journal of Nematology* 27 (S) (4): 535-543.

MICHAELS, T.E. 1991. The Bean Plant. *In*: Compendium of Bean Diseases. (ed: R. Hall). APS Press, USA. pp 1-4.

MKANDAWIRE, A.B.C., MABAGALA, R.B., GUZMAN, P. GEPTS, P. & GILBERTSON, R. L. 2004. Genetics Diversity and Pathogenic Variation of Common Blight Bacteria (*Xanthomonas campestris* pv. *Phaseoli* and *X. campestris* pv. *Phaseoli* var. *fuscans)* suggests Pathogen Coevolution with the Common Bean. *Bacteriology* 94 (6): 593-603.

MURRAY, J.J. POOLE, T.E. & OSTAZESKI, S.A. 1986. Techniques for determining reproduction of *Meloidogyne graminis* on Zoysiagrass and Bermudagrass. *Plant Disease* 70: 559-560.

NETSCHER, C. & SIKORA, R.A. 1993. Nematode parasites of vegetables. *In*: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. (eds: M. Luc, R.A. Sikora, J. Bridge). CAB International, Wallingford, UK. pp 237-238.

NGUNDO, B.W. 1977. Screening of bean cultivars for resistance to *Meloidogyne* species in Kenya. *Plant Disease Reporter* 61: 991-993.

NGUNDO, B.W. & TAYLOR, D.P. 1974. Effects of *Meloidogyne* species on beans yields in Kenya. *Plant Disease Reporter* 58 (11): 1020-1023.

NIBLACK, T.L., HUSSEY, R.S. & BOERMA, H.R. 1986. Effects of enviroment, *Meloidogyne incognita* inoculum levels and *Glycine max* genotype on root-knot nematode soybean interaction in field microplots. *Journal of Nematology* 18: 338-346.

NONNECKE, L. 1989. Vegetable production. Van Nostrand Reihold Publication. New York. pp 21-373.

NONO-WOMDIM, R., SWAI, I.S., MROSSO, L.K., CHADHA, M.L. & OPENA, R.T. 2002. Identification of root-knot nematode species occuring on tomatoes in Tanzania and resistant lines for their control. *Plant Disease* 86 (2): 127-130.

OMWEGA, C.O., THOMASON, I.J., ROBERTS, P.A. & WAINES, J.G. 1989. Identification og new sources of resistance to root-knot nematodes in *Phaseolus*. *Crop Science* 29: 1463-1467.

OOSTENBRINK, M. 1972. Evaluation and integration of nematode control methods. *In*: Economic Nematology. (ed: J.M. Webster). Academic Press Inc, London. pp 497-514.

ORNAT, C., VERDEJO-LUCAS, S. & SORRIBAS, F. 2001. A population of *Meloidogyne javanica* in Spain virulent to the resistant gene in tomato. *Plant Disease* 85: 271-276.

OVERMAN, A.J. 1991. Diseases caused by nematodes. *In*: Compendium of tomato diseases. (eds: J.B. Jones, J.P. Jones, R.E. Stall & T.A. Zitter). APS Press. USA. pp 49-52.

PACHICO, D. 1993. The demand for bean technology. *In*: Trends in CIAT commodities 1993. (ed: G. Henry). CIAT, Colombia. pp 60-73.

PAGE, L.J. & BRIDGE, J. 1993. Plant nematodes and sustainability in tropical agriculture. *Experimental Agriculture*. 29 (2): 139-154.

PAINTER, R.H. 1951. The economic value and biological significance of insects resistance in plants. *Journal of Economic Entomology* 34: 358-367.

PEET, M. 2001. Nematode Management. NCSU. [Available on internet:] http://www.cals.ncsu.edu/sustainable/peet/Ipm/nematodes/c06nematodes.html [Access date: 19/06/06]

PIERCE, L.C. 1987. VEGETABLES. Characteristics, production, and marketing. John Wiley & Sons, Inc, USA. pp 208-381.

PIOTTE, C., CASTAGONE-SERENO, P., UIJTHOF, J., ABAD, P., BONGIOVANNI, M. & DALMASSO, A. 1992. Molecular characterization of species and populations of *Meloidogyne* from various geographic origins with repeated-DNA homologous probes. *Fundamental and Applied Nematology* 15 (3): 271-276.

POTTER, J.W. & OLTHOF, T.H.A. 1993. Nematode pests of vegetable crops. *In*: Plant parasitic nematodes in temperate agriculture. (eds: K. Evans, D.L. Trudgill & J.M. Webster). Cambridge: University Press. pp 171-208.

POWERS, T.O. & HARRIS, T.S. 1993. A polymerase chain reaction method of identification of five major *Meloidogyne* species. *Journal of Nematology* 25: 1-6.

POWERS, T.O., PLATZER, E.G., & HYMAN, B.C. 1986. Species-specific restriction site polymorphism in root-knot nematode mitochondrial DNA. *Journal of Nematology* 18: 288-293.

RIEKERT, H.F. 1995. An adapted method for extraction of root-knot nematodes from maize root samples. *African Plant Protection* 1: 41-43.

ROBERTS, P. 1990. Resistance to nematodes: Definitions, concepts and concequences. *In*: Methods for evaluating plant species for resistance to plant-parasitic nematodes. (ed: J.L. Starr). The Society of Nematology, Maryland, USA. 1-15 pp.

ROBERTS, P.A. 1992. Current status of the availability, development and use of host plant resistance to nematodes. *Journal of Nematology* 24 (2): 213-227.

ROBERTS, P.A. 1994. Integration and protection of novel management strategies. *Fundamental and Applied Nematology* 17(3): 203-210.

ROBERTS, P.A. 2002. Concepts and Consequences of Resistance. *In*: Plant Resistance to Parasitic Nematodes. (eds: J.L. Starr, R. Cook & J. Bridge). CAB International, Wallingford, UK. pp 23-41.

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

ROBERTS, P.A., MATTHEWS, J.R., W.C. & EHLERS, J.W. 2005. Root-knot nematode resistant cowpea cover crops in tomato production systems. *Agronomy Journal* 97: 1626-1635.

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

ROSA, E.A.S., HEANEY, R.K., FENWICK, G.R. & PORTAS, C.A.M. 1997. Glicosinalates in crop plants. *Horticultural Reviews* 19: 99-215.

SEKHAR, N.S. & GILL, J.S. 1990. Penetration and multiplication of *Meloidogyne incognita* as influenced by *Pasteuria penetrans*. *Indian Journal of Nematology* 20 (2): 213-218.

SHARMA, R.D, DE ARAUJO, V. I., CAVALCANTE, M.D.B. & GOMEZ, A.D. 2005. Reaction of long pepper genotypes to *Meloidogyne javanica*, *M. incognita* race 1 and *Rotylenchus reniformis*. *Nematologia Brasileira* 29 (1): 83-86.

SHEPHERD, R.L. 1979. A quantitative technique for evaluating cotton for root-knot nematode resistance. *Phytopathology* 69: 427-430.

SIKORA, R.A. & FERNANDEZ, E. 2005. Nematode Parasites of Vegetables*. *In*: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. 2nd edition. (eds: M. Luc, R.A. Sikora & J. Bridge). CAB International, Wallingford, UK. pp 319-392.

SIKORA, R.A. & GRECO, N. 1993. Nematode Parasites of Food Legumes. *In*: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. (eds: M. Luc, R.A. Sikora & J. Bridge). CAB International, Aberystwyth, UK. pp 181-235.

SIKORA, R.A., GRECO, N. & SILVA, J.F.V. 2005. Nematode Parasites of Food Legumes*. *In*: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture-2nd edition. (eds: M. Luc, R.A. Sikora & J. Bridge). CAB International, Wallingford, UK. pp 259-318.

SIKORA, E., HAGAN, A., KEMBLE, J. & GAZAWAY, W. 2000. Nematode control in the home vegetable garden. Aces Publication. http://www.aces.edu/pubs/docs/A/ANR-0030/[Acess date: 19/06/06]

SINGH, S.P. 2001. Broadening the genetic base of common bean cultivars: A review. *Soil Science* 41: 1659-1675.

STARR, J.L., COOK, R. & BRIDGE, J. 2002. Resistance to Plant-parasitic Nematodes: History, Current Use and Future Potential. *In*: Plant Resistance to Plant Parasitic Nematodes. (eds: J.L. Starr, R. Cook & J. Bridge). CAB International, Wallingford, UK. pp 1-22.

STIRLING, G.R. & STIRLING, A.M. 2003. The potential of *Brassica* green manure crops for controlling root-knot nematode (*Meloidogyne javanica*) on horticutural crops in a subtropical environment. *Australian Journal of Experimental Agriculture* 43: 623-630.

SUBRAMANIYAN, S., RAGENDRAN, G. & VADIVELU, S. 1990. Estimation of loss in tomato due to *Meloidogyne incognita* and *Rotylenchus reniformis*. *Indian Journal of Nematology* 20 (2): 239-240.

SWANSON, T.A. & VAN GUNDY, S.D. 1984. Variability in reproduction of four races of *Meloidogyne incognita* on two cultivars of soybean. *Journal of Nematology* 16: 368-371.

SYDENHAM, G.M., McSORLEY, R. & DUNN, R.A. 1997. Effects of temperature on resistance in *Phaseolus vulgaris* genotypes and on development of *Meloidogyne* Species. *Journal of Nematology* 29 (1): 90-103.

TAYLOR, A.L. & SASSER, J.N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University Graphic, Raleigh NC, USA. pp 111.

THIES, J.A. 1996. Diseases caused by nematodes. *In*: Compendium of cucurbits diseases. (eds: T.A. Zitter, D.L. Hopkins & C.E. Thomas). APS Press. USA. pp 56-57.

THOMASON, I.J. & CASWELL, E.P. 1987. Principles of nematode control. *In*: Principles and practice of nematode control in crops. (eds: R.H. Brown, B.R. Kerry). Academic Press, Inc, Australia. pp 87-124.

TIGCHELAAR, E.C. 1991. Tomato diseases. *In*: Compendium of tomato diseases. (eds: J.B. Jones, J.P. Jones, R.E. Stall & Zitter, T.A). APS Press. USA. pp 2-4.

TIWARI, K.N., SINGH, A. & MAL, P.K. 2003. Effect of drip Irrigation on yield of cabbage (*Brassica oleracea*) under mulch and non-mulch condition. *Agricultural Water Management* 58 (1): 19-28.

TZORTZAKAKIS, E.A.,TRUDGILL, D.L. & PHILLIPS, M. 1998. Evidence for a dosage effect of *Mi* gene on partially virulent isolates of *Meloidogyne javanica*. *Journal of Nematology* 30: 76-80.

VAN ARK, H. 1981. Eenvoudige biometriese tegnieke en proefontwerpe met spesiale verwysing na entomologiese navorsing. Wetenskaplike pamflet. No 396. Departement Landbou en Visserye, Republiek van Suid Afrika. Pretoria: Staatsdrukker. pp 117.

VAN DER WAL, A.F. 1999. Vegetables and their nematodes. Integrated Nematode Management and Damage Control. University of Natal. Pietermaritzburg. pp 250-284.

WEBSTER, J.M. 1972. Nematodes and biological control. *In*: Economic Nematology. (ed: J.M. Webster). Academic press. London. pp 469-496.

WINDHAM, G.L. & WILLIAMS, W.P. 1987. Host suitability of commercial corn hybrids to *Meloidogyne arenaria* and *Meloidogyne incognita*. *Journal of Nematology* 19 (S): 13-16.

WINDHAM, G.L. & WILLIAMS, W.P. 1988. Reproduction of *Meloidogyne javanica* on corn hybrids and inbreds. *Annals of Applied Nematology* 2: 25-28.

WORTMANN, C.S. & ALLEN, D.J. 1994. African bean production environments: their definition, characteristics and constraints. Network on Bean Research in Africa Occasional Publication Series, no. 11. CIAT, Kampala, Uganda. pp 47.

XU, J., NARABU, T., MIZUKUBO, T. & HIBI, T. 2001. A molecular marker correlated with selected virulence against the tomato resistance gene *Mi* in *Meloidogyne incognita*, *M. javanica,* and *M. arenaria*. *Nematology* 91 (4): 377-382.

XUE, B., BAILLIE, D.L., BECKENBACH, K. & WEBSTER, J.M. 1992. DNA hybridization probes for studying the affinites of three *Meloidogyne* populations. *Fundamental and Applied Nematology* 15: 35-41.

YOUNG, L.D. 1992. Problems and strategies associated with long-term use of nematode resistance cultivars. *Journal of Nematology* 24 (2): 228-233.

ZIJLSTRA, C. 2000. Identification of *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* based on SCAR-PCR: a powerful way of enabling reliable identification of populations or individuals that share common traits. *European Journal of Plant Pathology* 106: 283-290.

ZIJLSTRA, C., DONKERS-VENNE, D.T.H.M. & FARGETTE, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR. *Nematology* 2: 847-853.

ZITTER, T.A. 1996. Cucurbit Diseases. *In*: Compendium of cucurbits diseases. (eds: T.A. Zitter, D.L. Hopkins, D.L. & C.E. Thomas). APS Press. USA. pp 1-2.