

# Chapter 7

## Alternative Nematode Management Strategies

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### 7.1 Introduction

South Africa (SA) has approximately nearly 56 million inhabitants of which 48 % live in rural areas (Anonymous 2016). A large portion (35 %) of this rural population lives below the poverty line (Labadarios et al. 2012). For their food, most of these communities depend on vegetables as well as grain and leguminous crops produced mainly in household or communal gardens (Fig. 7.1) (Fourie and Schoeman 1999; Van der Berg 2006; Aliber 2009; Coyne et al. 2009; Fourie et al. 2012; Ntidi et al. 2012). Available land is often limited and, therefore, frequently reused, which aggravates soil degradation as well as soil disease and pest problems (Van der Berg 2006; Aliber 2009). All these factors have a direct and negative effect on food security and cash income (Aliber 2009; Ntidi et al. 2012).

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**Fig. 7.1** A typical setup of intercropping by smallholding farmers in South Africa, with most of the crops being good hosts to plant-parasitic nematodes (Johnnie van den Berg, North-West University, Potchefstroom, South Africa)

In a commercial agricultural production, more than 10 % of crop yields can be lost due to diseases and pests, including plant-parasitic nematodes (Keetch 1989; Kleynhans et al. 1996; Sikora et al. 2005). However, in rural areas these percentages are much higher, and, in some circumstances, diseases and pests can cause total crop failures (Kleynhans et al. 1996; Sikora et al. 2005; Coyne et al. 2009).

During the past decade, substantial crop losses as a result of nematode infection were frequently reported from the local smallholding farming sector. A preliminary survey was conducted in 1999–2001 in collaboration with provincial governments, selected communities and non-governmental organisations (Mtshali et al. 2002a). The survey revealed that in 49 out of 51 rural and peri-urban home, community and school gardens in the Eastern Cape, North-West, Limpopo, Mpumalanga and KwaZulu-Natal provinces, root-knot nematodes (*Meloidogyne* spp.) were the major biotic constraint for food production. This was especially the case in gardens where vegetables were the primary food crop grown.

Numerous surveys have shown that many crops, as well as weeds and non-food crops occurring in and around crop fields, grown by rural and peri-urban communities are highly susceptible to nematode pests and can maintain exceptionally high population densities of particularly *Meloidogyne* spp. (Fourie and Schoeman 1999; Mtshali et al. 2002a; Tefu et al. 2005; Marais and Swart 2007; Fourie et al. 2012; Ntidi et al. 2012). In many cases, especially in locations where root-knot nematodes occurred in high population densities, the production of vegetables had to be abandoned despite acceptable cultivation practices by the farmers (Mtshali et al. 2002a; Ntidi et al. 2012). In rural areas, communities and households depend on these crops for their food security, and the adverse impact caused by plant-parasitic nematodes on the livelihood of the rural population is, therefore, considerable (Fourie and Schoeman 1999; Mtshali et al. 2002a; Coyne et al. 2009; Ntidi et al. 2012).



**Fig. 7.2** In South Africa, as in most African countries, the awareness of smallholding farmers of the presence and damage caused by plant-parasitic nematodes is either totally lacking or very limited (Originally drawn by Koos van Rensburg, Agricultural Research Council–Grain Crops Institute, Potchefstroom, South Africa; Redrawn by Ebrahim Shokoohi, North-West University, Potchefstroom, South Africa)

The nematode problem is further aggravated by the lack of knowledge among smallholding farmers of diseases and pests, including plant-parasitic nematodes (Fig. 7.2). Typically, farmers believe that the damage caused by plant-parasitic nematodes are symptoms of nutrient deficiency, drought or other abiotic harmful factors (Chitwood 2002; Sikora et al. 2005; McSorley 2011; Ntidi et al. 2012). Also, extension officers advising smallholding farmers often have a limited knowledge of nematode pests. Therefore, effective nematode management is impossible without raising the awareness of smallholding farmers and increasing the knowledge of extension officers of nematode problems (Fourie and Schoeman 1999; Mtshali et al. 2002a; Fourie et al. 2012; Ntidi et al. 2012). To this end, already in 2000, a leaflet was produced by the Agricultural Research Council–Grain Crops Institute (ARC–GCI) and disseminated in rural areas (Fourie and Mc Donald 2000).

The use of nematicides to limit the build-up of nematode pest population densities below a damage threshold is, in general, effective but unsuitable for the smallholding farming sector as these chemicals are expensive, usually toxic and hence hazardous to humans, livestock and the environment. In addition, farmers need expensive equipment and protective clothing to apply such chemicals, and a prerequisite is that a secure location is available to store these toxic substances. Therefore, to alleviate the nematode problem, alternative low-input, cost-effective and environmentally friendly management strategies need to be developed and made available. Only this approach will enable smallholding farmers to regain and maintain

acceptable levels of food production and to generate some cash income (Chitwood 2002; Sikora et al. 2005; McSorley 2011).

In SA, research on these alternative nematode management strategies for the smallholding farming sector has focused on:

- (i) The discovery of local botanical nematicides (phytonematicides) and their use as soil amendments to manage root-knot nematodes
- (ii) The use of natural sources of resistance (the ability of a plant to limit nematode reproduction) or tolerance (the ability of a plant to limit nematode damage)
- (iii) The application of crop rotation and intercropping
- (iv) The use of organic amendments
- (v) The use of cover crops as biofumigants

## 7.2 Agricultural Research in South Africa and Government Policies

In SA, agricultural research is strongly influenced by government policies. Agriculture in SA is dualistic in nature since it includes, at the same time, a strong established commercial sector and a large developing sector. This situation is still prominent despite initiatives to uplift the smallholding, developing sector since the implementation of the new democratic dispensation in 1994. Unfortunately, in the beginning, a top-down approach was applied resulting in non-sustainable projects that typically failed. Recently, however, a bottom-up approach is being followed. Today, the policies and strategies of the ARC are guided by the national priorities referred to in Box 7.1.

### **Box. 7.1 National Priorities of the Agricultural Research Council (ARC)**

The ARC is the convenor of a task force on rural development, which is one of the seven Presidential Imperative Programmes. The other six imperatives are job creation, regional integration, urban renewal, human resource development, human immunodeficiency virus - acquired immunodeficiency syndrome (HIV/AIDS) and crime prevention.

The national minister of agriculture together with nine members of the executive committee (MECs), representing the nine provincial departments of agriculture, developed seven priorities for public entities in the agricultural sector to deliver on, viz.:

- (i) Farmer settlement
- (ii) Promotion of sustainable agricultural production of food gardens and opening up opportunities for industrial crop production
- (iii) Supporting agribusiness development aimed at job creation, black economic empowerment and expanding income opportunities by focusing on value-adding and responsible exploitation of indigenous resources

- (iv) Improving the availability of services to support diverse types of farming systems
- (v) Restructuring irrigation schemes in ex-homelands
- (vi) Nurturing of human and natural resources through capacity building and training and implementation of sustainable farming practices
- (vii) Rural development, alleviation of poverty and job creation

The delivery of research priorities of the Department of Agriculture, Forestry and Fisheries (DAFF) must also be in accordance with the policy directives in the White Paper on Agriculture (1995) which are summarised as follows:

- Promoting competitiveness and employment creation
- Enhancing the quality of life
- Developing human resources
- Working towards environmental sustainability
- Promoting an informed society

According to the White Paper on Agriculture (1995), a major constraint in the SA agricultural structure was the unequal distribution of income between commercial and smallholding farmers. Therefore, not only reallocation of land to smallholding farmers needed to be considered and implemented but also appropriate and relevant research methodologies needed to be developed to ensure optimal food production and food security on both national and household/community levels. The White Paper on Agriculture (1995) further listed that one of the main technical problems experienced by the smallholding agricultural sector was a lack of awareness of diseases and pests and, consequently, an inability to manage these production constraints adequately. According to the document, this problem was related to a lack of knowledge and understanding of crop management in general.

Also, according to the White Paper on Agriculture (1995), imperatives applicable to technology development in the smallholding agricultural sector should ensure research to be need driven. Furthermore, a so-called farming systems approach (in which all components of farming, land, labour, capital, etc. are integrated) should be used in collaboration with the extension services and farmers, and research capacity should be expanded to include applied on-farm research.

### **7.3 Plant-Parasitic Nematodes Associated with Crops Grown by Smallholding Farmers in South Africa**

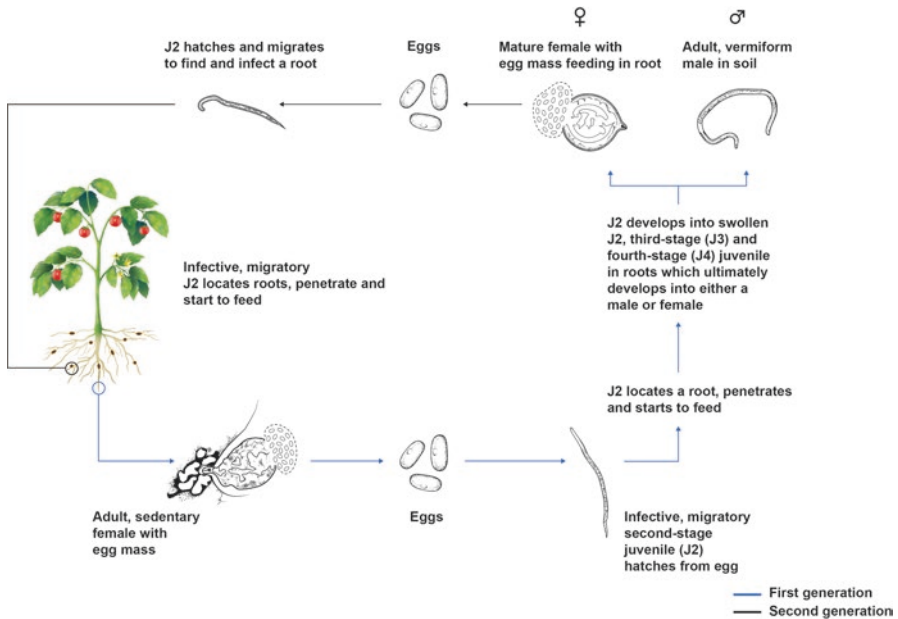
The same nematode pests that are of economic importance in commercial agriculture (see Chaps. 8–19) are also present in the fields of smallholding farmers, resulting in yield and quality losses. The most abundant and damaging genus is *Meloidogyne* (Mtshali et al. 2002a; Daneel et al. 2003; Ntidi et al. 2012).

During a survey in the early 2000s (see Sect. 7.1) in the Mpumalanga, KwaZulu-Natal and North-West provinces, the highest root-knot nematode population density (about 1 million 50 g<sup>-1</sup> roots) was recorded from potato (*Solanum tuberosum*) at Tshetshe near Ventersdorp (Mtshali et al. 2002a). The lowest root-knot nematode population density recorded (from turnip – *Brassica rapa* subsp. *rapa* roots at Bethal near Coligny) was 9,350 g<sup>-1</sup> roots, which is still high. Identification of the root-knot nematode species found during this survey revealed that *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, was predominant, followed by *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. Other root-knot nematode species identified from smallholding farming areas were *Meloidogyne arenaria* (Neal, 1889) Cobb, 1890; *Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley, 1980; *Meloidogyne fallax* Karssen, 1996; and *Meloidogyne hapla* Chitwood, 1949 (Coyné et al. 2009; Ntidi et al. 2012).

Together with *Meloidogyne*, many other plant-parasitic nematode genera identified from smallholding fields and gardens, as well as adjacent areas, were also reported (Tefu et al. 2005; Marais and Swart 2007; Ntidi et al. 2012). Surveys in the Bizana, Lusikisiki and Port St Johns area (Eastern Cape Province), for example, recorded the presence of 11 plant-parasitic nematode families, represented by 27 genera and 105 species (Marais and Swart 2007) on food and non-food crops, and natural vegetation. During these surveys the crops most sampled were maize (*Zea mays*), bean (*Phaseolus* spp.) and soybean (*Glycine max*) (38, 36 and 30 fields, respectively). Various vegetable and fruit crops were also sampled. The genera *Helicotylenchus*, *Meloidogyne*, *Scutellonema* and *Xiphinema* were present in more than 30 % of the localities sampled, whereas *Criconema*, *Criconemoides*, *Discocriconemella*, *Dolichodorus*, *Hemicycliophora*, *Longidorus*, *Nanidorus*, *Ogma*, *Paralongidorus*, *Paratrichodorus*, *Paratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Rotylenchus*, *Trophotylenchulus* and *Tylenchorhynchus* were present to a lesser extent. The genera *Aphelenchoides*, *Ditylenchus*, *Hemicriconemoides*, *Hoplolaimus*, *Radopholus*, *Trichodorus* and *Tylenchulus* were each identified from a single locality only. *Discocriconemella degrissei* Loof and Sharma, 1980, was reported as a first record for SA, while various nematode-plant associations were also reported for the first time. For example, for food crops only, associations of *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961, with butternut squash (*Cucurbita moschata*), chickpea (*Cicer arietinum*), lablab (*Dolichos* sp.), mung bean (*Vigna radiata*), pigeon pea (*Cajanus cajan*), sweet potato (*Ipomoea batatas*) and taro (*Colocasia esculenta*) represented first records for SA. The same applied to *Helicotylenchus martini* Sher, 1966, on maize; *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956, on bean; *Helicotylenchus parapatlyurus* Siddiqi, 1972, on soybean and sweet potato; and *Helicotylenchus serenus* Siddiqi, 1963, on butternut squash.

### 7.3.1 Root-Knot Nematodes

Vegetables, including beetroot (*Beta vulgaris*), brinjal (*Solanum melongena*), peppers (*Capsicum* spp.), spinach (*Spinacia oleracea*) and tomato (*Solanum lycopersicon*), host a wide variety of plant-parasitic nematode species, but predominantly



**Fig. 7.3** Life cycle of *Meloidogyne* (Hannes Visagie, North-West University, South Africa)

*Meloidogyne* spp. (see Chap. 10) (Mtshali et al. 2002a; Sikora and Fernandez 2005; Bridge and Starr 2007; Ntidi et al. 2012). Severe infection of good host crops by root-knot nematodes usually results in significant yield and/or quality loss and in various cases even in total crop failure (Mtshali et al. 2002a; Tefu et al. 2005). Worldwide in tropical and subtropical regions, *M. incognita* and *M. javanica* are the predominant root-knot nematode species on tomato (Nono-Womdim et al. 2002). Both species infect vegetable crops wherever they are grown which may result in substantial yield losses when proper nematode management strategies are not applied (Sikora and Fernandez 2005; Bridge and Starr 2007; McSorley 2011). Yield losses of 20–40 % (Bridge and Starr 2007) and in excess of 50 % (Nono-Womdim et al. 2002) have been reported on tomato because of infection by *Meloidogyne* spp. The ability of root-knot nematodes to produce high numbers of offspring, particularly in warmer areas where they go through several life cycles (Fig. 7.3) in one season, is a major contributing factor to the high crop losses caused by them.

Many smallholding farmers either buy commercial seed or use second- and even third-generation seed for planting vegetables in their home or communal gardens in which nematode pests may thrive. Most of the commercial crop varieties used by these farmers are susceptible to root-knot nematodes (Fourie et al. 2012). Roots of seedlings often become already infected in so-called home nurseries before they are transplanted in the field. The impact of root-knot nematode infection on tomato (and other crops) depends upon many factors, including abiotic and biotic factors, agronomic practices, etc., and therefore can vary considerably among localities (Nono-Womdim et al. 2002; Bridge and Starr 2007; Coyne et al. 2009).

## 7.4 Management Practices for Smallholding Farmers

### 7.4.1 Botanical Nematicides (*Phytonematicides*)

Research on phytonematicides was initiated inter alia to mitigate the drawbacks of conventional organic amendments in suppressing plant-parasitic nematode population densities (Mashela 2002). These drawbacks include:

- (i) Unavailability of the basic materials from which the amendments are derived
- (ii) The rather long period needed for the microbial decomposition of the amendments before they can be applied on the field
- (iii) The need for large quantities of the amendments (ranging from 10-500 metric tonnes (MT) ha<sup>-1</sup>)
- (iv) The high transportation costs to haul the amendments to the field where they will be used
- (v) Excessive lowering of soil pH
- (vi) Inconsistency of the results (Jafee et al. 1994; Bélair and Tremblay 1995; McSorley and Gallaher 1995; Mashela 2002; Kimpinski et al. 2003; Thoden et al. 2011; Stirling 2014)

The basic materials for the development of phytonematicides were usually collected from locally available plants (Muller and Gooch 1982; Akhtar and Malik 2000; Oka 2010; Mashela et al. 2011; Ahmad et al. 2013). The phytochemical compounds of these phytonematicides can be broadly classified as alkaloids, alkamides, carbohydrates, cyanogenic glycosides, fatty acids, glucosinolates, nonprotein amino acids, phenolic compounds (coumarins, flavonoids, phenylpropanoids, tannins), polyacetylenes, polyketides, terpenoids, thiophenes and waxes (Zasada and Ferris 2003; Wuyts et al. 2006; Oka 2010; Okwute 2012; Wink and Van Wyk 2014). Van Wyk et al. (2002) listed 390 plant species endemic to SA that are potentially toxic to humans and animals. For the purpose of this chapter, we can classify these potential toxins in seven groups based on their toxic effects (Table 7.1).

**Table 7.1** Plants with potential toxicities for uses in various industries in South Africa

Toxic effect	Number of plant species	percentage of total number of plant species
Deadly to humans and animals	25	6
Very poisonous to humans and animals	68	17
Poisonous to humans alone	84	22
Poisonous to animals alone	163	42
Poisonous to humans and animals	18	5
Skin allergies or contact dermatitis	18	5
Not poisonous to human and animals	14	4
Total	390	

Adapted from Van Wyk et al. (2002)



Wild cucumber (*Cucumis myriocarpus*) and castor bean (*Ricinus communis*), which produce phytonematicides that can suppress nematode population densities using the ground leaching technology (GLT) system (Mashela 2002; Mashela and Nthangeni 2002), are classified as being poisonous and very poisonous, respectively (Van Wyk et al. 2002). By contrast, crude extracts from oleander (*Nerium oleander*) leaves and tamboti (*Spirostachys africana*) bark did not have nematicidal properties against *Meloidogyne* spp. or the citrus nematode (*Tylenchulus semipenetrans* Cobb, 1913) (Mashela et al. 2011). The bark, twigs and leaves of oleander and tamboti are deadly to both humans and animals (Van Wyk et al. 2002). On the other hand, certain plants listed as not poisonous by Van Wyk et al. (2002), for example, fever tea (*Lippia javanica*) and *Brassica* spp., produce potent nematicides against *Meloidogyne* spp. (Mashela et al. 2007, 2012). The degree of toxicity to humans and animals, therefore, is not an indication that a plant or a plant organ also contains nematicidal substances.

#### 7.4.1.1 Chemical Properties of Cucurbitacin A and B

Cucurbitacins are oxygenated tetracyclic triterpenoids and are the bitterest chemical compounds known to humankind (Chen et al. 2005). Cucurbitacin A ( $C_{32}H_{46}O_8$ ) is unstable and disintegrates rapidly to cucumin ( $C_{27}H_{40}O_9$ ) and leptodermin ( $C_{27}H_{38}O_8$ ) which are both stable compounds (Jeffrey 1978). Cucurbitacin A is concentrated in the roots and fruits of *Cucumis myriocarpus* (Jeffrey 1978), with leaves being used as a vegetable food source (Mashela et al. 2011; Mashela and Dube 2014). By contrast, cucurbitacin B ( $C_{32}H_{48}O_8$ ) is concentrated in all organs of *Cucumis africanus* and is stable (Jeffrey 1978). The chemical is not equally concentrated in all organs, and significantly higher concentrations occur in the fruit (Shadung and Mashela 2016). Cucurbitacins are widely used in African traditional medicine (Mphahlele et al. 2012). Pure cucurbitacins can be highly toxic to animals (Lee et al. 2010).

The efficacy of phytonematicides derived from *Cucumis* spp. in suppressing nematode pest population densities was similar to the efficacy of aldicarb and fenamiphos, which are systemic chemical nematicides (Mashela et al. 2008). *Meloidogyne incognita* eggs exposed to 0–2.5  $\mu\text{g ml}^{-1}$  cucurbitacin A and B dilutions in distilled water exhibited negative curvilinear quadratic relations (Dube et al. 2016). In other words, at low concentrations the cucurbitacins inhibited the hatching of second-stage juveniles (J2), whereas at high concentrations J2 hatching was stimulated. These results confirmed observations by Yu (2015) who reported that *M. incognita* eggs exposed to increasing crude extracts of mustard (*Sinapis arvensis*) levels stimulated the hatching of J2. Hatching of J2 of root-knot nematodes at various concentrations of phytochemicals can be explained on the basis of the extent to which J2 pick up chemical cues and their ‘interpretation’ of these cues. At low concentrations, the chemoreceptors of first-stage juveniles (J1) in eggs may interpret these phytochemicals as being analogous to chemical signals sent by plants entering senescence, resulting in survival stage setting of the nematode juveniles (McSorley 2003). In contrast, at high concentrations the J1 may be tricked to interpret the

phytochemicals as being analogous to chemical signals sent by actively growing plants, thereby resulting in further development of such juveniles and the hatching of the infective J2 in high numbers.

#### 7.4.1.2 Application Through Ground Leaching Technology (GLT)

The ground leaching technology (GLT) system was developed to ameliorate the above-mentioned drawbacks of the use of conventional organic amendments (Mashela 2002). Following an extensive search for botanical materials that would not reduce soil pH when used in small quantities ( $2\text{--}4\text{ g plant}^{-1}$ ) in powdered or fine particulate form, fruits of *C. myriocarpus* and *C. africanus* were observed to fit this requirement. This discovery was followed by in vitro (Muedi 2005) and in vivo (Mashela 2002; Mashela et al. 2008) trials to examine the effects of these materials on *Meloidogyne* spp. and *T. semipenetrans*. Preparations for the development of the GLT system involved collecting plants from fields under cultivation, cutting fruits into small pieces followed by drying of these pieces at  $52\text{ }^{\circ}\text{C}$  for 72 h. Thereafter the dried material was ground to pass through a 1-mm-aperture sieve (Mashela 2002). Drying of the plant material at a lower temperature will result in decay of the fruit pieces, whereas drying at temperatures higher than  $52\text{ }^{\circ}\text{C}$  will result in substantially lower cucurbitacin levels (Shadung et al. 2015).

In granular (G) formulation, the two cucurbitacins are referred to as nemarioc-AG and nemafric-BG, with the first part referring to the cucurbitacin species name and the second part to the formulation type. In tomato production, the granular material is applied at  $2\text{ g plant}^{-1}$  (Fig. 7.4). The material is applied around the crop



**Fig. 7.4** Application of crude material of a phytoneemacide (nemarioc-AG) after transplanting of a tomato seedling according to the ground leaching technology (GLT) system to suppress population densities of plant-parasitic nematodes (Zakheleni Dube, University of Limpopo, Polokwane, South Africa)

seedling at transplanting or postemergence after which it is covered by soil. Not all plant species are suitable for use in a GLT system. *Brassica* spp., which are widely used in biofumigation for nematode management, require microbial degradation first to release the active substance (a.s.) with nematicidal properties (Bello 1998). Along with chilli (*Capsicum* spp.), oleander and tamboti, materials derived from *Brassica* spp. were not effective in suppressing root-knot nematode population densities when used in a GLT system (Thovhakale et al. 2006; Mashela et al. 2012). Generally, in a GLT system, the efficacy of the plant materials to suppress nematode population densities depends upon the solubility of the a.s. to enable its leaching into the soil rhizosphere (Mashela and Nthangeni 2002).

### 7.4.1.3 Application Through Botinematation

Although the GLT system is labour intensive and requires at least two applications per tomato growing season, the system has become popular with the smallholding farmers. In large commercial farming systems, phytonematicides can be applied through drip irrigation, a technology which is being referred to as botinematation (Mashela 2014). By definition, botinematation is using botanicals (phytonematicides) to manage nematode population densities through irrigation. The a.s. is extracted from plant organs through fermenting with anaerobic microorganisms. Technically, fermentation specifically refers to the anaerobic breakdown of glucose through pyruvic acids to lactic acids. To prepare phytonematicides in liquid formulation, the basic inputs are simple (Table 7.2). In Nemaalan, a biologically-derived product developed locally and which exhibits antinematodal properties, the plant materials are used in fresh form (see Sect. 7.4.1.7) (Daneel et al. 2014a), whereas in *Cucumis* spp. fruits are used in ground or granular form (Mashela 2002). Care should be taken to ensure that the entire fermentation process is taking place in an airtight container since a large quantity of gases are released during fermentation. An escape route for the gas should be provided by inserting a small tube into a bottle half filled with water in order to avoid explosions. In an airtight container, the

**Table 7.2** Ingredients of selected phytonematicides, before fermentation, to be used for botinematation

Ingredient	Amount (g) or volume (ml or l) of ingredient added	Nemalan <sup>a</sup>	Nemafric-BL	Nemarioc-AL
Plant material	g	120	40	80
Brown sugar	g	100	100	100
Molasses	ml	300	300	300
EM <sup>b</sup> stock	ml	300	300	300
Chlorine-free H <sub>2</sub> O	l	16	16	16

<sup>a</sup>A biologically-derived product with antinematodal properties developed by the South African leading fresh produce company ZZ2, Polokwane, South Africa; <sup>b</sup>A product based on microorganisms produced by Microzone, Polokwane, South Africa

process is usually completed within 14 days, with the pH of the mixture having declined to at least 3.7 (Mashela et al. 2011; Mashela 2014).

#### 7.4.1.4 Non-phytotoxic Concentrations

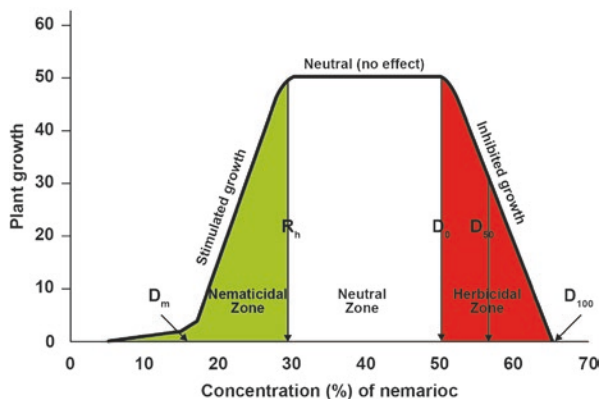
Under in vitro conditions, the use of phytonematicides usually provides consistent results (Mashela et al. 2011; Mashela 2014), but under field conditions most phytonematicides (being allelochemical compounds; Rice 1984) are highly phytotoxic (Mafeo and Mashela 2010; Mafeo et al. 2011a, b). In many countries, as exemplified by the European and Mediterranean Plant Protection Organisation regulations (EPPO 2010), there is a zero tolerance for agricultural inputs that have a phytotoxic effect on crops. Consequently, non-phytotoxic concentrations for each phytonematicide and its application time interval(s) should be empirically established. Generally, at low concentrations cucurbitacins stimulate cell division of the affected plant part (Lee et al. 2010). In a number of studies, it was consistently demonstrated that various plant variables, when subjected to increasing concentrations of phytonematicides, invariably showed quadratic relations which characterise the existence of density-dependent growth (DDG) patterns (Zasada and Ferris 2003; Wuyts et al. 2006; Mafeo and Mashela 2010; Mafeo et al. 2011a, b; 2012; Pelinganga and Mashela 2012; Pelinganga et al. 2012, 2013a, b, c).

#### Curve-Fitting Allelochemical Response Dosage Model

Using data collected to assess the responses of organisms to increasing allelochemical concentrations, Liu et al. (2003) developed a three-phase curve-fitting allelochemical response dosage (CARD) model. The CARD model quantifies three phases (i.e. stimulated, neutral and inhibited phase) and three zones (i.e. nematocidal, neutral and herbicidal zone) using biological indices (Fig. 7.5). Mashela (2014) conceptualised the three phases in terms of plant growth responses to increasing concentrations of phytonematicides using the mean values of the biological indices (Table 7.3). The latter was feasible since indices are numbers without units and could therefore be added and averaged. Mashela et al. (2015) demonstrated that plant responses can either be stimulated, neutral or inhibited, with the degree of the response being dependent on the concentration of the phytonematicides. In the neutral zone, growth of untreated and treated plants can statistically not be differentiated resulting in the conclusion that the phytonematicide has no effect.

#### Mean Concentration Stimulation Point

Using the 50 % inhibition ( $D_{50}$ ) concept, Mashela (2014) investigated the possibility of the existence of its counterpart (50 % stimulation) in the stimulated growth phase, which was possibly situated midway between  $D_m$  and  $R_h$  (Fig. 7.5). The values



**Fig. 7.5** The curve-fitting allelochemical response dosage (CARD) model that quantifies the effect of phytonematicides on plant growth (Mashela 2014)

**Table 7.3** Mean biological indices generated from dry shoot mass (DSM), dry root mass (DRM), plant height (PHT) and stem diameter (SDM) of tomato seedlings over six nemarioc-AL phytonematicide concentrations (Pelinganga et al. 2013a)

Biological index <sup>a</sup>	DSM	DRM	PHT	SDM	Mean
Threshold stimulation ( $D_m$ )	2.53	2.20	2.73	1.53	2.22
Saturation point ( $R_h$ )	0.71	0.32	2.00	0.08	0.77
0 % inhibition ( $D_0$ )	11.48	9.21	19.94	5.42	10.96
50 % inhibition ( $D_{50}$ )	164.90	59.00	2899.74	1603.20	957.17
100 % inhibition ( $D_{100}$ )	703.50	170.30	2902.47	1604.74	1110.84
$k$ (sensitivity ranking)	4	1	2	4	–
Overall sensitivity ranking: $\sum k = 11$					
$P \leq$	0.01	0.01	0.01	0.05	–

<sup>a</sup>All biological indices are without units and can be added to calculate the integrated mean

between the actual  $D_m$  and  $R_h$  define the concentration stimulation range (CSR) which is representative of the concentration range at which plant growth is stimulated (Fig. 7.5). The midpoint of CSR is arbitrarily referred to as the mean concentration stimulation point (MCSP) and can be considered as the concentration that will stimulate plant growth while consistently suppressing nematode population densities (Mashela 2014). Using the relation  $MCSP = D_m + (R_h/2)$ , MCSP values for nemarioc-AL and nemafric-BL were established at 2.63 (Table 7.3) and 2.89 %, respectively (Pelinganga and Mashela 2012; Pelinganga et al. 2013a). A point worth mentioning is that with an MCSP of 2.63 %, application of nemarioc-AL on tomato resulted in a  $D_0$ ,  $D_{50}$  and  $D_{100}$  of 13.96 ( $D_m + R_h + D_0$ ), 971.12 ( $D_m + R_h + D_0 + D_{50}$ ) and 2081.97 % ( $D_m + R_h + D_0 + D_{50} + D_{100}$ ), respectively (Table 7.3). Incidentally, when the MCSP concept is correctly used, it is unlikely that the concentrations of phytonematicides that will suppress nematode population densities will be phytotoxic at the same time. In principle, for every 263 ml nemarioc-AL and 289 ml

nemafri-BL phytonematicide applied, respectively, 10 l of chlorine-free water should be used for the botinematation of a tomato field. However, these values differ for each crop. For African geranium (*Pelargonium sidoides*), MCSP values were 6.2 and 2.9 % for nemarioc-AL and nemafri-BL, respectively (Sithole 2016). Similarly, on *Citrus volkameriana* seedling rootstocks, the values were 8.6 and 6.3 %, respectively (Mathabatha et al. 2016). These values, although they would stimulate plant growth, were rather high when compared to those calculated for tomato (Pelinganga et al. 2012, 2013a, b). In both studies, lower MCSP values than the actual stimulating values consistently suppressed nematode population densities and therefore were adopted instead of the relatively higher empirically derived MCSP values.

The MCSP values should be interpreted alongside the overall  $k$ -values of the phytonematicide on the crop (Table 7.3). Generally, the closer the overall sensitivity index ( $\sum k$ ) of the plant is to zero, the higher the sensitivity of the plant to the phytonematicide and vice versa (Liu et al. 2003). Basically,  $\sum k$  is specific for several factors including the phytonematicide concentration, application rate, plant species and growth phase and nematode life stage. For example, experimental data show that seedlings are less sensitive than mature plants to phytonematicides derived from fruits of *Cucumis* spp. (Mafeo et al. 2011a, b; Pelinganga et al. 2013a, b, c).

#### 7.4.1.5 Application Interval of Phytonematicides

Maile et al. (2013) demonstrated that the response of plants to phytonematicides, in addition to being concentration specific, was application time interval specific as well. Trial results suggested that treatment of tomato plants infected with root-knot nematodes with nemarioc-AL and nemafri-BL should be made at 17- and 19-day intervals, respectively, over a 56-day period (Pelinganga et al. 2013a, c).

#### 7.4.1.6 Dosage in Phytonematicides

Dosage ( $D$ ) is the product of concentration (equivalent to MCSP value) and the application frequency ( $T_f$ ), summarised as  $D (\%) = C (\%) \times T_f$ . The  $T_f$  is the proportion of the crop cycle (days) to the application interval (days) and is constant for a particular crop. For example, when the crop cycles of two tomato varieties were 56 and 112 days,  $T_f$  values for 2.63 % nemarioc-AL would be 3.3 and 6.6, respectively. Therefore, for nemarioc-AL in the 56- and 112-day tomato crop cycles, the dosage would be 8.68 and 17.36 %, respectively. Bearing in mind that MCSP values are empirically derived to avoid phytotoxicity, increasing the concentration, e.g. to 4 %, would not have any added effect on the suppression of the nematode population densities, but would increase the residues of the phytonematicide in the soil, which is undesirable in terms of the soil allelochemical residue (SAR) concept (Mashela and Dube 2014). The SAR concept assesses the effects of soil allelochemical residues of phytonematicides on the following crop(s). It has been shown that

allelochemical residues in the soil had phytotoxic effects on cowpea (*Vigna unguiculata*) as well as inhibitory effects on the development of *Bradyrhizobium japonicum* nodules and residual nematode population densities (Mashela and Dube 2014; Mashela et al. 2012).

#### 7.4.1.7 Phytonematicides Used on a Commercial Scale

Effective application of phytonematicides in large commercial and smallholding farming systems in SA involves the use of crude extracts in granular and liquid formulations (Nzanza and Mashela 2012; Mashela 2014). Plant materials commonly used in the phytonematicide Nemalan include lantana (*Lantana camara*) shoots and wild garlic (*Tulbaghia violacea*) (Daneel et al. 2014a). Fruits from *C. africanus* and *C. myriocarpus* (Mashela 2014) are used in the production of nemafric-BL and nemarioc-AL, respectively (Mashela 2014). The centre of biodiversity of the two *Cucumis* spp. is the Limpopo Province (Kristkova et al. 2003), whereas lantana is an invader plant (Daneel et al. 2014a). Active substances in lantana leaves are saponins and cucurbitacins in fruits of the two *Cucumis* spp. (Van Wyk and Wink 2004). Combining plant organs with different a.s., for example, *C. myriocarpus*, *L. javanica* and *R. communis*, resulted in synergistic effects on the suppression of nematode population densities (Mashela et al. 2007).

#### 7.4.2 Medicinal Plants ('Muti') Used as Phytonematicides

Plant parts (powdered leaf meals) of non-crop plant species used in traditional medicine in SA were selected and examined for their nematicidal activity as soil amendments on *M. incognita* race 2 (Khosa 2013). Parts of these non-crop species are locally known as 'muti' as they are considered to have certain medicinal properties. Traditional healers in SA frequently use these mutis to treat human and domestic animals for various ailments. Living specimens of these plant species, as well as supplies of dried and finely ground material made from them, can be found in abundance in SA in the rural areas and communities of the lowveld in the Mpumalanga, Limpopo and KwaZulu-Natal provinces. These plant species contain chemicals such as alkaloids, diterpenes, diterpenoids, esters, fatty acids, ingenol, oxalic acid and terpenoids. The observed general effects of these traditional medicines on humans at prescribed dosage rates suggested that they might be toxic to small multicellular organisms such as plant-parasitic nematodes.

Nine plant species were identified, collected and examined for their nematicidal activity and plant growth enhancement in glasshouse trials. Five of these plant species, namely, cactus vine (*Cissus cactiformis*), Candelabra tree (*Euphorbia ingens*), Bushveld bead-bean tree (*Maerua angolensis*), Dead-man's tree (*Synadenium cupulare*) and Toad tree (*Tabernaemontana elegans*) were further tested under field conditions (Table 7.4) (Khosa 2013). Soil amendments of powdered leaf meals of

**Table 7.4** Effect of soil amendments of powdered leaf meals of five plant species on the number of root-knot nematode eggs and second-stage juveniles (J2) in roots of tomato grown under glasshouse and field conditions (Khosa 2013)

Source of amendment	Rate (g)	Glasshouse	Field
		No. of eggs and J2 root system <sup>-1</sup>	No. of eggs and J2 root system <sup>-1</sup>
Control (no amendment)	0	4.42 <sup>a</sup> (30,063) <sup>b</sup> a <sup>c</sup>	4.4 (26,800) a
<i>Cissus cactiformis</i>	5	3.20 (1,688) bcd	3.5 (4,267) d
	10	3.20 (1,950) bcd	3.6 (5,867) cd
	15	2.82 (2,975) cd	3.7 (6,933) cd
<i>Euphorbia ingens</i>	5	3.10 (1,838) cd	3.6 (5,067) cd
	10	3.04 (1,350) cd	4.0 (13,600) bc
	15	2.63 (1,113) d	3.4 (2,733) d
<i>Maerua angolensis</i>	5	3.77 (6,688) b	3.7 (8,200) cd
	10	3.79 (6,625) ab	3.6 (5,150) cd
	15	3.37 (3,763) bc	3.7 (7,333) cd
<i>Synadenium cupulare</i>	5	3.25 (2,588) bcd	3.8 (8,200) cd
	10	3.20 (1,838) bcd	3.7 (9,633) cd
	15	3.01 (1,300) cd	3.6 (6,933) cd
<i>Tabernaemontana elegans</i>	5	3.44 (3,588) bc	3.7 (7,067) cd
	10	3.32 (2,275) bc	3.5 (6,200) cd
	15	3.09 (1,375) cd	3.7 (7,300) cd
<i>Cucumis myriocarpus</i>	5	2.86 (2,550) cd	3.4 (4,267) d
Fenamiphos	5	1.17 (138) e	3.3 (4,267) d
LSD	–	0.64	0.45
P-value	–	0.001	0.002
F-ratio	–	7.79	2.59

<sup>a</sup>Log transformed ( $x + 1$ )

<sup>b</sup>Non-transformed means in parenthesis

<sup>c</sup>Means followed by different letters in the same column differ significantly at LSD ( $P \leq 0.05$ )

*C. cactiformis*, *M. angolensis* and *T. elegans* were shown to reduce the number of eggs and J2 of *M. incognita* race 2 in tomato (Khosa 2013). The effect of the leaf meals was similar to that of fenamiphos and the *C. myriocarpus* treatment (Mashela 2002) which was also included in the study.

A previous study showed that ground fruit of *C. myriocarpus* suppressed root-knot nematode population densities under glasshouse, microplot and field conditions (Mashela 2002; Mashela and Mphosi 2002). Tomato plants treated with soil amendments of powdered leaf meals of *C. cactiformis*, *M. angolensis* and *T. elegans* had larger root systems, greater shoot mass and were taller when compared with the untreated control plants. This effect in turn resulted in a higher number of fruit and greater fruit mass. Similar results with botanical soil amendments were obtained by Akhtar (2000), Akhtar and Malik (2000), Mashela and Mpati (2002), Mashela and Nthangeni (2002) and Sikora and Fernandez (2005). Miami and Rodríguez-Kábana (1982) demonstrated that improved plant growth responses could be due to the



absorption of carbon compounds that leached from plant residues into the soil. The soil C:N ratio of the amendments varied, which could explain why plant growth responses were different. Tomato growth response observed in the glasshouse, microplots and field indicated some phytotoxic effects with the applications of *E. ingens* and *S. cupulare*. This was, however, not consistent in all the trials and with all variables measured. These negative effects casted doubt on the possible large-scale use of such phytonematicides as soil amendments in crop production. After screening and further selection, the extracts of *M. angolensis* and *T. elegans* that showed significant nematotoxic and/or growth-stimulating potential are being tested at a more advanced level of chemical separation (MC Khosa, 2016, personal communication).

Possible human, animal and environmental risks of the use of phytonematicides in crop production should be determined, preferably in consultation with the traditional healers that use these materials for medicinal purposes. However, these materials have a potential to be used as soil amendments in integrated nematode management strategies, in domestic garden, smallholding and large commercial farming in SA. Although some of these plants are quite common, e.g. *C. cactiformis*, *M. angolensis* and *T. elegans*, and grow abundantly in the Mopani and Vhembe districts of the Limpopo Province as well as in the Lowveld regions of the KwaZulu-Natal and Mpumalanga provinces, overexploitation of these natural resources should be avoided.

## 7.5 Organic Amendments

As stated above, organic amendments have several drawbacks limiting their use in smallholding farming. Nonetheless, when planting crops in small areas such as home and communal gardens and small fields, the use of organic amendments is an important alternative practice to assist in the management of nematode pests and to increase crop yields. Nematologists of the ARC-Institute for Tropical and Subtropical Crops (ITSC) conducted a series of trials to determine the effects of several pre-planting treatments on natural occurring plant-parasitic nematode complexes (dominated by mixed *M. incognita* and *M. javanica* populations). The treatments represented chicken (45 MT ha<sup>-1</sup>) and cattle manure (45 MT ha<sup>-1</sup>), compost (30 t (m<sup>2</sup>)<sup>-1</sup>), marigold (*Tagetes minuta*), oilcakes of sorghum (*Sorghum bicolor*) and soybean (2–4 kg plot<sup>-1</sup>), plastic cover (solarisation) and permaculture which consists of a pit (about 60 cm deep) filled with waste material from branches, leaves, fruits and weeds (Fig. 7.6). In SA, the main sources of fertiliser used by smallholding farmers include kraal manure, chicken litter, organic waste and compost (Masarirambi et al. 2002). The trials were conducted during 2004–2005 with vegetable/maize/vegetable crop rotation sequences. Maize was planted in all plots, while the same vegetable crops were planted in the original plots in the 2005 season. Vegetables included tomato, spinach and beetroot/green pepper. The application of animal manures resulted in yield increases in both seasons, while nematode

**Fig. 7.6** Permaculture is practised by filling trenches (approximately 2.5–3 m long × 1 m wide × 1 m deep) with different types of organic material (Mieke Daneel, Agricultural Research Council–Institute for Tropical and Subtropical Crops, Mbombela)



control was inconsistent. However, permaculture provided substantial better yields especially in the second season, viz. between 63 and 81 % compared to the untreated plants. Nematode control varied among the different crops being unsatisfactory in especially the tomato crop (Daneel 2007; Tefu et al. 2009). Following these initial results, permacultures with different waste materials were compared mainly to attempt improving nematode control. Permaculture that contained mainly fruit waste materials resulted in the highest vegetable yields and nematode control ranging between 50 and 80 % (Tefu et al. 2009). Further investigation showed that permaculture with citrus fruit gave the best yield and nematode control, and this permaculture is now under further investigation (Tefu et al. 2014). It is important to mention that permaculture is renewed (i.e. the pit filled with fresh waste material) once every 3–4 seasons and not every time a new crop is planted. Also, citrus and other fruits are widely available in the subtropical areas of SA throughout the year. Most smallholding farmers living in the subtropical areas of SA possess one or a few citrus, avocado, guava, mango or other fruit trees in their home gardens (Fig. 7.7). Waste fruits can hence be used for permaculture and contribute towards both higher crop yields and improved nematode management.

Nematologists of the ARC-Grain Crops Institute (ARC-GCI) conducted a series of on-farm (smallholdings) trials during several summer-growing seasons at Coligny, Potchefstroom and Morokweng (North-West Province) and Dingleydale (Mpumalanga Province) to investigate the effect of animal manures, compost and



**Fig. 7.7** Smallholding farms with fruit trees growing in small gardens in the Mpumalanga and Limpopo provinces of South Africa (Mieke Daneel, Agricultural Research Council–Institute for Tropical and Subtropical Crops, Mbombela)

marigold amendments as well as solarisation on root-knot nematode population densities (i.e. single species as well as mixed populations of either *M. incognita* or *M. javanica*) (Fourie and Schoeman 1999; Mtshali et al. 2002a; Ntidi et al. 2012). A 52 % reduction of the root-knot nematode population densities in tomato roots (susceptible cv. Rodade) (Fourie et al. 2012), 60 days after planting, in plots amended with chicken manure (equivalent to 5 MT ha<sup>-1</sup>) was recorded at Potchefstroom. Root-knot nematode population densities after cattle manure amendment (20 MT ha<sup>-1</sup>) were reduced by 28 and 92 % at Potchefstroom and Morokweng, respectively, and 41 and 74 % (45 MT ha<sup>-1</sup>) at Dingleydale and Coligny, respectively. When cattle manure amendment was combined with solarisation, a 99 % reduction in the root-knot nematode population densities was obtained at Morokweng, which was 7 % higher than when cattle manure amendment was applied alone. At Morokweng, the amendment of only compost (equivalent to 20 MT ha<sup>-1</sup>), consisting of residues of various plants grown by the community, as well as a combination of compost and solarisation reduced the root-knot nematode population densities by 90 %. Reductions of 49 and 99 % in the root-knot nematode population densities in roots of maize (cv. SR52), 60 days after planting, in plots amended with cattle manure (equivalent to 45 MT ha<sup>-1</sup>) were observed at Dingleydale and Coligny, respectively.

Unfortunately, yield data could only be obtained at Morokweng where a 370 and 500 % increase in tomato yield was recorded for plots amended with compost and cattle manure, respectively. For the combined treatment of either cattle manure or compost and solarisation, a 17 and 146 % increase, respectively, in tomato yield was obtained. Another amendment tested consisted of shoot cuttings of mature marigold plants (equivalent to 12 MT ha<sup>-1</sup>) growing alongside agricultural fields on the ARC-GCI campus. This treatment resulted in a 71 % reduction in the root-knot nematode population densities 60 days after planting of the tomato plants (cv. Rodade).

## 7.6 Host Plant Resistance

Smallholding farmers buy seeds or seedlings of crops that are readily available. Commercially available varieties of grain, leguminous and vegetable crops were hence obtained from seed companies and evaluated for their host response to *M. incognita* and *M. javanica*. Crops included in these evaluations were *Amaranthus* spp., beetroot, cabbage (*Brassica oleracea* var. capitata), dry bean (*Phaseolus vulgaris*) (Mtshali et al. 2002b), chilli, carrot (*Daucus carota*), cowpea (Riekert 1999), green bean (*Phaseolus* spp.), pumpkin (*Cucurbita* spp.), maize (Ngobeni et al. 2011), spinach and tomato (Riekert 1999; Mothata 2006; Steyn et al. 2013, 2014). A limited number of genotypes were identified with resistance to *M. incognita* race 2, viz. *Amaranthus* sp. accession Local 33 (Steyn et al. 2013), green pepper cv. Tabasco (Steyn et al. 2013) and tomato cvs. Rhapsody, MFH 9324, FA 1454 and FA 593 (Fourie et al. 2012). The superior *M. incognita* resistance in the tomato cv. Rhapsody was further verified in a microplot trial using a range of initial nematode population densities (Pi). The results of this trial showed that final nematode population densities (Pf) and reproduction factor (Rf) values were consistently significantly lower compared to those recorded for the susceptible tomato cv. Moneymaker.

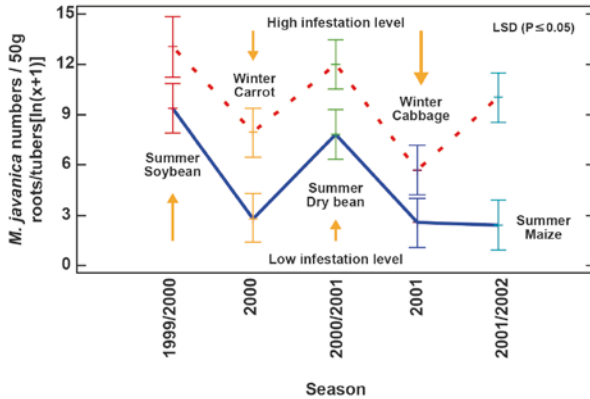
Resistance to *M. incognita* and *M. javanica* was observed in some maize (Ngobeni et al. 2011; see also Sect. 8.2.4.2) and cabbage (Mothata 2006) genotypes and to *M. javanica* in two cowpea lines (T182b-889 and R6A; Riekert 1999). Nonetheless, no root-knot nematode resistance was identified in the beetroot, carrot, green bean, pumpkin and spinach germplasm screened (Mothata 2006; Steyn et al. 2014).

The results of all this research underline the importance of the continuous screening of new crop varieties that enter the local market so that farmers can be updated about the host status of these crops to abundant root-knot nematode species. This up-to-date information about genotypes, together with the accurate identification and monitoring of the root-knot nematode species populations in fields, will enable farmers and extension officers to make informed and thus the best possible decisions regarding nematode management.

## 7.7 Crop Rotation and Intercropping

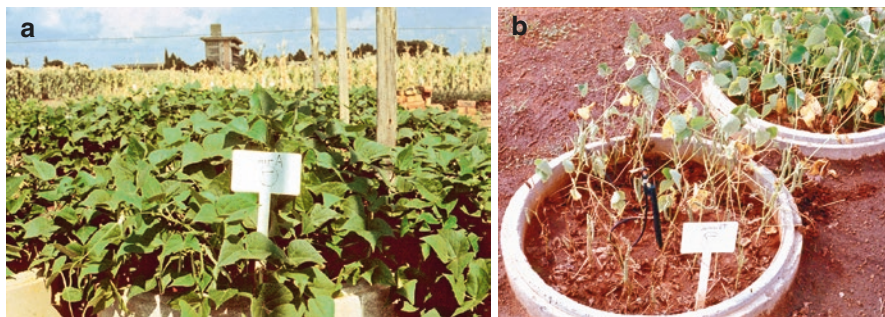
Limited research has been conducted in the smallholding farming sector in particular with regard to the use of crop rotation sequences and intercropping as practices to manage important nematode pests.

The effect of crop rotation on grain, legume and vegetable crops was examined during a long-term on-farm microplot trial carried out on the premises of the ARC-GCI (Venter et al. 2004) over four (consecutive) growing seasons. At the start of the trial, microplots with either a low or high infestation level of *M.*



**Fig. 7.8** The effect of crop rotation over four consecutive growing seasons on *Meloidogyne javanica* population densities (Venter et al. 2004)

*javanica* were created by growing resistant (cv. A7119 - low infestation) and susceptible (cv. Prima2000 - high infestation) soybean cvs in microplots (Fourie et al. 1999, 2006). The following crop sequence was examined: carrot (cv. Chantenay Karoo), dry bean (cv. Mkuzi), cabbage (cv. 3306) and maize (cv. SC701). At the start of the trial, the plants were inoculated with a range of Pi levels (viz. 0, 100, 500, 1000, 5000, 10,000 and 20,000 *M. javanica* eggs and J2 plant<sup>-1</sup>) to study also the effect of pest pressure on nematode reproduction and yield. Final nematode population densities in the rhizosphere and roots were determined for each crop 60 days after nematode inoculation during each of the growing seasons. Data were ultimately pooled over Pi levels to demonstrate the collective value of the crop sequence. The results (Fig. 7.8) showed that the first winter planting of carrot resulted in a significant 99 % decrease (from a Pf of 12,625 eggs and J2 50 g roots<sup>-1</sup> in soybean cv. A7119 to a Pf of 75 in carrot) in *M. javanica* population densities in the low infestation microplots. A similar trend was evident for the high infestation microplots with a 91 % reduction (from a Pf of 268,786 eggs and J2 in soybean cv. Prima2000 to a Pf of 24,033 in carrot). Growing of cabbage during the second winter growing season resulted in a reduction of 54 and 50 % in *M. javanica* population densities in cabbage compared with dry bean grown during the preceding summer season in the low and high infestation microplots, respectively. The high residual Pf in both carrot and cabbage roots in microplots with a high infestation showed that, even in winter, inclusion of these crops is not adequate to reduce the population densities of *M. javanica*. However, the value that the inclusion of winter plantings of carrot and cabbage may have in the reduction of *M. javanica* population densities in fields infested with a lower population density of *M. javanica* was demonstrated. The results also showed that inclusion of dry bean during the second summer season



**Fig. 7.9** (a, b) Healthy dry bean plants (cv. Mkuzi) (a) in microplots where the *Meloidogyne javanica*-resistant soybean cultivar A7119 was grown the previous season compared to stunted plants (b) with chlorotic leaves in plots where the susceptible soybean cultivar Prima was grown (Driekie Fourie, Agricultural Research Council–Grain Crops Institute, Potchefstroom, South Africa)

increased the *M. javanica* population densities to levels comparable to those in the resistant and susceptible soybean cvs at the start of the trial. Interestingly, inclusion of maize during the third summer season kept *M. javanica* population densities in the low infestation microplots similar to those recorded on the preceding cabbage crop. By contrast, in the high infestation microplots inclusion of maize during the third summer season increased the *M. javanica* population densities significantly by 37 %. At the termination of the trial, the four season crop sequence had reduced the *M. javanica* population root densities by 70 and 20 % in low and high infestation microplots, respectively. The yield of dry bean grown in the high infestation microplots was significantly lower compared with the low infestation microplots, but for carrot, cabbage and maize, no yield differences were observed (Fig. 7.9a, b).

The effect of intercropping with *Tagetes erecta* (cv. Lemon Drops) on root-knot nematode population densities was studied in two on-farm experiments at Coligny and Dingleydale (Fourie and Schoeman 1999). The results showed a substantial reduction in nematode numbers in the roots of tomato cv. Rodade (by 57 and 30 % at Coligny and Dingleydale, respectively) and maize cv. SR52 (by 91 % at Dingleydale) when the crops were intercropped with *T. erecta*.

The effect of intercropping on soil and plant fertility, nematode population composition and yield of sugarcane grown in a sandy soil on a smallholding farm was also studied (Berry et al. 2009). Groundnut (*Arachis hypogaea*) and sugar bean (*Phaseolus limensis*) were intercropped between the sugarcane rows in an irrigated experiment, while the same was done with velvet bean (*Mucuna deeringiana*) and sweet potato (*Ipomoea batatas*) in a rain-fed experiment. These practices were compared with a standard nematicide (aldicarb) treatment and an untreated control. Intercropping with velvet bean, groundnut and sweet potato increased the population densities of *M. javanica* and *Pratylenchus zaei* Graham, 1951, in sugarcane sett roots. By contrast, intercropping with sugar bean reduced the nematode numbers. Intercropping with velvet bean, sugar bean and sweet potato had no effect on sugar-

cane yield, whereas intercropping with peanut reduced sugarcane yield by 22 % and sucrose yield by 29 %. Also, intercropping with velvet bean increased the levels of some nutrients in the soil and leaves of sugarcane. These results showed that intercropping can be used by smallholding farmers to manage nematode pests, in this case on sugarcane. Furthermore, intercropping provided nutrients to the sugarcane crop when velvet bean in particular was used. Ultimately, the use of intercropping provided an alternative food source and/or income, viz. sweet potato, and improved the overall productivity of the land without being detrimental to sugarcane cultivation.

## 7.8 Cover Crops Used for Biofumigation

Brassicaceae crops have been and are continuously evaluated for their cover and biofumigation properties as an alternative strategy to control plant-parasitic nematode pests and more specifically root-knot nematodes worldwide (Fourie et al. 2016). Biofumigation is defined as a process during which volatile compounds (e.g. isothiocyanates and thiocyanates) with pesticidal effects are released during the decomposition and biodegradation of plant parts or animal products incorporated in the soil. Brassicaceae crops contain such compounds that are collectively referred to as glucosinolates (Youssef 2015).

Climate, soil conditions, plant density and other factors play an important role in the efficacy of biofumigation using Brassicaceae crops. In Box 7.2, the modes of action of biofumigation are summarised. Cultivars of *Brassica* spp. that are commercially available showed variable results in terms of reducing nematode population densities (Fourie et al. 2015). A field experiment with mustard (*Eruca sativa* cvs. Rocket Trio and Nemat), Indian mustard (*Brassica juncea* cvs. Calienté and Fumigreen) and radish (*Raphanus sativus* cvs. Doublet and Terranova) showed an increase (115 %) in *M. incognita* population densities in the roots of potato (cv. Mondial) at tuber initiation when Indian mustard cv. Calienté was first grown, and its aerial parts subsequently incorporated in the soil for biofumigation. At this specific experimental site, the high root-knot nematode population ( $\pm 25,000$  eggs and J2 50 g<sup>-1</sup> potato tubers in the season preceding the experiment) proved to be too high for biofumigation to be successful (Engelbrecht 2012). Nonetheless, in another experiment (at Mbombela), when tomato (cv. Monica) plants were grown after biofumigation with the same Brassicaceae cvs. as mentioned above, substantial reductions in the number of eggs and J2 50 g root<sup>-1</sup> (a mixture of *M. javanica* and *M. incognita*) and significantly higher yields were recorded compared with the uninfected control plants. The increases in tomato yield were, for example, between 103 and 163 % higher in plots cover cropped and subsequently biofumigated with aerial parts of the radish cvs. Terranova and Doublet, respectively, compared with the untreated control. By contrast, for plots cover cropped with cv. Calienté, a yield decrease of 26 % was observed (Daneel et al. 2014b).

### **Box. 7.2 Modes of Action of Biofumigation with Brassicaceae to Protect Crops Against Plant-Parasitic Nematodes**

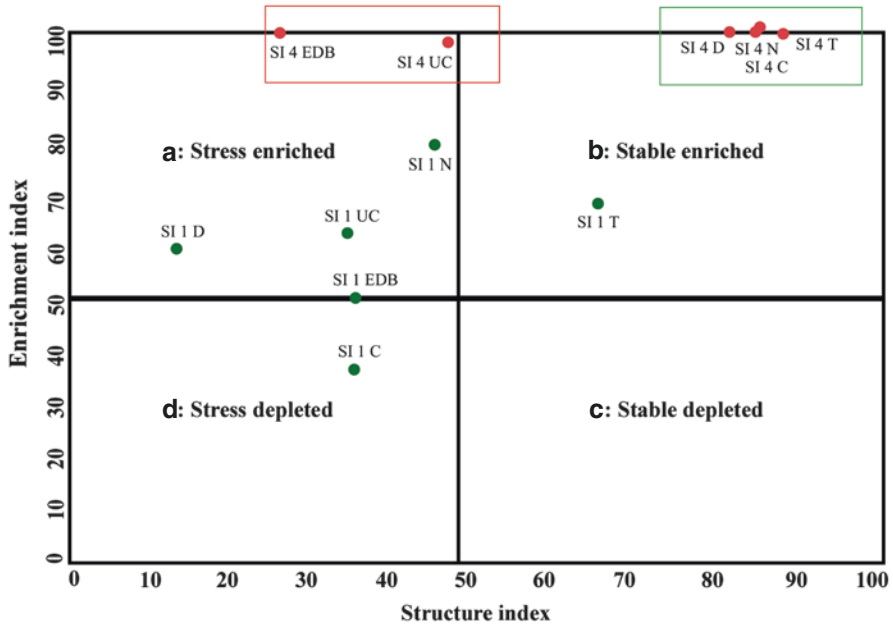
Modes of action:

- (i) Production of nematotoxic glucosinolate (GSL) degradation products, viz. isothiocyanates (ITCs), thiocyanates, nitriles or oxazolidinethiones
- (ii) Stimulation of antagonistic microbial communities
- (iii) Production of nitrogenous compounds that are toxic to plant-parasitic nematodes

Degradation products are formed as a result of the hydrolysis of sulphur-containing secondary metabolites by the enzyme myrosinase (stored separately in plant cells), yielding nitriles, epithionitriles and thiocyanates. To optimise ITC release from aerial parts of Brassicaceae crops, the plant cells of these aerial parts must be damaged by slashing and/or rupturing and immediately incorporated into the soil. Such actions are most effective when ITC levels are highest which is usually during flowering in aerial plant parts of Brassicaceae crops. Popular Brassicaceae crops are *Brassica oleracea* (broccoli, Brussels sprouts, cabbage, cauliflower), *Brassica oleracea acephala* (kale), *Brassica napus* (canola and rape seed), *Brassica rapa* (turnip), *Raphanus sativus* (radish) and a variety of mustards such as *Brassica juncea* (Indian mustard) and *Sinapis alba* (white mustard). More than 200 GSLs, contained in the vegetative parts and seeds of cultivated and wild plant species, have been identified mainly from plants belonging to the Brassicaceae family. The majority of these GSLs are unique in their chemical characteristics, and the types and quantities of GSL vary among individual plant species, plant organs, developmental stages and environmental factors (e.g. drought, availability of sulphur in the soil, sulphate and nitrogen nutrients, seasonal and diurnal cycles). Canola cv. Hyola 401, for example, contains low levels of GSLs, while rapeseed cv. Dwarf Essex, turnip cv. Purple Top and yellow mustard cv. Ida Gold contain moderate levels. The growth stage of Brassicaceae crops and the amount of biomass slashed and incorporated into the soil are the two main factors that contribute towards the success of biofumigation. Also, the Brassicaceae crop planted and its adaptability to the environment depict its biofumigation efficacy. Except for green manures, seed meals of Brassicaceae crops also may have biofumigation effects. Seed meals can be easily spread and incorporated into soil with no risk of frost damage as is the case with green manure crops. Also, seed meals do not serve as hosts to plant-parasitic nematodes as the roots and tubers of cover crops may do.

Literature consulted: Zasada and Ferris (2003), Bellostas et al. (2004), Kirkegaard and Matthiessen (2004), De Pascale et al. (2007), Larkin and Griffin (2007), Van Dam et al. (2009), Winde and Wittstock (2011), Borgen et al. (2012), Lelario et al. (2012), Kruger et al. (2013) and Fourie et al. (2016).





**Fig. 7.10** Enrichment index (EI) and structure index (SI) of free-living nematode assemblages (according to soil food web analyses – Ferris et al. 2001) demonstrating the effect of cover cropping and biofumigation with four Brassicaceae crops after four consecutive sampling intervals during the 2010/2011 growing season. Soils of treated plots (green box) plotted in the ‘stable and enriched’ quadrant opposed to soils of untreated and EDB-fumigated plots (red boxes). Only data for sampling interval (SI) 1 (SI 1, green dots in red block) and 4 (SI 4, red dots in green block) are shown: EDB ethylene dibromide treated, UC untreated control, C cultivar Calienté, D cultivar Doublet, N cultivar Nemat, T cultivar Terranova (Adapted from Engelbrecht 2012)

Results obtained from local studies demonstrated that Brassicaceae crops could be used for their cover and biofumigation effects to reduce root-knot nematode population densities, increase crop yields and contribute towards soil health (Engelbrecht 2012; Daneel et al. 2015). In Fig. 7.10, improvements of the quality of soils (assessed in terms of free-living nematode assemblages) in which Brassicaceae crops were grown and biofumigated are shown. For example, at the termination of the experiment, the soil of plots biofumigated with Brassicaceae crops attained high so-called enrichment (EI) and structure indices (SI). The number of free-living nematodes increased in the soils cover cropped and biofumigated with the Brassicaceae compared with the untreated and EDB-treated soils. The correct choice of the Brassicaceae crop species and cvs is crucial since different species and cvs have different levels of GLS, which are the a.s. responsible for the nematicidal effects obtained as a result of biofumigation (Daneel et al. 2015; Fourie et al. 2016).

## 7.9 Conclusions

Research aimed at managing the adverse impact of plant-parasitic nematode pests in fields/gardens of smallholding farmers are being done on a relatively large scale and contributed already to combatting these pests in this agricultural farming sector. Furthermore, knowledge generated to date on the incidence and abundance of nematode pests in the smallholding agricultural sector yielded value data also on nematode-plant interactions. Practically applied research should be continued to ensure that these farmers reap the benefits and be able to produce crops in the presence of reduced nematode pest population levels.

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