



**Soybean host status to *Meloidogyne incognita*
and nematode biodiversity in local soybean
cropping systems**

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I would like to dedicate this thesis to two special ladies in my life who could not see its completion. I hope this would have made you proud.

my mother Nomthunzi Mbatyoti (25 March 1964 to 04 August 2015)

and

my aunt Vuyiseka Gxolo-Twani (09 November 1976 to 30 October 2017)

We all miss you

DECLARATION

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Abstract

Root-knot nematodes (*Meloidogyne* spp.) are the major nematode pests of local soybean crops, resulting in up to 100 % yield losses. The host response of locally adapted soybean genotypes to the predominant *Meloidogyne incognita* was determined, while nematode (plant-parasitic and non-parasitic) abundance, diversity and occurrence in local soybean cropping systems and especially in glyphosate-treated versus non-treated (conventional soybean and natural vegetation) soils were also assessed. The host status of 36 soybean genotypes was determined for *M. incognita* in glasshouse experiments under two temperature regimes. Substantial variation existed amongst the cultivars for all nematode parameters. Only line PRF-GC17 and the resistant standard cultivar LS 5995 had reproduction factors (R_f) < 1 , while DM 6.2i RR had an $R_f = 1$ and percentage resistance ($\%R$) ≤ 2 %, indicating resistance. These genotypes retained their resistance even at the higher temperature regime. From the surveys conducted, *M. incognita*, *Meloidogyne javanica*, *Pratylenchus brachyurus* and *Pratylenchus zaeae* were the predominant endoparasites. Ectoparasitic nematode genera that dominated in soil samples were *Helicotylenchus*, *Scutellonema*, *Criconema*, *Criconemoides*, *Tylenchorhynchus* and *Nanidorus*. Seven species, viz. *Pratylenchus flakkensis*, *Pratylenchus scribneri*, *Pratylenchus vulnus*, *Rotylenchulus brevicaudatus*, *Telotylenchus avaricus*, *Tylenchorhynchus brevicaudatus* and *Quinisulcius capitatus* are first reports for soybean in South Africa. The highest plant-parasitic nematode diversity was associated with conventional soybean cultivars followed by natural vegetation and the glyphosate-tolerant soybean cultivars. A total of 32 non-parasitic nematode genera were also listed for the three ecosystems, viz. 21 for glyphosate-tolerant soybean, 23 for conventional soybean and 28 for natural vegetation. Bacterivore genera (*Acrobeles*, *Acrobeloides*, *Eucephalobus* and *Panagrolaimus*) generally dominated in soils of all three ecosystems, followed by fungivore genera (*Aphelenchus* and *Aphelenchoides*). Low abundance, diversity and occurrence were recorded for predators and omnivores. No correlations were apparent for non-parasitic nematode genera and the three ecosystems. According to soil food web analyses, soils from all three ecosystems were disturbed and degraded. A field experiment was also conducted to determine the response of nematode communities to glyphosate. This was done over two consecutive

growing seasons for a soybean/maize cropping cycle. The abundance of six plant-parasitic nematode genera, *Criconema*, *Helicotylenchus*, *Meloidogyne*, *Nanidorus*, *Pratylenchus* and *Tylenchorhynchus* did not differ between glyphosate-treated and non-treated plots. Of the 14 non-parasitic nematode genera identified (*Acrobeles*, *Acrobeloides*, *Aphelenchoides*, *Aphelenchus*, *Aporcelaimellus*, *Cephalobus*, *Discolaimium*, *Ditylenchus*, *Eucephalobus*, *Teratocephalus*, *Leptonchus*, *Panagrolaimus*, *Tylencholaimus* and *Tylenchus*), only a few differed significantly in abundance between the glyphosate-treated and non-treated plots. Faunal analyses showed that soils from glyphosate-treated plots were degraded, less enriched and fungal-mediated. Conversely, soils from non-treated plots were disturbed and enriched, and bacterial-mediated. This study re-emphasised the challenges posed by plant-parasitic nematodes, in particular *Meloidogyne* and *Pratylenchus*, to local conventional and genetically-modified soybean crops. It also gave an insight regarding the importance of non-parasitic nematodes as bio-indicators of soil quality in soybean cropping agro-ecosystems. Ultimately, it showed that nematode assemblages generally did not differ among glyphosate-treated and non-treated plots. More research over longer study periods should, however, be conducted to determine whether glyphosate has an effect on both plant-parasitic and non-parasitic nematodes that prevail in local soybean-based cropping systems.

Key words: bio-indicators, conventional, *Glycine max*, glyphosate-tolerant, nematodes.

Chapter 1

General introduction

1.1 Background, scientific hypothesis and objectives, research outline

1.1.1 Background

In 2008, the World Bank projected a 35 % increase in the human population by 2050 (Alexandratos & Bruinsma, 2012), implicating a similar increase in the demand for food. Legumes are ranked second to cereals in terms of nutritional importance for human consumption and represent a major source of proteins worldwide (Kristensen *et al.*, 2016). Amongst legumes, soybean (*Glycine max* L.) occupies the most prominent position in terms of production and source of proteins, particularly in developing countries (Marsh *et al.*, 2012). Hence, soybean is considered an important protein and oilseed crop, with the area planted in South Africa continuously expanding. From the 2011/12 to the 2016/17 growing seasons the area planted with soybean increased with 91 % (PRF, 2017). The expansion of soybean production has as a consequence that the crop is being exposed to new and existing diseases and pests that have the potential to seriously hamper its production. Plant-parasitic nematodes, of which root-knot nematodes (*Meloidogyne*) are considered the most damaging (Fourie *et al.*, 2017), pose a problem to local soybean producers. Therefore, to ensure sustainable food production the need for nematode research on soybean warrants urgent attention.

An important factor to bear in mind regarding soybean production in South Africa is that the cultivation of genetically-modified (GM) glyphosate-tolerant soybean cultivars dominate the local market (Dlamini *et al.*, 2014). The intensive cultivation of glyphosate-tolerant soybean and the extensive use of glyphosate have raised concerns about their impact on soil health (Liphadzi *et al.*, 2005). Furthermore, limited information is available about the impact of glyphosate on the occurrence, abundance and diversity of both plant-parasitic and non-parasitic/beneficial nematode communities.

1.1.2 Scientific hypothesis

Based on previous studies, it can be expected that most locally available soybean cultivars are susceptible to *Meloidogyne incognita* (Kofoid & White, 1919) (Venter, 2014; Fourie *et al.*, 2015). Differences in plant-parasitic nematode communities in terms of abundance and diversity are not foreseen to exist in glyphosate-treated fields where glyphosate-tolerant soybean cultivars are grown compared to fields where their conventional soybean cultivars are cultivated (Yang *et al.*, 2002; Liphadzi *et al.*, 2005). Bacterial-feeding nematodes will be the most prevalent beneficial nematodes in soils from glyphosate-treated and conventional soybean, and natural vegetation areas (Hu & Qi, 2010). Soils treated with glyphosate tend to have high incidences of different fungi species due weed root tissue decomposition (Meriles *et al.*, 2006). Consequently, glyphosate-treated fields are expected have high numbers of fungal feeding nematodes because of food abundance (Liphadzi *et al.*, 2005). Finally, greater colonizer-persister (c-p) value nematodes are generally associated with low stress and undisturbed environments (Bongers, 1999; Ferris *et al.*, 2001). Therefore, natural vegetation sites are foreseen to have a higher diversity of omnivorous and predatory nematodes than glyphosate-tolerant and conventional soybean fields.

1.1.3 Objectives

The overall aim of our study was to determine the host status of available soybean cultivars to *M. incognita* and to generate knowledge on nematode assemblages (both plant-parasitic and non-parasitic nematodes) present in soybean agro-ecosystems, treated with glyphosate *versus* non-treated, under South African environmental conditions.

The specific aims were to assess: i) the host response of commercially-available South African soybean cultivars to *M. incognita*, ii) the abundance and diversity of plant-parasitic nematodes assemblages associated with commercially-available local soybean cultivars, iii) the abundance and diversity of nematodes (parasitic and non-parasitic) in fields where glyphosate-tolerant- and conventional soybean cultivars were

grown as well as the nematode assemblages in adjacent natural veld areas and iv) the effects of glyphosate on nematode assemblages in a soybean-maize rotation system.

In this way, researchers can assist soybean producers and the related industries in combatting nematode pests that parasitise glyphosate-tolerant-, and the few conventional soybean crops still planted. The study also aimed to enable growers to be aware of plant-parasitic nematodes (with focus on *Meloidogyne* spp.) that prevail in soybean production areas, which may increase the risks of reduced yields and/or quality of soybean and other rotation crops. Ultimately, growers will be informed about soybean cultivars that are resistant/poor hosts to *M. incognita*, which can rather be grown (opposed to their susceptible counterparts) to reduce population densities of these pests. Growers will in this way be enabled to effectively practice one of the pillars of integrated pest management, namely genetic host plant resistance, to alleviate nematode problems in the long term.

1.1.4 Research outline

The first objective was achieved by assessing the host response of 36 commercially-available local soybean genotypes to *M. incognita* in glasshouse (evaluated for their agronomical characteristics in the National Cultivar Trials of the Agricultural Research Council's Grain Crops Institute (ARC–GCI), Potchefstroom (Chapter 2). The second objective was achieved by three surveys carried out in the major soybean production provinces across the country (viz. Free State, Kwa-Zulu Natal, Limpopo, Mpumalanga and North West). One survey was carried out for plant-parasitic nematodes associated with soybean crops during 2014/15 cropping season (Chapter 3). Two other surveys were carried out for plant-parasitic nematode communities associated with glyphosate-tolerant *versus* conventional soybean during the 2011/12 and 2012/13 cropping seasons (Chapter 4). The third objective was achieved by two surveys carried out for beneficial nematode communities associated with glyphosate-tolerant *versus* conventional soybean in South Africa during 2011/12 and 2012/13 cropping seasons (Chapter 5). Finally, the fourth objective was achieved by determining the effects of glyphosate application on nematode communities in a small field trial (Chapter 6).

1.2 Literature review

1.2.1 Soybean

1.2.1.1 Origin and distribution

Soybean, a summer legume crop (Hymowitz *et al.*, 1998), emerged as an edible crop in north-eastern China around 1 700 BC and was widely cultivated in south-eastern Asia by the 16th century. Cultivation of the crop spread to Europe, the United States of America (USA) and, subsequently, South America during the 18th Century. It was, however, only during the late stages of 19th Century discovered by the West as a dual-purpose protein and oil crop for both human and animal consumption (Hartman *et al.*, 2011; Yadava *et al.*, 2011). Through soybean breeding programs new varieties for various adaptive traits (*i.e.* agronomical, disease and pest resistance) have been developed that enabled global cultivation of the crop in climatic conditions ranging from temperate to subtropical (Kinloch, 1998; Hymowitz *et al.*, 2008).

1.2.1.2 Taxonomy, basic anatomy and growth phases

Soybean is a dicotyledonous crop (family Fabaceae) with erect, bushy herbaceous diploid ($2n = 40$) plants (up to 1.2 m tall) (Hymowitz *et al.*, 1998; Doyle *et al.*, 2003; Gill *et al.*, 2009). The primary leaves of soybean are unifoliate, opposite and ovate, while secondary leaves are trifoliate. The nodulated root system consists of a taproot from which a lateral root system emerges. Pods are straight or slightly curved, ranging in length from 2-7 cm, and consist of two halves of a single carpel (joined by a dorsal and ventral suture). The seed shape is usually oval, but may vary among cultivars from almost spherical to elongate and flattened (Liu, 2004).

Soybean seedlings emerge under favourable environmental conditions from 4-5 days after planting onwards (Nelson & Larson, 1984) (Fig. 1.1). Cotyledons open, supplying the new seedling with stored energy through absorbing light energy. The growing point, located above-ground between the two cotyledons, can be damaged or killed by spring frost or physical damage. The first true vegetative, unifoliolate leaves form directly opposite one another above the cotyledonary node. The rest of the other leaves are trifoliolates and consist of three leaflets (V1- stages) (Kouchi & Hata, 1993).

Soybean development is characterised by two distinct growth phases (Liu, 2004), viz. the vegetative stage (VE) that commences with the emergence of young seedlings from the soil surface and ends with the start of flowering, and the reproductive (R) stage that encompasses flowering to maturation (Fig. 1.1). Plant stages are determined by the classification of leaf, flower, pod, seed development and node characterisation.

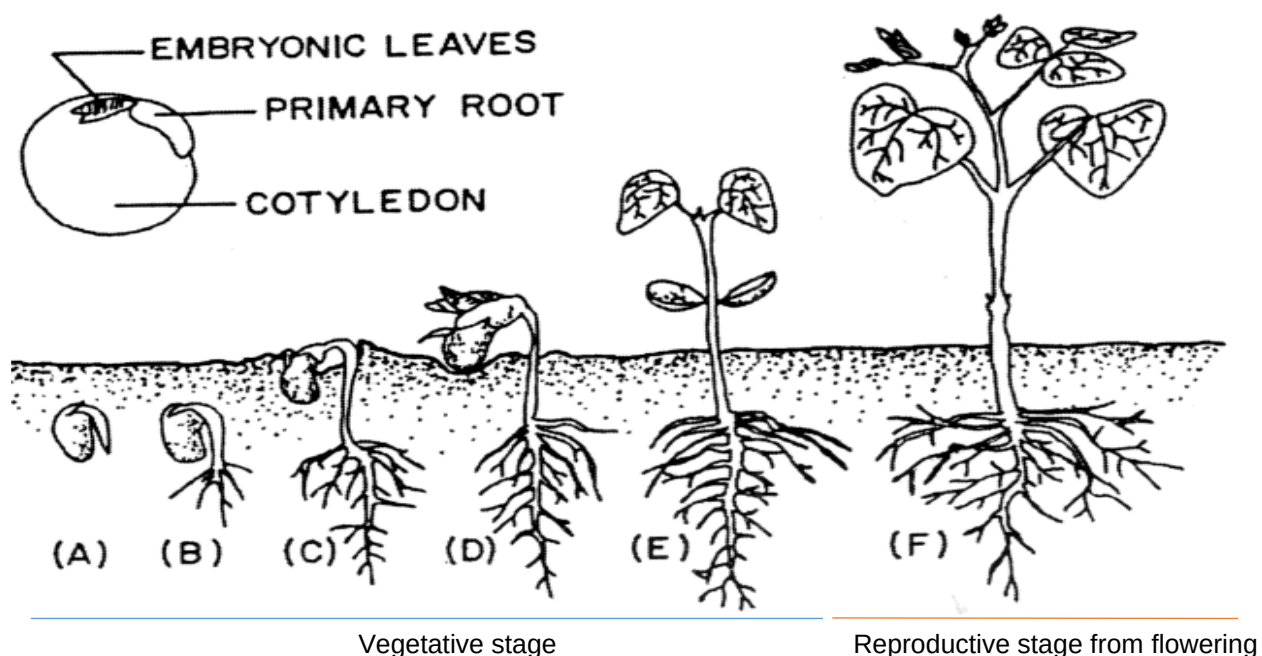


Figure 1.1. Germination, seedling development and growth of a soybean plant, with (A) emergence of the radicle to form the primary root, (B) development of secondary roots, (C) elongation of the active hypocotyl with the hypocotyl arch penetration through the soil surface, (D) seedling becoming erect, (E) before drying and (F) falling from autotrophic seedling (A-E = vegetative stage and F onwards = reproductive stage; Fehr *et al.*, 1971) (Illustration adapted from Nelson & Larson, 1984).

Flowering may commence 25 days after germination or may be delayed until 50 days after germination depending on the cultivar and environmental conditions (Whigham, 1983). Flower stigmas are receptive to pollen approximately 24 h before anthesis and remains receptive for 48 h thereafter. The anthers mature in the bud and directly pollinate the stigma of the same flower with a high percentage of self-fertilisation occurring while cross-pollination is usually less than 1 % (Abernethy *et al.*, 1977). After fertilisation, pods develop and reach maximum length after 15-20 days (Whigham, 1983).

1.2.1.3 Crop production practices

Soybean growth is influenced by climate, day length (in particular) and soil characteristics (Kantolic & Slafer, 2007). In South Africa, the crop may be grown in all the provinces but it thrives well in warmer regions. Locally soybean is usually planted from November after the first rains occurred. Planting in some instances may be extended to January due to variable rainfall and climate conditions (De Beer & Prinsloo, 2013). Planting at this late stage may present a risk of lower harvest potential since soybean is a short-day plant and flowers in response to daylight length (Kantolic & Slafer, 2007). Soybean thrives well in soils with a pH range between 5.5 and 6. Production requirements to optimise soybean yield are summarised in Table 1.1.

Table 1.1. A concise summary of production requirement to optimise soybean yield under South African climatic conditions.

1. Climatic requirements	Optimal temperature for all growth stages of soybean is 25 °C. However, at planting, soil temperature of approximately 15 °C may occur and still stimulate germination. Rainfall of 500 to 900 mm is required for optimal yield and quality (DAFF, 2010).
1. Cultivar selection	A cultivar that best suits the farmer's agro-ecological area should be selected. The maturity period is the first consideration when choosing a cultivar. Treating seeds with fungicides before planting protects the crops against soil-borne fungal diseases (Zilli <i>et al.</i> , 2009). The use of glyphosate-resistant cultivars dominate the local market and is elaborated on in paragraph 1.3.
3. Planting and spacing during sowing	Narrow rows increase yield, however, spacing is determined by the irrigation type and water availability. Plant densities of 250 000-400 000 plants per hectare are generally recommended depending on the yield potential of the area (DAFF, 2010).
4. Application of fertilisers	Soybean seeds must be inoculated with nitrogen-fixing bacteria, <i>viz. Bradyrhizobium japonica</i> , since they cannot fix atmospheric nitrogen on their own. Phosphorus and potassium are major elements required by soybean for growth. Minor elements, e.g. calcium, magnesium and sulphur also play a vital role for this crop's growth (Leggett & Frere, 1971; Keino <i>et al.</i> , 2015).
5. Irrigation	Although soybean requires less water in the late reproductive growth stage, water plays an essential role in the translocation of nutrients. Most soybean crops are produced under rain-fed conditions, but sprinkler and drip irrigation are also used where water sources are available (DAFF, 2010).
6. Weed control	Weed control is either manual or chemical, or both. If weed is controlled manually, the first weeding should be done 2 weeks after planting and the second 5-6 weeks later. Chemical weed control, <i>viz.</i> application of herbicides, is effective in controlling weeds if done properly. The predominant weed species and the availability of the herbicide usually dictate the choice of herbicide to be used (Beckie & Gill, 2006; Yao <i>et al.</i> , 2010).
7. Disease and pest management	Integrated practices such as chemical (<i>viz.</i> use of registered pesticides and fungicides), mechanical, biological and other cultural practices can be used to control diseases and pests (DAFF, 2010).

1.2.1.4 Nutritional, economic and social value

Soybean is among the top-traded commodities and a most important crop used as a food source for humans and animals (Hartman *et al.*, 2011; Soyatech, 2016). The seeds contain approximately 40 % protein, 20 % oil and 26 % carbohydrates (Khalequzzam, 2003; Qiu & Chang, 2010). Today, the crop accounts for approximately 80 % of the

world's production of annual oilseed crops including Bambara groundnut (*Vigna subterranea* L.), dry bean (*Phaseola vulgaris* L.), cow pea (*Vigna unguiculata* L.), groundnut (*Arachis hypogaea* L.) and lupin (*Lupinus mutabilis* L.) (FAO, 2017). Soybean has also been considered to be among the most promising crops for producing biofuel (biodiesel) in the future (Sparks *et al.*, 2011).

1.2.1.5 Worldwide production figures

Worldwide, 306.5 million metric tonnes (MMT) of soybean were produced during the 2014 season, with the dominating countries in terms of production being the USA (107 MMT), Brazil (86 MMT), Argentina (53 MMT), China (12 MMT) and India (11 MMT) (FAO, 2017). South Africa is a relatively small producer of soybean and has been ranked 13th worldwide during 2014 (FAO, 2017), with 1 070 000 MT being produced during the 2015/16 growing season (PRF 2017). However, on the African continent South Africa is the lead producer, followed by Nigeria (679 000 MT) and Zambia (214 179 MT) (FAO, 2017).

1.2.1.5.1 Soybean production in South Africa

Soybean production is a relatively new and small-growing component of the agricultural economy in South Africa (De Beer & Prinsloo, 2013). The first report on local production of soybean is found in the Cedara Memoirs (1903), which revealed that seeds were imported from China. However, poor germination and shattering of pods prior to harvesting in initial trials led to the implementation of a breeding programme at the Agricultural Research Station in Potchefstroom in the early 1950s (DAFF, 2010). This programme resulted in the development of cultivar Geduld (grown by most producers until the early 1980s) (Noble *et al.*, 1984) that was adapted to local conditions and also exhibited resistance to premature shattering of pods. Although soybean production in South Africa was localised until recently, gradual expansion of the crop in rotation systems with maize and wheat has been experienced (Riekert, 1996; Smit, 2000; DAFF, 2010) and during the late 1990s production started to gain momentum (PRF, 2016). Soybean is produced mostly in the eastern parts of the country (Fig. 1.2), with significant production in the Free State and Mpumalanga provinces.

Recently, there is a notable growing interest in soybean production in SA due to producers and the soybean industry realising the importance of this protein-rich crop (De Beer & Prinsloo, 2013; PRF, 2016). Hence, the area planted to soybean locally has increased by 98 % from 134 000 ha planted in 2001/02 to 696 400 ha in 2015/16 (PRF, 2017). A record of 1 316 370 MT was produced during the 2016/17 growing season, indicating the progressive increase in soybean production during the past 15 years (PRF, 2017). Due to severe drought experienced during the preceeding 2015/16 growing season production of soybean and other food crops were substantially lower (Grain SA, 2017). The biggest soybean producer is Mpumalanga Province (54 %), followed by Kwa-Zulu Natal (19 %), Free State (15 %), Limpopo (5 %), North West (4 %), Gauteng (2.6 %) and others (0.3 %) (Fig. 1.2) (AgriSA, 2015).

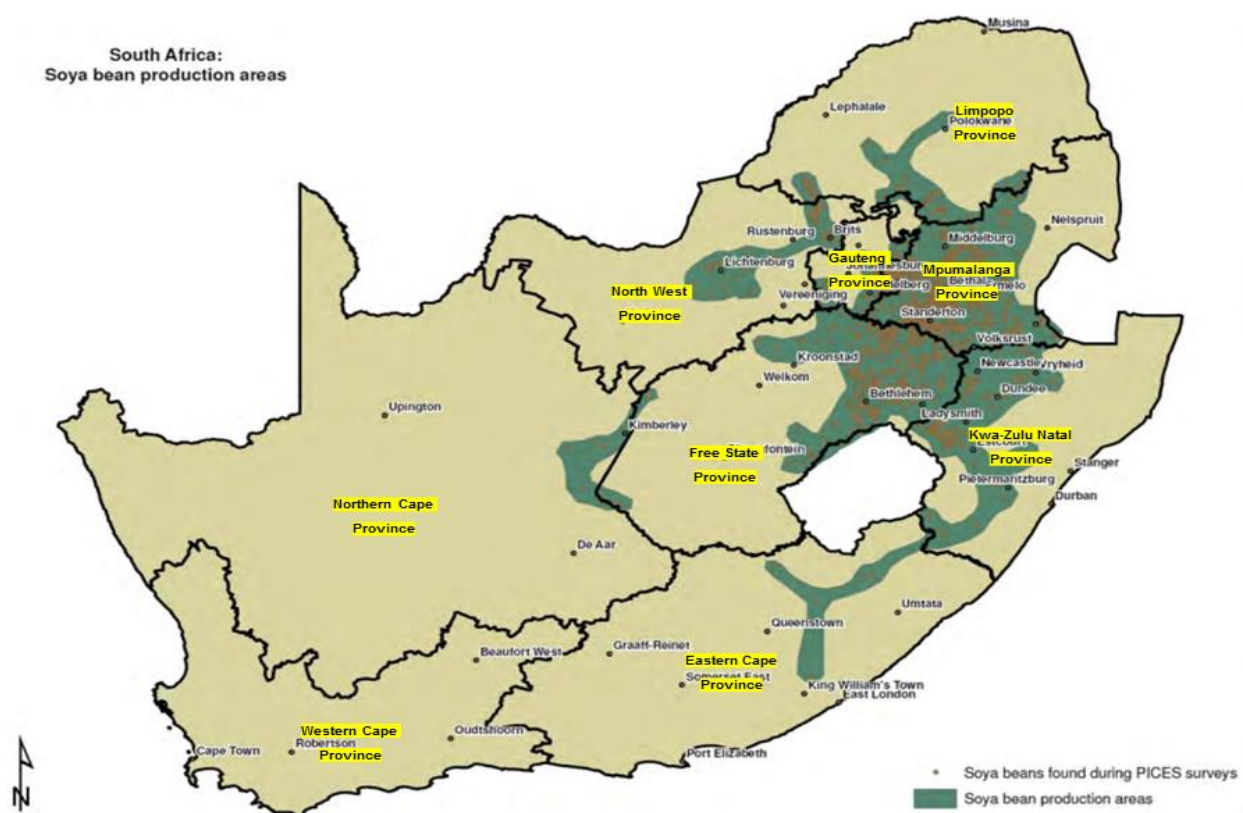


Figure 1.2. An adapted map of South Africa indicating the main soybean-producing areas in the Eastern Cape, Gauteng, Kwa-Zulu Natal, Limpopo, Mpumalanga, Northern Cape and North West provinces (Illustration: Department of Agriculture, Forestry and Fisheries, South Africa: www.daff.gov.za).

The progressive increase in local soybean production follows the realisation by producers that soybean is a beneficial rotation and high-income crop. Soybean crops leave substantial residual levels of nitrogen in the soil after cultivation. Growing soybean thus improves soil fertility without a need to add additional nitrogen fertilisers, limiting the application of such chemicals (Bullock, 1992; Peoples *et al.*, 2009). The price per MT of soybean seed is also relatively high, increasing from ZAR 3 684 during 2012 to ZAR 6 513 during the 2016/17 growing season (DAFF, 2014; Grain SA, 2016).

The increasing local demand for protein-rich food sources has also contributed to an increase in soybean production, especially for the livestock, poultry and aquaculture industries, since this crop is regarded as a cheap source of good quality protein feed (Nicol *et al.*, 2011; Joubert & Jooste, 2013; PRF, 2016). Moreover, production of soybean also has been made easier and more cost effective with the availability of glyphosate-tolerant cultivars, because all the weeds can be eliminated without harming the soybean plants grown in that particular field (Graham & Carrol, 2003; Khalezuqqam, 2003). Even though glyphosate-tolerant soybean cultivars now dominate the local market, some farmers still grow conventional soybean cultivars to gain access to high profitable markets that regularly demands non-GM soybean produce (Dlamini *et al.*, 2014).

1.3 Conventional *versus* genetically-modified (GM) glyphosate-tolerant soybean production

Weed control practices in soybean fields have evolved since the earliest production of the crop (Carpenter & Gianessi, 1999). Before the introduction of herbicides, weeds were controlled by mechanical and cultural means. Herbicides began to replace tillage and cultivation practices as the primary weed control method in the 1960s. Herbicides are generally applied before or at planting and are often followed by selective post-emergence products (Beckie & Gill, 2006). The use of post-emergence, conventional herbicides was widely practised by soybean growers in the 1980s. The domination of conventional herbicides was, however, over-shadowed by the commercialisation of glyphosate-tolerant soybean cultivars in the USA in the mid-1990s. Since this major

inception in soybean production, glyphosate use increased substantially wherever the crop was grown. Before the adoption of glyphosate-tolerant crops, glyphosate was mainly used before planting to avoid contact with the crop or other non-target vegetation (Franz *et al.*, 1997).

Soon after the release of glyphosate-tolerant soybean cultivars, local and international markets were dominated by these crops (Bøhn *et al.*, 2014; Dlamini *et al.*, 2014). This situation led to a marked decline in the application of conventional herbicides on soybean (Carpenter & Gianessi, 1999; Duke *et al.*, 2012). It is estimated that approximately 84.5 million ha was planted with glyphosate-tolerant soybean crops worldwide in the 2013 growing season (Anonymous, 2013b; Bøhn *et al.*, 2014). The first record of glyphosate-tolerant soybean being grown in South Africa dates back to 2001 (Wolson, 2007), when cultivar A5409RG was grown in fields near Delmas, Groblersdal and Ermelo (Mpumalanga Province), Dundee and Greytown (KwaZulu-Natal Province), Koedoeskop (Limpopo Province), Potchefstroom and Rustenburg (North West Province) for experimental purposes (De Beer, 2001) (Fig. 1.3). At present, glyphosate-tolerant soybean cultivars are being grown on more than 80 % of the fields used for soybean production in South Africa (Dlamini *et al.*, 2014)

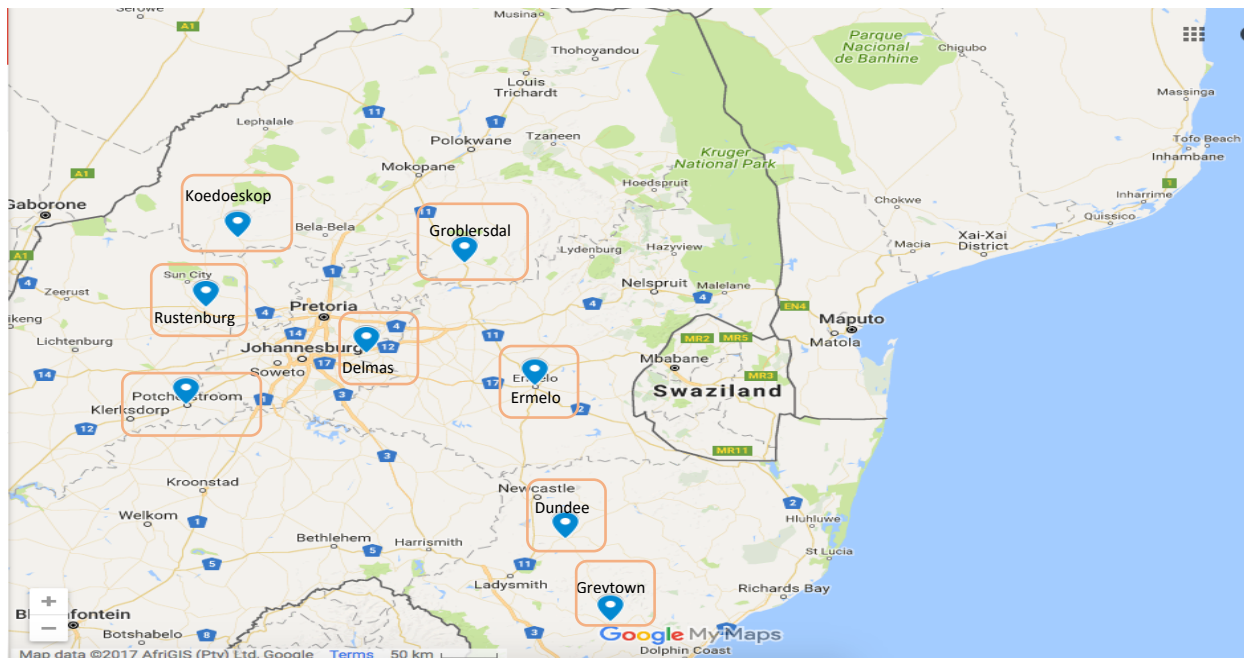


Figure 1.3. The eight localities (Delmas, Dundee, Ermelo, Greytown, Groblersdal, Koedoeskop, Potchefstroom and Rustenburg) where genetically-modified, glyphosate-tolerant soybean cultivar A5409RG was grown for the first time in 2001 in South Africa.

1.3.1 Glyphosate

Glyphosate (N-(phosphonomethyl) glycine) is the active substance of herbicides such as Roundup® and others which are highly effective, non-selective herbicide. Numerous products containing this a.s. are registered in South Africa for use on glyphosate-resistant crops such as soybean (Van Zyl, 2012). It is considered one of the less toxic herbicides (Duke & Powles, 2008) and producers prefer to use such a single herbicide to control a broad spectrum of weeds and grasses, resulting in minimal crop injury and great economic benefits (Carpenter & Gianessi, 1999; Hurley *et al.*, 2009). Glyphosate is considered as less toxic and environmentally benign due to its low mammalian toxicity, relatively short persistence and extremely low activity in the soil due to its binding to soil minerals (Duke & Powles, 2009). Ultimately, the use of glyphosate provides farmers with more crop rotation options, because this chemical has no residual activity (Carpenter & Gianessi, 1999; Marra *et al.*, 2004). Its use far outweighs the application of several herbicides throughout a growing season to accomplish adequate

weed control. Additionally, the trend towards reduced or minimum soil tillage (improving surface water quality and soil water retention) in turn reduces soil erosion and leaching of herbicides (Young, 2006; Fawcett *et al.*, 1994).

Glyphosate is applied onto soybean leaves. It is taken up and transported to the plants' growth tissues where it inhibits (Shaner, 2009) the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme (present in all green plants) (Duke *et al.*, 2012) of the shikimate pathway. Glyphosate competes with the phosphoenolpyruvate (PEP) binding site on the EPSPS which is the catalyst for the transfer of the enolpyruvyl moiety of PEP to shikimate-3-phosphate (S3P), forming EPSP and phosphate. The former is a key step in the synthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan (Dill, 2005; Duke & Powles, 2008; Duke *et al.*, 2012; Hove-Jensen *et al.*, 2014).

In glyphosate-tolerant soybean cultivars the *CP4* gene has been inserted into the crop's genome by means of genetic engineering (Duke & Powles, 2008). The *CP4* gene is a bacterial EPSPS enzyme isolated from *Agrobacterium* spp. that has a (herbicide) binding site identical to that of EPSPS (Cromwell *et al.*, 2002). Hence glyphosate-tolerant soybean cultivars contain both EPSPS and *CP4*-EPSPS enzymes. When treated with glyphosate, the glyphosate bind with EPSPS, PEP is able to by-pass EPSPS and bind with *CP4*-EPSPS, resulting in a shikimate pathway that will function normally and the plant will maintain aromatic amino acid levels (Reddy, 2001; Dill, 2005).

To understand the potential side effects of glyphosate in the rhizosphere of treated plants, it is necessary to understand its persistence in the soil and how it interacts with the root system of plants and soil micro-organisms. Glyphosate is applied by means of foliar sprays, therefore, its presence in the soil is from direct interception of spray by the soil surface or from runoff or leaching of the herbicide and/or its breakdown products from plants. It can also be translocated to roots from foliar tissues and exuded by the roots into the soil (Laitinen *et al.*, 2007). Once glyphosate interacts with soil, the active

substance strongly adsorbs to soil particles and is rapidly degraded by micro-organisms even if the product was applied at high dosages (Duke & Powles, 2009; Jochimsen *et al.*, 2011). Glyphosate is regarded a lesser soil contaminant if applied at recommended dosages with activity period of approximately 47 days in soil. This period can, however, be as long as 174 days in some soils under certain conditions (Cerdeira & Duke, 2010). The degradation of glyphosate's active substance is variable and significantly influenced by various factors such as application methods, soil properties, and environmental conditions (particularly moisture and temperature) (Borggaard & Gimsing, 2008). According to Neumann *et al.* (2006), glyphosate transfer to non-target plants is facilitated by the rhizosphere. Information about the effects of glyphosate on soil organisms (bacteria, fungi, nematodes *etc.*) are, however, scarce and fragmented and are summarised in Section 3.2.

1.4 Threats and challenges facing soybean production in South Africa

Soybean crops are adapted to a wide range of soils, and environmental conditions (Gibson & Benson, 2005) and, hence, production in SA is progressively being expanded outside the traditional growing areas to lighter-textured soils where maize used to be the dominant crop (Riekert, 1996; Dlamini *et al.*, 2014). As the number of hectares planted to soybean increase, so does the occurrence and severity of diseases and pests associated with the crop (Sinclair & Hartman, 2008). Researchers, producers and related industries are, however, addressing these challenges to improve and ensure sustainable soybean production. Some strategies applied to increase yields include the use of fertilisers and pesticides, and the optimised use of *Rhizobium/Bradyrhizobium* inoculants for optimal nitrogen nodulation. The development of new cultivars that are adapted to local environmental conditions and best suit the needs of producers are also gaining field in South Africa (Hartman *et al.*, 2011; Liebenberg, 2012). Nonetheless, increases in crop production due to varietal enhancements of genetic material are often restrained by diseases (viruses, bacteria and fungi) and pests (nematodes and insects) (Liebenberg, 2012).

¹² Nematode pests associated with soybean, with focus on root-knot nematodes (*Meloidogyne* spp.)

Plant-parasitic nematodes are considered an important limiting factor in crop production, particularly in the tropics and sub-tropics (Luc *et al.*, 2005; Jones *et al.*, 2013). On soybean, approximately 100 plant-parasitic nematode species have been reported to cause serious losses worldwide (Jones *et al.*, 2013). In South Africa, approximately 49 species have been associated with soybean crops (Fourie *et al.*, 2001a; Fourie *et al.*, 2015; SAPPNS¹).

Although the soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) is a devastating pest to soybean crops in several countries such as the USA (Chitwood, 2003; Tylka *et al.*, 2015), Canada (Tylka & Marett, 2014), Brazil (Vitti *et al.*, 2014), Nigeria (Ishaq & Ehirim *et al.*, 2014) and China (Wang *et al.*, 2014), it has not been detected in local soybean production areas to date (personal communication Dr Mariette Marais). Species of the genus *Meloidogyne* Göldi, 1889 are generally considered the economically most damaging nematode pests of soybean worldwide (Sikora *et al.*, 2005; Bridge & Starr, 2007; Fourie *et al.*, 2015). Lesion nematode species of the genus *Pratylenchus* Cobb, (1917) and reniform species of the genus *Rotylenchulus* Linford & Oliveira, (1940) nematodes generally follow as important pests of the crop (Sikora *et al.*, 2005; Bridge & Starr, 2007; Oyekanmi & Fawole, 2010). These genera are distributed worldwide and its species attack and can cause significant losses to numerous agricultural crops (Karssen & Moens, 2006; Jones *et al.*, 2013).

Meloidogyne spp. are also known to affect soybean production in South Africa (Fourie *et al.*, 2001a; Fourie *et al.*, 2015; Liebenberg, 2012). For example, during the 2011 growing season exceptionally high root-knot nematode population densities, ranging between 4 252 to 11 401 eggs and second-stage juveniles (J2) per 50 g roots, were reported from plants (cv. PAN737RR) that were grown in the Bothaville area (Free State Province) (Fourie *et al.*, 2011). The damage potential of *Meloidogyne* spp. is, unfortunately, underestimated by many farmers, particularly

¹Dr Mariette Marais of the Nematology Unit, Biosystematics Division, Agricultural Research Council – Plant Health and Protection is thanked for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database; E-mail: maraism@arc.agric.za

those in developing countries (Coyne *et al.*, 2009). This is due to symptoms caused symptoms, which are easily and often misdiagnosed and/or confused with those caused by other constraints (Oyekanmi & Fawole, 2010; Onkendi *et al.*, 2014).

Worldwide, six *Meloidogyne* spp., namely *Meloidogyne arenaria* Neal, 1889 (Chitwood, 1949), *Meloidogyne enterolobii* Yang & Eisenback, 1983, *Meloidogyne ethiopica* Whitehead, 1968, *Meloidogyne hapla* Chitwood, 1949, *M. incognita* and *M. javanica* are considered the major pests of soybean (Ibrahim *et al.*, 2011; Machado, 2014). The thermophilic species *M. arenaria* and *M. incognita* have the widest distribution worldwide (Moens *et al.*, 2009), while *M. javanica* is regarded as the most destructive and most frequently occurring species in Brazil and other soybean producing tropical and subtropical regions (Kinloch & Rodríguez-Kábana, 1999). *Meloidogyne hapla*, a cryophilic species, is reported as the least damaging species of soybean compared to other root-knot nematode species that infect the crop (Hirunsalle *et al.*, 1995; Strajnar *et al.*, 2011).

In South Africa, five *Meloidogyne* spp. are associated with soybean, viz. *M. arenaria*, *M. ethiopica*, *M. hapla*, *M. incognita* and *M. javanica* (Van der Linde *et al.*, 1959; Coetzee, 1968; Fourie *et al.*, 2001a, 2015). According to the latter two studies, *M. incognita* is the economically most important species, followed by *M. javanica*. These two species either occur in single or mixed species populations in local soybean production areas. *Meloidogyne ethiopica* and *M. hapla* were reported as being restricted to certain areas in the Kwa-Zulu Natal and Mpumalanga provinces (Fourie *et al.*, 2001a).

The next part of this chapter refers to the general biology, life cycle, symptoms inflicted, associations with disease-causing bacteria and fungi as well as yield losses caused by *Meloidogyne* spp. in soybean crops.

2.1 General classification, morphology, life cycle and reproduction of *Meloidogyne* spp.

The genus *Meloidogyne* is classified under the Phylum Nematoda Potts, 1932, Class: Chromadorea Inglis, 1983, Order: Rhabditida Chitwood, 1933, Family: Hoplolaimidae Filipjev, 1934 and Subfamily: Heteroderinae Filipjev & Schuurmans Stekhoven, 1941.

The word *Meloidogyne* originated from Greek and means an ‘apple-shaped female’ (Moens *et al.*, 2009). The bodies of *Meloidogyne* spp. individuals, which are pseudocoelomate, unsegmented aquatic microscopic roundworms, are covered with a transparent cuticle (Treonis & Wall, 2005). The body length of motile J2 (Fig. 1.4A) and males (Fig. 1.4B), respectively, range from 250 to 600 μm , and 600 to 2 500 μm (Karssen *et al.*, 2013). However, other life stages (third- and fourth-stage, and adult females; Figs 1.4C & D) are swollen and sedentary inside the roots of soybean. This phenomenon is being referred to as sexual dimorphism (Moens *et al.*, 2009). Eggs (Fig. 1.4E) are deposited in a gelatin matrix through the vulva of the female.

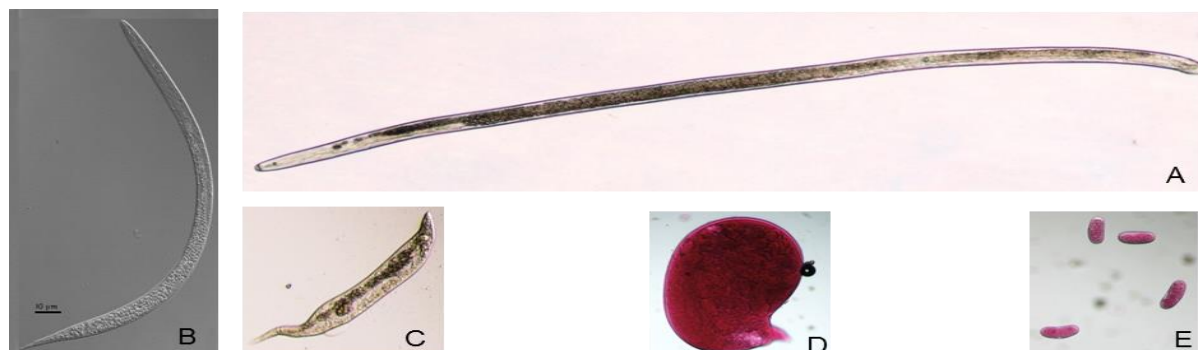


Figure. 1.4A–E. A vermiform *Meloidogyne* sp. Male (A) and second-stage juvenile (B), with a swollen third-/fourth stage juvenile (C) and a red-stained swollen female (D) as well as red-stained eggs (E) that have been isolated from an egg mass (Photo's: A: Ebrahim Shokoohi, NWU; B–E: Suria Bekker, NWU).

As with other plant-parasitic nematode genera, *Meloidogyne* exhibits four basic systems namely digestive, musculature (only longitudinal muscles), nervous, and reproduction (Decraemer & Hunt, 2013), which will not be elaborated on for the purpose of our study. Important to note is that nematodes have no circulatory system but the fluid-filled pseudocoelom transports substances in the nematode body (Decraemer & Hunt, 2013). *Meloidogyne* spp. are sedentary endoparasites that have

hollow stomatostylets with which they pierce the cell walls of plant roots to invade their host plants (Perry *et al.*, 2013).

The life cycle of plant-parasitic nematodes generally includes the following stages: egg, four juvenile stages and the mature male and females, which is also representative of *Meloidogyne* (Decraemer & Hunt, 2013; see Fig. 1.4A-E). After embryogenesis, the adult female produces eggs into a gelatinous egg mass that can be either deposited on the surface of the infected plant part or within the plant tissues. The first-stage juvenile (J1) develops within a protective egg shell and then moults to produce the J2 that can enter diapause and only hatch later when conditions are favourable again. During favourable conditions, most J2 hatch rapidly and move through the soil in moisture films surrounding soil particles to reach and infect plant roots. The invasive J2 begin to feed, usually behind the root tip, and move through the root to initiate a permanent feeding site consisting of so-called giant cells in the vascular cylinder. Here they moult to third- (J3) and fourth-stage (J4) juveniles and ultimately adults (either females or males) (Perry *et al.*, 2013). Males may be produced in parthenogenetic species when unfavourable conditions prevail such as high population densities which lead to limited food supply or when poor hosts are grown. Both J3 and J4 lack a functional stylet and do not feed, while the stylet is reinstated in females and males. However, males do not feed and leave the roots of soybean plants to reside in the soil. Females feed on modified giant cells (Fig. 1.5) in plant roots (Moens *et al.*, 2009).

Different types of reproduction have evolved in *Meloidogyne* spp. Parthenogenesis is the most common for this genus (Moens *et al.*, 2009; Decraemer *et al.*, 2014) and depending on the species the mechanism may either be mitotic or meiotic. In meiotic parthenogenesis (e.g. *M. chitwoodi* Golden, O'Bannon, Santos & Finley (1980), *M. exigua* Goeldi, (1892) , *M. fallax* Karssen (1996), *M. graminophila* Golden & Birchfield (1965) and *M. minor* Karssen, Bolk, Van Aelst, Van den Beld, Kox, Korthals, Moldendijk, Zijlstra, Van Hoof & Cook (2004), the second nuclear division does not occur so that the normal somatic chromosome number is restored and embryogenesis can proceed. In mitotic parthenogenesis (*M. arenaria*, *M. incognita* and *M. javanica*), the mature oocyte undergoes a single mitotic division forming a diploid egg (Jones & Goto, 2011; Perry *et al.*, 2013).

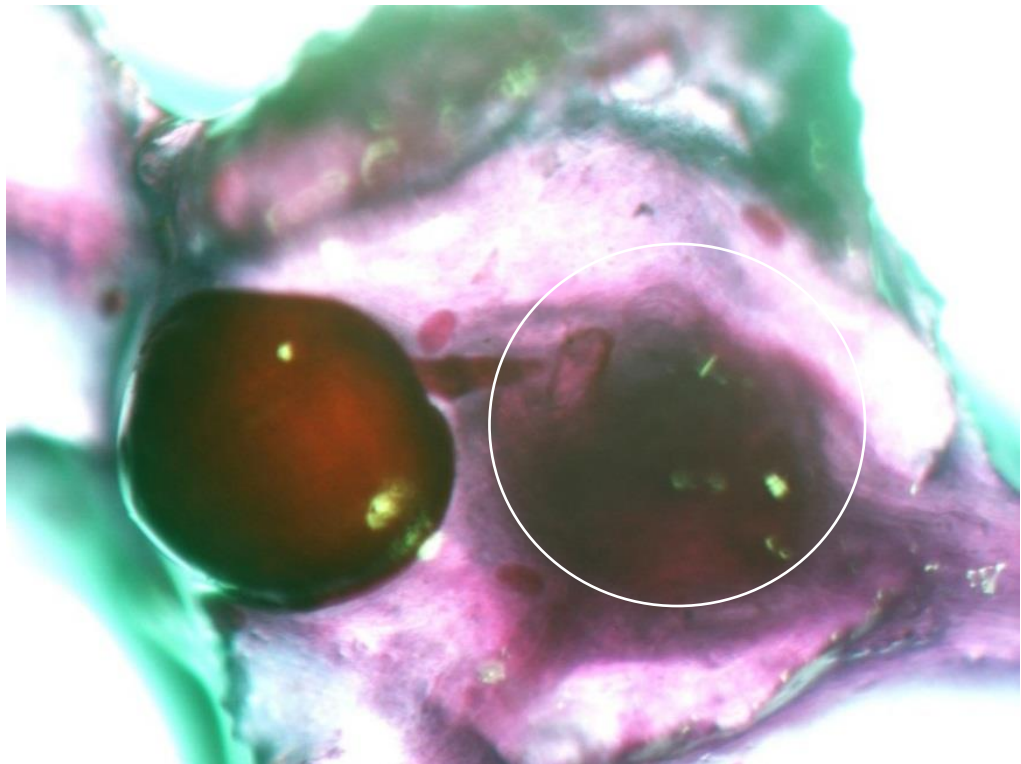


Fig. 1.5. A stereomicroscope photo of a red-stained, swollen *Meloidogyne* female (left side of picture) feeding from a cluster of giant cells (encircled in white) in the root of a soybean (cv. Superboon) plant (Photo: Driekie Fourie, NWU, 40x magnification).

2.2 Symptoms caused by *Meloidogyne* spp.

Damage, e.g. galls/knots (Fig. 1.6A) on soybean roots, caused by *Meloidogyne* spp. (Jones & Fosu-Nyarko, 2014) generally varies with the population density. Damage to plants are generally visible when the nematode pest population exceeds the damage threshold level. Root-knot nematode galls can be distinguished from *Rhizobium* nodules in that the latter can easily be rubbed off the root surface opposed to galls that cannot be rubbed off, but form an integral part of the root. Inside *Rhizobium* nodules are furthermore either pinkish or greenish in colour, depending on their development stages. However, nodules may be infected and galled due to root-knot nematode infection (Bridge *et al.*, 2005). Other below-ground symptoms include reduced and abnormal distorted growth of infected roots (Guerena, 2006). Of importance, however, is that producers and extensionists should remove soybean plants from the soil to investigate the roots for the presence of galls to make a diagnosis. Conversely, aerial symptoms may be absent or confused with those caused by other abiotic and biotic pathogenic factors, *i.e.* drought, excessive

heat, and nutrient uptake (Powell *et al.*, 1971). Severe stunting, wilting, yellowing and sometimes the death of soybean plants may occur as a result of *Meloidogyne* infection (Fig. 1.6B).



Figure 1.6A & B. Galling caused by root-knot nematode infection of soybean roots (A) and a soybean field with pronounced areas with poor stand and yellowish plants (B) that survived root-knot nematode parasitism (Pictures courtesy: www.lsuagcenter.com).

2.3 Interactions of *Meloidogyne* spp. with disease-causing bacteria and fungi

Back *et al.* (2002) and Manzilla-López & Starr (2009) reported that the concurrent presence of root-knot nematodes with pathogens such as bacteria and fungi in crop fields may incite and aggravate diseases. For example, when a root-knot nematode J2 pierces the cell walls of soybean roots with its stylet, the resultant tissue damage may serve as an entry point for bacteria and fungi (Sikora *et al.*, 2005; Siddiqui *et al.*, 2012). According to Agarwal and Goswami (1973), when *M. incognita* and the fungus *Macrophomina phaseolina* were inoculated simultaneously, the mortality of soybean plants was highest (30 %), whereas 25 % of plants showed collar rot symptoms and wilted completely. Also, root and shoot lengths of infected plants showed a marked decrease compared to plants inoculated with either *M. incognita* or fungi. When *M. javanica* J2 were inoculated one week before *Rhizoctonia solani* and *M. phaseolina*, a significant reduction in soybean plant height (48 %) and dry plant mass (65 %) was recorded (Stephan *et al.*, 2006). Bacterial diseases such as bacterial blight (caused by *Pseudomonas syringae* pv. *glycinea*), bacterial pustule (*Xanthomonas campestri* pv. *phaseoli*) and wildfire (*Pseudomonas syringae* pv. *tabaci*) are reported to significantly

suppress soybean yield (Susianto *et al.*, 2014; Murithi *et al.*, 2015). Various fungal diseases may cause the sudden death syndrome on soybean. These include *Fusarium solani* f. sp. *glycines*, stem rot (*Sclerotinia sclerotium*), seed rot and seedling death (*R. solani*), charcoal root rot (*M. phaseolina*) and soybean rust (*Phakopsora pachyrhizi*).

No documented information is available on the complex of diseases caused by root-knot nematodes and bacteria or fungi in South Africa. It is suggested, that by controlling root-knot nematode pests, soybean crops will to a certain extent also be protected against secondary infections by these pathogens.

2.4 Potential soybean crop yield losses caused by *Meloidogyne* spp.

Soybean yield losses of \$US 50 million per annum have been reported worldwide (Musarrat *et al.*, 2012). In South Africa, infection by plant-parasitic nematodes was estimated to cause 9 % yield losses of soybean crops in the 1980s (Keetch, 1989). This figure, however, did not distinguish between the contribution of a particular nematode pest and represented the damage caused by all plant-parasitic communities present. Total yield losses in 1998 as a result of crop destruction by root-knot nematodes in soybean cultivar trials of the ARC-GCI (Smit & De Beer, 1998). Fourie and Mc Donald (2001b) and Fourie *et al.* (2010) reported yield losses ranging between 25 and 70 % in field and glasshouse experiments as a result of single-species populations of *M. incognita* and *M. javanica*. Despite these recorded yield losses, it is believed that the impact of root-knot nematodes on soybean production is most probably underestimated because these results emanated from research conducted when soybean production was still relatively small compared to the current situation.

2.5 Nematode management strategies

The main strategies used to manage root-knot nematode pests in soybean fields worldwide are chemical control, crop rotation and genetic host plant resistance (Bridge & Starr, 2007; Nyczepir & Thomas, 2009).

2.5.1 Chemical control

Chemical control constitutes the use of synthetically-derived nematicides that was and still is a very effective and popular nematode control tool to optimise soybean yields (Mueller, 2015). However, lately the use of several nematicides has been restricted, or in some instances forbidden, due to their toxicity and adverse effects on animals, humans and the environment (Castillo *et al.*, 2006; Kearns *et al.*, 2014). For example, ethylene dibromide (EDB) was withdrawn by the Environmental Protection Agency (EPA) in 1983 due to possible groundwater contamination (Rich *et al.*, 2004). Ultimately, undesirable levels as an air pollutant and possible contribution to ozone layer depletion lead to the ban of methylbromide in 2001 (Reitze, 2001).

Although a range of nematicides are registered for use on soybean in other countries such as the USA (Mueller, 2015), no products are registered for use in soybean locally (Van Zyl, 2013). Although nematicides were evaluated for use on soybean to reduce root-knot nematode population densities in the early 2000s, their application and dosage rates were not cost-effective (Fourie & McDonald, 2001; Fourie & McDonald, 2007). However, with new-generation cultivars being released this situation may no longer be valid since substantially higher yields are nowadays obtained compared to those of traditional cultivars in earlier years. Although the production per hectare for soybean is higher (mean of 0.814 tons/ha during 2005 compared to a mean of 1.492 tons/ha in 2015/16) and the application of nematicides may be economically viable (Dlamini *et al.*, 2014; Grain SA, 2017), improvement in yield of soybean cultivars should be realised to make synthetic nematicides a viable option. Although seed-coat products, which are a cost-effective option to reduce nematode pest populations, have been registered on maize in South Africa (Van Zyl, 2013) no such product(s) have been registered to date for soybean.

2.5.2 Crop rotation

Crop rotation is the oldest and arguably one of the most important approaches to reduce root-knot nematode damage to soybean roots since it is aimed at reducing initial population densities of these pests (McSorley, 2011). This approach generally enables the succeeding crop to become established and complete early development before being heavily attacked by root-knot nematode pests. This, however, only applies should the previous crop be a poor or resistant host of the

target nematode pests (*i.e.* *Meloidogyne* spp.) (Nusbaun & Ferris, 1973; Trivedi & Barker, 1986). For example, velvet bean (*Mucuna deeringiana* L.) in rotation with soybean reduced mixed populations of *H. glycines* and *M. arenaria* in the USA (Weaver *et al.* 1993). Also, in a 3-year soybean-groundnut (*Arachis hypogaea* L.) rotation study (Rodríguez-Kábana *et al.* 1988), groundnut following either one or two years of soybean resulted in a 50 % reduction of *M. arenaria* J2 population densities. Soybean yield also increased during the the latter study compared to that of plots where monoculture groundnut crops were planted.

The situation in South Africa is, however, different in that crop rotation has seldom (if ever) been successfully applied to reduce root-knot nematode population densities. The reason is that all crops, e.g. dry bean (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogaea* L.) (Riekert & Henshaw, 1998), maize (*Zea mays* L. (De Waele & Jordaan, 1988; Riekert & Henshaw, 1998), potato (*Solanum tuberosum* L. (Engelbrecht, 2012; Agenbag, 2016), sunflower (*Helianthus annuus* L. (Bolton *et al.*, 1989) and other vegetables (Fourie *et al.*, 2012) grown in soybean-based cropping systems are susceptible to highly susceptible to prevailing, predominantly *M. incognita* populations (Fourie *et al.*, 2015). It is hence agreed with Weaver *et al.* (1988) and Moens *et al.* (2009) that crop rotation to combat root-knot nematode pests is still a challenge due to the polyphagous nature of these nematodes. Ultimately, the use of poor or non-host crop cultivars included in crop rotation sequences is regarded the most effective method to limit root-knot nematode reproduction (Karssen *et al.*, 2013; Van Biljon *et al.*, 2015). Unfortunately, only a few (if any) poor-host and resistance crop cultivars have been identified against the predominant root-knot nematodes that occur in South African soils (Ngobeni *et al.*, 2010; Fourie *et al.*, 2013; Steenkamp *et al.*, 2014; Fourie *et al.*, 2015).

2.5.3 Genetic host plant resistance

Genetic host plant resistance is described as the inability of the target nematode pest to produce functional feeding sites in the host plant after invasion and subsequently to develop and reproduce optimally. This way optimal development and reproduction of these pests are hampered and their damage potential decreased in soybean (Davis, 1998; Fourie *et al.*, 2015). According to Lilley *et al.* (2011), the introgression of genetic resistance is achieved by exploiting and transferring natural-

occurring resistance genes from wild relatives through conventional or advanced breeding. As mentioned above, genetic host plant resistance is most probably the most practical, popular and economical management strategy to control *Meloidogyne* spp. that damage soybean crops. Most important, it is an eco-friendly strategy (Williamson, 1999; Starr & Mercer, 2009). However, it is paramount that the identity of the target *Meloidogyne* spp. is known to apply genetic host plant resistance effectively.

Success in identifying and developing root-knot nematode resistant soybean cultivars has been reported worldwide, particularly to the thermophilic species *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*, (Hussey *et al.*, 1991; Roberts, 1992; Starr *et al.*, 2002; Das *et al.*, 2010; Beneventi *et al.*, 2013; Jiao *et al.*, 2015; Fourie *et al.*, 2015). Locally, different levels of resistance to root-knot nematodes have been reported (Van den Berg & McDonald, 1991; Van Biljon, 2004; Fourie *et al.*, 2006; Fourie *et al.*, 2013; Venter, 2014). Interestingly, only two locally adapted commercially available cultivars with different levels of resistance to *M. incognita*, viz. Egret and DM 6.2i RR, are available for use by South African producers (Venter, 2014; Fourie *et al.*, 2015). It is, however, important to bear in mind that continuous planting of the same resistant cultivar(s) must be avoided because virulent biotypes of the target *Meloidogyne* spp. may occur and render the strategy unsuccessful (Halbrendt & LaMondia, 2004). Some studies have demonstrated that resistance-breaking populations of *Meloidogyne* spp. can arise following continuous exposure to the same root-knot nematode resistant soybean cultivars (Swanson & Van Gundy, 1984; Windham & Barker, 1986; Noe, 1992; Trudgill & Blok, 2001). Pedrosa and Moura (2001) demonstrated in a glasshouse experiment that an increase in *M. arenaria* race 1 reproduction occurred after growing resistant soybean genotypes CNS, Jackson and PI 200538 in four consecutive experiments. Conserving the root-knot nematode resistance genes identified and used in soybean crops is hence of utmost importance to ensure sustainable production of the crop as well as other rotation crops.

3 Nematodes as bio-indicators of soil quality

Any bio-indicator (which is nematodes for the purpose of our study) must reflect the structure and function of ecological processes, and respond to changes in soil

conditions that result from agricultural management practices (Neher, 2001; Neher & Darby, 2009). Moreover, there must be adequate taxonomic knowledge to identify bio-indicators accurately and efficiently. Doran and Zeiss (2000) suggested that indicators of soil health should meet the following criteria: i) be sensitive to variations in management, ii) be well correlated with beneficial soil functions, iii) be useful for elucidating ecosystem processes, iv) be comprehensible and useful to land managers and v) be easy and inexpensive to sample and measure. Soil nematodes meet these criteria as they particularly respond to changes in food supply and anthropogenic disturbances caused by agricultural management practices (Porazinska *et al.*, 1999; Yeates, 1999a; Sánchez-Moreno *et al.*, 2006; Ferris *et al.*, 2012; Stirling, 2014). The use of nematodes as bio-indicators for aquatic environments, for example, dates back to the 1970s (Samoiloff, 1987). However, only since the 1980s more interest was shown in using nematode communities as indicators for monitoring of terrestrial ecosystems (Freckman, 1988; Bongers, 1990). During that period, researchers focused mainly on plant-parasitic nematodes because of their economic importance (Barker *et al.*, 1994; Bird & Kaloshian, 2003). In recent years, however, a better understanding of the beneficial role played by non-parasitic nematodes in terms of soil ecosystems became evident and inspired nematologists to focus on the entire nematode community, representing both plant-parasitic and freeliving, beneficial nematodes (Yeates, 1987; Bongers, 1990; Yeates *et al.*, 1993). Conventionally, all nematodes that are not animal parasites are referred to as non-parasitic, including the herbivores or plant-parasitic nematodes (Poinar 1983). However, in this study the term non-parasitic will be used in a more restricted sense to refer only to non-plant-parasitic, terrestrial nematodes.

Environmental disturbances due to agricultural practices can be divided into two groups, e.g. chemical and physical. Chemical disturbances include nutrient enrichment (fertilisers) and chemical pollutants such as herbicides (e.g. glyphosate and pesticides (Fiscus & Neher, 2005), whereas physical disturbances include practices associated with the cultivation of crops (Bongers & Bongers, 1998; Neher, 2001). For example, population densities of omnivorous- and predatory nematodes were reduced in a Polish study by the application of mineral fertiliser or organic fertiliser such as sheep manure (Wasilewska, 1989).

Soil nematodes, particularly non-parasitic species, are important role players in soil food web communities (Sochová *et al.*, 2006). Nematodes can be divided into at least five trophic or functional groups, viz. herbivores (classified as plant-parasites), bacterivores (feeding on bacteria), fungivores (feeding on fungi), omnivores (feeding on micro-organisms) and predators (feeding on prey such as other nematodes, mites and other micro-organisms) (Yeates *et al.*, 1993). In agro-ecosystems, nematodes are the basis of important ecosystem processes since they interact directly or indirectly with plants and provide different important ecological services in soil such as nutrient recycling, suppression of undesirable organisms, decomposition and biodegradation of harmful compounds (Bongers, 1990; Neher, 2001; Ferris & Bongers, 2006). For example, protozoa, bacteria, fungi and algae are primary decomposers of organic matter. Nematodes feed on micro-organisms that occur in decaying organic matter, thus regulating the decomposition rate of the substrate (Bongers & Bongers, 1998). Bacterial-feeding and predatory nematodes were reported to contribute directly and indirectly 8 to 19 % to nitrogen mineralisation in soils (Beare, 1997). When bacterivores grazed on bacteria in a microcosm experiment, soil mineral nitrogen levels increased by 20 % or more (Ferris *et al.*, 1998). Chen & Ferris (1999), observed an increase in nitrogen mineralisation when fungi were grazed by the fungivore *Aphelenchus avenae* (Bastian, 1865) Cobb, 1927 and *Aphelenchoides composticola* Fischer, 1894.

3.1 Measuring nematode diversity and community structure

The abundance of each nematode functional group in a soil community can be transformed into ecological indices and parameters. These are used to measure community changes in diversity and trophic structure and ultimately assess soil disturbance levels and decomposition pathways (Bongers, 1990; Ferris, 2010). Nematode communities can be studied on the basis of trophic functional groups (see above) which are regarded as having similar effects on the ecosystem processes (Ferris *et al.*, 2001; Neher, 2001). The calculations that need to be done to determine indices and parameters for use in nematode faunal analysis are challenging. However, less sophisticated programs such as an automated calculation system for nematode-based biological monitoring (NINJA) to interpret data in a more comprehensive manner (Sieriebriennikov *et al.*, 2014) have been developed recently. This specific programme provides food web indicators, resource availability,

status and functionality of an ecosystem, is easily assessable online and is user-friendly.

Ecological indices based on the proportional contribution of each nominal nematode taxon were initially proposed for assessing the stability or disturbance in terrestrial ecosystems (Bongers, 1990). Ecological indices condense information regarding the structure and composition of communities and are based on the following: Maturity Index (MI), Enrichment Index (EI), Structure Index (SI), Channel Index (CI) and Metabolic footprints (Bongers, 1990; Ferris *et al.*, 2001; Neher & Darby, 2006). The MI is used as a measure of the ecological successional status of a soil nematode community. It is based on the principle that different taxa have contrasting sensitivities to stress or disruption of the successional sequence because of their life history characteristics. Bongers (1990) initially suggested separate MI calculations for non-parasitic and plant-parasitic nematodes because the latter may or may not have positive correlations with MI (Neher & Campbell, 1994; Bongers *et al.*, 1997). For the calculation of MI, nematodes are assigned to classification at family level along coloniser-persister (c-p) guilds, using a scale from 1 to 5 (Table 1.2).

Table 1.2. Description of coloniser-persister (c-p) scale classification of nematodes as used in soil food web diagnostics (Bongers & Ferris, 1999; Ferris *et al.*, 2001; Neher, 2001).

cp-value	Nematode description	Example
1	Short life cycles, small eggs, high fecundity, mainly bacterivores, feed continuously in enriched media, form dauer larvae as microbial blooms outside and relatively tolerant to pollution-induced stress.	Bacterial feeders such as Rhabditids, Diplogastrids and Panagrolaimids.
2	Longer life cycles and lower fecundity than c-p1 group, very tolerant to adverse conditions and may become cryptobiotic. Feed more deliberately and continue feeding as resources decline.	Mainly smaller Tylenchids feeding on epidermal cells; fungivores (Aphelenchoids and Anguinids); and bacterivores (Cephalobes, Plectids and Monhysterids).
3	Nematodes with characteristics that fall between c-p groups 2 and 4, longer life cycles and greater sensitivity to adverse conditions.	Mainly bacterivores (Araeolaimids and Chromadorids); larger Tylenchids feeding on deeper root cells; the Diphtherophorids and carnivorous Tripylids.
4	Nematodes with longer life cycles, lower fecundity and greater sensitivity to disturbance.	Small omnivore species, small Dorylaimids and large non-Dorylaimids; larger carnivores; bacterial feeding (Alaimids and Bathyodontids); and plant-feeding Trichorids.
5	Large dorylaimids with longest life cycles, largest body sizes, lowest fecundity and greatest sensitivity to disturbance.	Predominantly herbivores, carnivores and omnivores.

Bongers (1990) only assigns c-p values to nematode families, due to a lack of information on the biology and sensitivity of certain nematode genera to soil contamination or changes. By contrast, the NINJA programme allows the assignment of c-p values to genus level (Sieriebriennikov *et al.*, 2014). With the NINJA output, disturbed or enriched environments are indicated by low MI values whereas high MI values indicate stable environments (Figure 1.7). This system assumes that taxa within one c-p class are similar in their responses to environmental disturbances. For example, ‘colonisers’, (*r* strategists) rapidly reproduce under favourable conditions, have a short life cycle, release large numbers of small eggs, have a high colonisation ability and are tolerant to disturbance, eutrophication and anoxymbiosis. Conversely, ‘persisters’, (*K* strategists) have low reproduction rates, high longevity, low colonisation abilities, are sensitive to disturbance, hardly dominate in a sample, rarely fluctuate during cold periods of the year, produce large eggs and few offspring and live in habitats with fewer disturbances (Bongers, 1990; Bongers & Bongers, 1998; Bongers & Ferris, 1999).

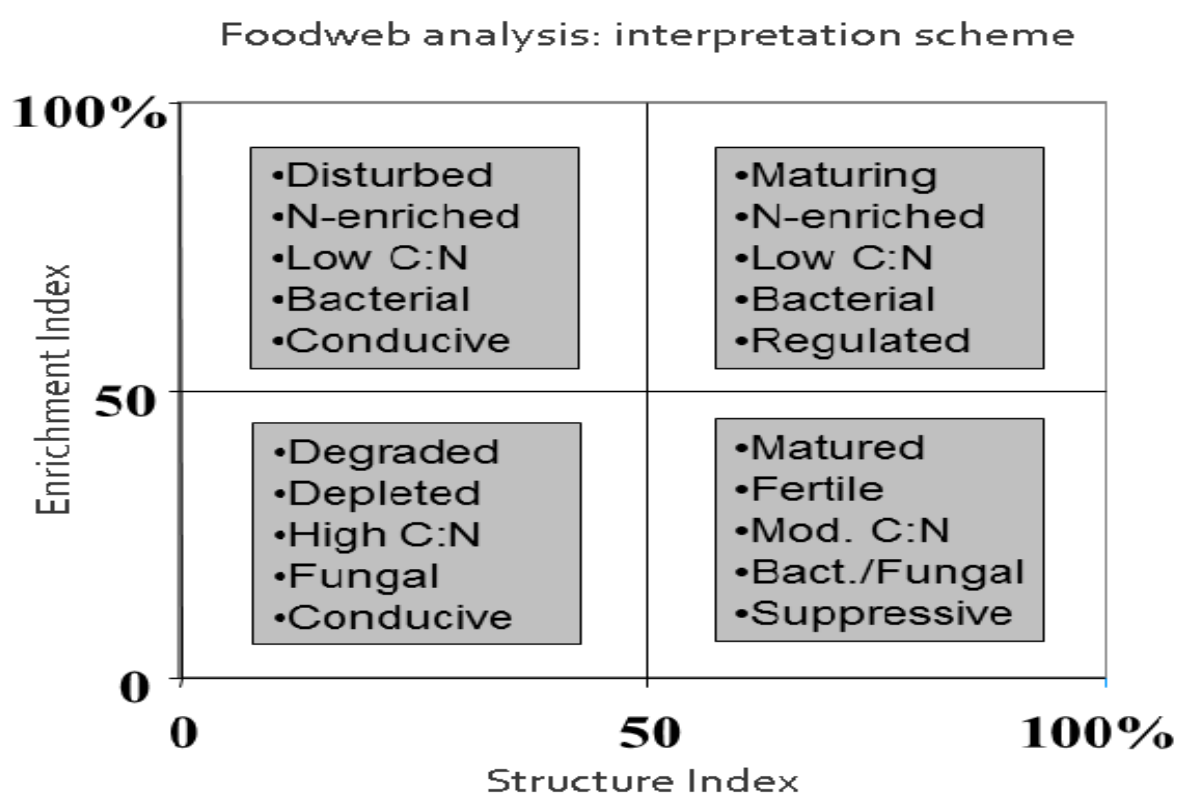


Figure 1.7. A food web structure interpreted on enrichment index (EI) and structure index (SI) for free-living nematodes using ‘An automated calculation system for nematode-based biological monitoring’ (NINJA) program (Sieriebriennikov *et al.*, 2014).

The combination of nematode feeding groups and c-p scaling into functional guilds resulted in a more powerful technique for determining soil health/quality and food web status (Bongers & Bongers, 1998; Ferris *et al.*, 2001). For example, Ferris *et al.* (2001) used nematode functional guilds to calculate EI, SI and CI. The EI is based on how non-parasitic nematode guilds respond to food resource enrichment; SI represents an aggregate of longevity, body size and disruption sensitivity of functional guilds (Fig. 1.7); CI indicates the decomposition channel through the soil food web with low CI values suggesting a primarily bacterial decomposer community and high values a fungal dominated decomposition. The point in the middle of a rhombus represents the intersection of EI and SI, and length of vertical and horizontal axes of the rhombus corresponds to the footprints of enrichment and structure components, respectively (Bongers, 1990; Bongers & Bongers, 1998; Ferris *et al.*, 2001; Berkelmans *et al.*, 2003; Liang *et al.*, 2005; Ferris, 2010; Sieriebriennikov *et al.*, 2014). Ferris (2010), in addition proposed the measuring of the metabolic footprint of terrestrial nematodes, which quantifies the amplitude of carbon utilisation by different food web components.

3.2 The effects of glyphosate on soil health, with reference to nematode communities

As explained earlier in Section 3, soil nematodes respond to changes (positive or negative) in the soil substrate. Application of products, e.g. pesticides, hence impact on either fauna (including nematodes) or flora, or both.

Genetically-modified crops may affect non-target micro-organisms, either directly or indirectly, as is the situation with glyphosate application to tolerant crops (Noel & Wax, 2009). However, limited research has been done on the impact of glyphosate on soil-inhabiting organisms, with contradicting findings being reported (Kremer & Means, 2009; Duke *et al.*, 2012; Lane *et al.*, 2012). Some scientists argue that intensive application of glyphosate to control weeds impacts on soil ecology, leading to either negative or positive alterations in soil microflora and/or -fauna populations (Gimsing *et al.*, 2004; Battaglin *et al.*, 2005; Neumann *et al.*, 2006; Kremer & Means, 2009; Benamú *et al.*, 2010; Zaller *et al.*, 2014). The following part of this chapter summarises both negative and positive results reported on the application of glyphosate on soybean, even though it may not necessarily be on soil nematodes.

In field trials conducted by Kremer & Means (2009) in the USA, colonisation of *Fusarium* spp. was higher on glyphosate-tolerant soybean roots within one week after glyphosate application. The magnitude of this *Fusarium* infection was generally two to five times higher than that recorded from soybean that was cultivated in untreated soil (no glyphosate application but application of non-glyphosate herbicides). Sanogo & Scherm (2000), reported that both in growth chamber and glasshouse experiments, a significant increase in disease severity and frequency of isolation of *F. solani* f. sp. *glycines* was recorded from roots of different soybean cultivars after application of glyphosate compared to the non-treated control plants. In another study, glyphosate was reported to be toxic to *Rhizobium* (nitrogen-fixing bacteria) and mycorrhizal fungi (*Rhizophagus*), which are known for their beneficial effects in terms of the physiology and health of soybean plants (Moorman & Dowler, 1991). Zobiolo *et al.* (2010) observed decreased nitrogen fixation in glyphosate-tolerant soybean which resulted directly from toxicity of glyphosate to nitrogen-fixing micro-organisms. Moreover, photosynthetic variables such as chlorophyll, photosynthetic rate, transpiration and stomatal conductance were significantly reduced in glyphosate-tolerant soybean plants. Liphadzi *et al.* (2005) reported that after three years of continuous glyphosate application in a soybean/maize crop sequence, the abundance of soil micro-organisms was not altered in glyphosate-treated plots compared to those to which conventional herbicides were applied.

A dearth of information exists regarding the effect of glyphosate on soil nematodes. In the United Kingdom, population densities (eggs in soil) of the potato cyst nematodes *Globodera pallida* (Stone, 1973) Behrens, 1975 and *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 in soybean fields were significantly reduced (by 65-72 %) compared to their initial population densities after application of glyphosate (Dewar *et al.*, 2000). Noel & Wax (2009) reported that glyphosate application increased the final population densities of *H. glycines* eggs in a field study, while a glasshouse study by Yang *et al.* (2002) did not show an interaction between glyphosate and population densities of the soybean cyst nematode. Liphadzi *et al.* (2005) observed no differences in total nematode population densities and trophic groups among soybean/maize cropping systems over a three-year, treated with glyphosate and those treated with other conventional herbicides. When glyphosate was used in combination with 2-methyl-4-

chlorophenoxyacetic acid (MCPA®), its application negatively affected the penetration of infective juveniles of the entomopathogenic nematode species *Steinernema feltiae* Filipjev, 1934 and *Heterorhabditis bactriophora* Poinar, 1976 into larvae of the wax moth *Galleria mellonella* (García-del-Pino & Morton, 2010).

The only study conducted under South African environmental conditions on the effects of glyphosate on nematodes and micro-organisms was done by Jansen (2014). The comparative effects of glyphosate on microbial and nematode communities in conventional and glyphosate-tolerant soybean ecosystems at six sites in local production areas showed that no statistical difference existed between the abundance and diversity of these two biological entities. It is, however, important to note that this study was conducted during one season only and during drought year, which may have affected the results.

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Chapter 2

Host response of South African soybean genotypes to *Meloidogyne incognita* infection

Abstract

The host response of 36 locally adapted soybean genotypes to *Meloidogyne incognita* infection was evaluated in two separate glasshouse experiments. The experiments were terminated 56 days after inoculation of ca. 1 000 eggs and second-stage juveniles (J2; Pi) of an *in vivo* reared *M. incognita* population. The final nematode population density (Pf) per root system, reproduction factor (Rf) and relative percentage susceptibility (%R) were used as parameters to classify the host response of the soybean genotypes. Substantial variation of the parameters measured was observed among the 36 soybean genotypes and between the two experiments. A significant interaction existed between the two experiments, due to differences in the ambient temperature ranges. In the experiment with the highest ambient temperature range, higher population densities of *M. incognita* were recorded. In this experiment, only line PRF-GCI7 and the resistant reference cultivar LS 5995 had a Rf value < 1. DM 6.2i RR had a Rf value = 1 and %R value ≤ 2 %. Since DM 6.2i RR is the only commercially available cultivar in South Africa it could be grown in areas where *M. incognita* may cause or is already causing yield losses. Alternative options to use are cultivars with a Rf value ≤ 5 and/or cultivars that had a %R value ≤ 10 %. The host response of local soybean cultivars to *M. incognita* and other *Meloidogyne* spp. that are prevalent in the local soyabean-based production areas in South Africa should be done on a regular bases to provide producers with the most up-to-date information.

Key words: genotypes, *Glycine max*, resistance, root-knot nematodes, screening.

2.1 Introduction

Root-knot nematodes (*Meloidogyne* spp.) are the most widespread and damaging nematode pests that parasitise agricultural crops worldwide (Jones *et al.*, 2013) including South Africa (Kleynhans *et al.*, 1996; Fourie *et al.*, 2015a). Locally, estimated soybean yield losses due to *Meloidogyne* spp. range between 25 and 100 % (Riekert & Henshaw, 1998; Fourie *et al.*, 2015b). *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, is the most common root-knot nematode species in soybean fields, followed by *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Fourie *et al.*, 2001a). These two species either occur in single or mixed populations in local soybean-based cropping systems (Riekert & Henshaw, 1998; Liebenberg, 2012; Fourie *et al.*, 2015a). Recently, Agenbag (2016) also identified *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, and *Meloidogyne enterolobii* Yang & Eisenback, 1983, in maize production areas where soybean is increasingly grown as a rotation crop.

In the past, the most effective strategy used to control plant-parasitic nematodes was achieved by the use of chemical nematicides (Niblack *et al.*, 2004; Nyczepir & Thomas, 2009). During the past decade, nematicides have been increasingly withdrawn from world markets or their use restricted due to their adverse effects on the environment and their toxicity to animals and humans (Rich *et al.*, 2004; Haydock *et al.*, 2013). The withdrawal of several Class I nematicides encouraged the development and implementation of eco-friendly nematode management strategies (Stirling, 2011). Evaluation of various synthetically-derived nematicides at different dosages during the early 2000s to control *M. incognita* and *M. javanica* on soybean did not yield economically viable results (Fourie & McDonald, 2001, 2007). To date, no nematicide has been registered for use on soybean in South Africa (Van Zyl, 2013). However, with the current substantial higher value of soybean the situation has changed and it may be economically feasible to evaluate the use of nematicides again.

An alternative management strategy that is being used with success in various countries to reduce the adverse impact of root-knot nematodes on soybean and follow-up crops is host plant resistance (Sikora *et al.*, 2005; Bridge & Starr, 2007; Oyekanmi & Fawole, 2010; Matsuo *et al.*, 2015). Host plant resistance is defined as

the ability of a host plant to inhibit or limit the reproduction of a pathogen (Bos & Parlevliet, 1995). The relative level of resistance is based on the comparison with a highly susceptible reference plant. A highly resistant plant will allow only a very low nematode reproduction level while, for instance, a moderately resistant plant will allow an intermediate reproduction level (Roberts, 2002; Matsuo *et al.*, 2015). In less developed countries and in low cash crop systems, host plant resistance is considered the most viable solution to nematode problems (Roberts, 2002).

In annual cropping systems where soybean is included as a rotation crop, root-knot nematode resistant cultivars can reduce the population densities of these pests to levels that are non-damaging to the soybean as well as the follow-up crops (Sharma *et al.*, 2012). Since the 1970s, considerable progress has been made in identifying resistant cultivars and breeding for *M. incognita* resistance in soybean (Hussey & Boerma, 1981; Luzzi *et al.*, 1997; Davis *et al.*, 1998; Fourie *et al.*, 2006). Since the late 1990s, numerous soybean cultivars with resistance to *M. incognita* have been reported in countries such as the United States of America (USA) (Allen *et al.*, 2005), Brazil (De Oliveira *et al.*, 2015), China (Jiao *et al.*, 2015) and South Africa (Fourie *et al.*, 1998; Mienie, *et al.*, 2002; Van Biljon, 2004; Fourie *et al.*, 2008). The situation in South Africa is, however, not promising since only two soybean cultivars with resistance to *M. incognita* are available (Venter *et al.*, 2014; Fourie *et al.*, 2015b). These are the conventional cultivar Egret and the genetically-modified (GM) cultivar DM 6.2i RR that is tolerant to glyphosate. Another highly resistant cultivar (LS 5995), identified in the early 2000s, is no longer available for commercial production (Fourie *et al.*, 2015b). Although *M. incognita* resistance has been introgressed from LS 5995 into seven conventional lines that are adapted to local environmental conditions, this material has not been commercialised since the lines do not contain the gene inducing glyphosate tolerance (Fourie *et al.*, 2015b).

From a nematologists' view, the aim of breeding for root-knot nematode resistance in crops should be to reduce the population densities of the nematode pest in soil over time while concurrently ensuring acceptable yields (Tripathi *et al.*, 2015). For soybean, this has been demonstrated by various authors who reported increased soybean yields using *M. incognita*-resistant cultivars compared to their susceptible counterparts (Niblack *et al.*, 1986; Allen *et al.*, 2005; Carpentieri-Pípolo, 2005;

Kinloch, 2012; De Oliveira *et al.*, 2015). Under South African environmental conditions, Fourie *et al.* (2013) showed an increase of 22 % in the yield of the *M. incognita*-resistant cultivar LS 5995 compared to a susceptible reference cultivar. This part of the study focused on determining the host response of 36 soybean genotypes (including 30 commercially available South African cultivars) to *M. incognita* with the objective to find much-needed additional sources of resistance.

2.2 Materials and methods

2.1.2.1 *Meloidogyne incognita* inoculum

A population of *M. incognita* was obtained from infected maize roots in the Vryburg region in the Northern Cape Province. A single egg mass population (Fourie *et al.*, 2012) of this species was established and maintained in 25 000 cm³ plastic pots on the *M. incognita*-susceptible tomato cultivar Rodade (*Lycopersicon esculentum* L.) (Fourie *et al.*, 2012) in a greenhouse at the Eco-Rehab facilities of the North-West University, Potchefstroom. To obtain the nematode inoculum, eggs and second-stage juveniles (J2) of this population were extracted from the roots of six 30 to 40-day-old tomato plants using the adapted NaOCl method (Riekert, 1995). The root system was excised, the roots gently rinsed free of soil and adhering debris under running tap water, and 1 cm root segments shaken for 4 min in a 1 % NaOCl solution. Eggs and J2 released from the root tissues were collected on a 20 µm mesh sieve.

2.2.2 Plant material

Thirty commercially available glyphosate-tolerant soybean cultivars, (evaluated for their agronomical characteristics in the 2014/15 National Soybean Cultivar Trials of the Agricultural Research Council–Grain Crops Institute, Potchefstroom, South Africa) (De Beer, 2015) and four soybean lines with varying levels of *M. incognita* resistance (*viz.* PRF-GCI4, PRF-GCI5, PRF-GCI6 and PRF-GCI7; Fourie *et al.*, 2015b) were included in our study. The highly resistant cultivar LS 5995 (Fourie *et al.*, 2006) and the *M. incognita*-susceptible cultivar LS 6248 R served as the reference cultivars (Mbatyoti *et al.*, 2013; Venter, 2014).

2.2.3 Host response assessment

Fifteen seeds of each soybean genotype were planted in 4 000 cm³ plastic pots filled with Telone II-fumigated soil (dosage rate of 150 l/ha; active substance 1,3 dichloropropene at 1110 g/l). The sandy loam soil (5.3 % clay, 93.6 % sand, 1.1 % silt and 0.47 % organic matter content) had a pH (H₂O) of 7.5. Ten days after emergence, one seedling of each of the 36 genotypes was transplanted to 500 cm³ plastic tubes (Fig. 2.1) that contained the same soil as used to grow the seedlings. During transplanting of the seedlings in the plastic tubes, ca. 1 000 *M. incognita* eggs and J2 were inoculated by pipetting 2 ml of water (in which the eggs and J2 were suspended) onto the exposed root system of each seedling according to the procedure described by Corbett *et al.* (2011).

Nitrogen-fixing bacteria (*Bradyrhizobium japonicum*) obtained from Soygro Pty Ltd. (Potchefstroom) were inoculated concurrently with the eggs and J2 at a dosage rate of 300 µl/seed to optimise nitrogen fixation. The experiment was repeated once. A 14L:10D hour photoperiod and an ambient temperature range of 18–24 ±1 °C were maintained for the duration of the first experiment, while the temperature range for the second (repeat) experiment was 21–26 ±1 °C. Temperatures were recorded throughout the duration of each experiment using iButton®DataLogger ([www.https://www.ibuttonlink.com/collections/temperature-logging-ibuttons](https://www.ibuttonlink.com/collections/temperature-logging-ibuttons)). The lay-out of both experiments was a randomised complete block design (RCBD) with six replicates of each genotype. The plastic tubes containing the plants were rotated bi-weekly to ensure that the plants were exposed to similar light, temperature and humidity conditions in the greenhouse since gradients could occur. Plants were watered as needed, usually three times a week, and weekly supplied with 250 ml of Hoagland's nutrient solution/tube throughout the duration of the experiments (Hoagland & Arnon, 1950). The experiments were terminated 56 days after inoculation (DAI). Eggs and J2 were extracted from the roots of each genotype (Riekert, 2015) and counted using a stereomicroscope (60× magnification).



Figure 2.1. Set-up of the host response greenhouse experiment showing 500 cm³ plastic tubes filled with a fumigated sandy soil in which the soybean seedlings were grown for 56 days after inoculation with ca. 1 000 eggs and second-stage juveniles(J2) of *Meloidogyne incognita*.

The reproductive potential of *M. incognita* in the roots of each genotype was calculated using Oostenbrink's reproduction factor (Rf) according to the following equation: $Rf = \text{final number of eggs} + J2 \text{ (Pf)} / \text{inoculated number of eggs} + J2 \text{ (Pi)}$ (Windham & Williams, 1987). In addition to this, the percentage of the Pf in the roots of each genotype in relation to the Pf in the roots of the most susceptible genotype (including the susceptible reference cultivar) was calculated and used as an additional parameter (%R = relative percentage susceptibility) to classify the level of resistance (Hussey & Janssen, 2002).

2.2.4 Statistical analyses

Data of both experiments first analysed using Analyses of Variance (ANOVA). Thereafter, the data were submitted to Factorial Analysis of Variance (Statistica, Version 13) and means were separated with Tukey's HSD Test ($P \leq 0.05$). Treatments (genotypes) represented Factor 1 and experiments (first and second) Factor 2. Nematode counts were $\log(x+1)$ transformed before statistical analysis to reduce variation. Real means are included in Table 2.1 and A1 and in Annex 1.

2.3 Results

2.3.1 Final population densities (Pf; eggs + J2) per root system

No interaction ($F = 1.1$, $P = 0.381$) was observed for Pf/root system in terms of genotypes x experiments. However, a significant interaction existed between the two experiments with regard to the host response of the 36 genotypes to *M. incognita* ($F = 338.3$, $P \leq 0.001$) (Fig. 2.2; Table 2.1). This was the result of a higher Pf in Experiment 2 compared to Experiment 1. The Pf of the genotypes also differed significantly ($F = 8.2$, $P \leq 0.001$) from each other in each of the two experiments.

For Experiment 1, egg and J2 numbers per root system ranged from 42 (PRF-GCI7) to 7 563 (DM 5.1i R), while it was substantially higher in Experiment 2 in which it ranged between 556 (PRF-GCI7) to 57 068 (DM 5953 RSF) (Table 2.1).

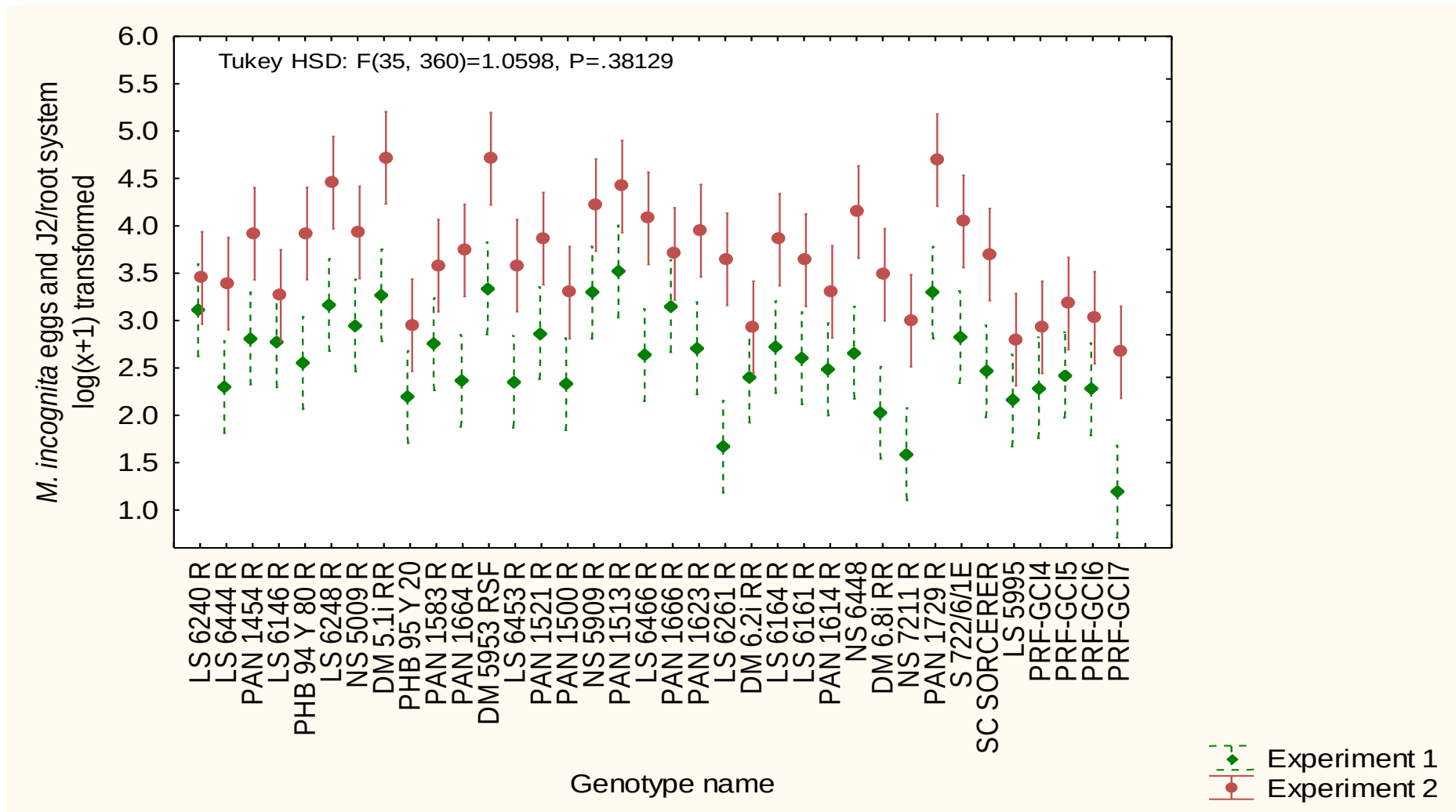


Figure 2.2. *Meloidogyne incognita* eggs and J2 per root system extracted from the roots of 36 soybean genotypes adapted to South African climatic conditions 56 days after inoculation with ca. 1 000 eggs and J2. The data of two separate glasshouse experiments are shown. Data are log(x+1) transformed.

2.3.2 Reproduction factor (Rf)

An interaction existed for Rf in terms of genotypes x experiments, with the Rf values differing significantly ($F = 4.7$, $P \leq 0.001$) from each other each genotype for the two experiments (Fig. 2.3). The Rf values of the genotypes differed also significantly ($F = 16.9$, $P \leq 0.001$) from each other in each of the two experiments.

For Experiment 1, Rf values ranged from 0.04 (PRF-GCI7) to 7.56 (DM 5.1i RR) (Table 2.1). This cultivar and others, viz. DM 5953 RSF, LS 6240 R, LS 6248 R, NS5009 R, NS 5909 R, NS 6448 R, PAN 1500 R, PAN 1513 R, PAN 1521 R, PAN 1583 R, PAN 1666 R and PAN 1729 R had Rf values > 1 , indicating susceptibility. The other genotypes had Rf values < 1 , indicating resistance.

For Experiment 2, the Rf values of all the genotypes were generally higher compared to that of Experiment 1 and ranged from 0.57 (PRF-GCI7) to 57.07 (DM 5953 RSF) (Table 2.1). Only two genotypes (PRF-GCI7 and LS 5995) showed resistance to *M. incognita* with Rf values < 1 , while all the other genotypes were classified as susceptible (Rf > 1).

2.3.3 Relative percentage susceptibility (%R)

In Experiment 1, the root systems of genotypes DM 6.8i RR, PRF-GCI4, PRF-GCI6, PRF-GCI7, LS 5995, LS 6161 R, LS 6261 R, LS 6444 R, LS 6453 R, NS 7211 R, PAN 1614 R, PAN 1623 R, PHB 94 Y 80 R and SC SORCERER maintained less than 10 % of the *M. incognita* population density present in the root system of the most susceptible cultivar DM 5.1i RR (Table 2.1).

In Experiment 2, genotypes DM 6.2i RR, DM 6.8i RR, PRF-GCI4, PRF-GCI5, PRF-GCI6, PRF-GCI7, LS 5995, LS 6146 R, LS 6240 R, LS 6444 R, LS 6453 R, NS 7211 R, PAN 1500 R, PAN 1583 R, PAN 1614 R, PHB 95 Y 20 sustained less than 10 % of the *M. incognita* population density present in the root system of the most susceptible genotype DM 5953 RSF (Table 2.1).

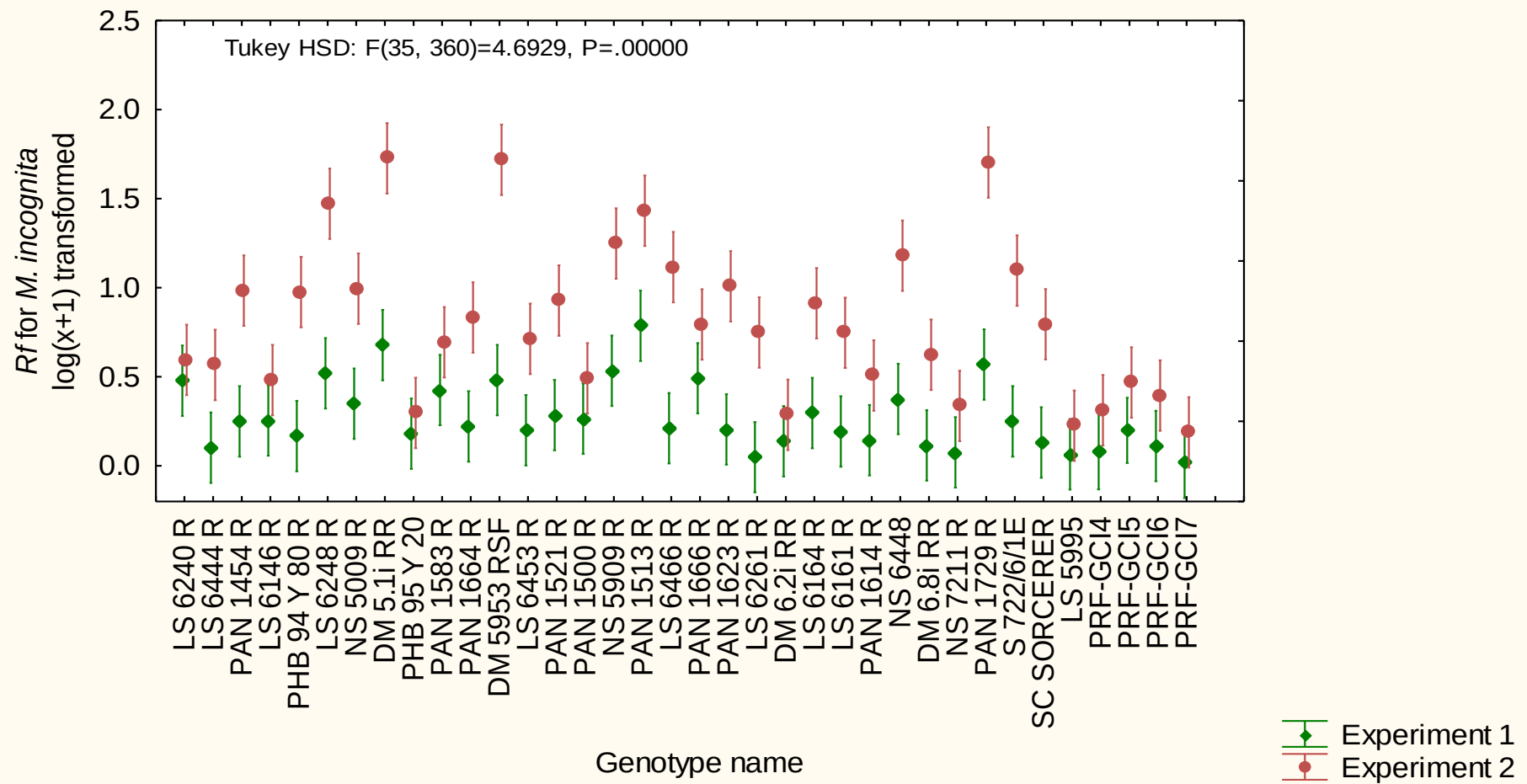


Figure 2.3. *Meloidogyne incognita* reproduction factor per root system extracted from the roots of 36 soybean genotypes adapted to South African climatic conditions 56 days after inoculation with ca. 1 000 eggs and J2. The data of two separate glasshouse experiments are shown. Data are log(x+1) transformed.

Table 2.1. Mean root mass, eggs and second-stage juveniles (J2)/root system, Reproduction factor (Rf) and Relative susceptibility percentages (%R) of 36 soybean genotypes inoculated with *Meloidogyne incognita*.

Genotype name	Experiment 1				Experiment 2			
	Root mass (g)	Eggs and J2/root system	Rf value	% R	Root mass (g)	Eggs and J2/root systems	Rf value	% R
DM 5.1i RR	4.4 ab	3.3 (7 563) defghijklmnopqrst	0.68 (7.56) cdefghijklmnop	100	10.1 jklmnopq	4.7 (54 543) t	1.73 (54.54) s	96
DM 5953 RSF	5.7 abc	3.3 (2 486) defghijklmnopqrst	0.48 (2.03) abcdefghijkl	27	12.6 pq	4.7 (57 068) st	1.72 (57.07) s	100
DM 6.2i RR	4.4 ab	2.4 (458) abcdefghi	0.14 (0.46) abcd	16	13.3 pq	2.9 (995) bcdefghijklmnop	0.29 (1) abcdefgh	2
DM 6.8i RR	4.9 ab	2 (427) abcd	0.12 (0.43) abc	6	11 opq	3.5(3 675) efghijklmnopqrst	0.62 (3.68) bcdefghijklmno	6
LS 5995*	5.1 ab	2.2 (167) abcde	0.07 (0.17) ab	2	9.9 ijklmnopq	2.8 (718) bcdefghijklmnop	0.23 (0.72) abcdefg	1
LS 6146 R	5.8 abcde	2.8 (983) bcdefghijklmnop	0.26 (0.98) abdcefg	13	11.3 pq	3.2 (2 372) defghijklmnopqrs	0.48 (2.37) abcdefghijkl	4
LS 6161 R	6.2 abcdefghi	2.6 (703) abcdefghijklm	0.19 (0.7) abcde	9	10.3 lmnopq	3.6 (5 641) fghijklmnopqrst	0.75 (5.64) efghijklmnop	10
LS 6164 R	4.7 ab	2.7 (1 424) bcdefghijklmno	0.3 (1.42) abcdefgh	19	9.4 defghijklmnop	3.9 (7 648) ijklmnopqrst	0.91 (7.65) ijklmnopq	13
LS 6240 R	6.7 bcdefghijklmn	3.1 (2 538) cdefghijklmnopqr	0.48 (2.54) abcdefghijkl	34	10.7 mnopq	3.4 (3 288) defghijklmnopqrst	0.6 (3.29) abcdefghijklmno	6
LS 6248 R**	5.8 abcde	3.2 (3 643) defghijklmnopqr	0.52 (3.64) abcdefghijklm	48	10.4 klmnopq	4.5 (31 238) rst	1.47 (31.24) qrs	55
LS 6261 R	4 a	1.7 (126) abc	0.05 (0.13) ab	2	11.2 pq	3.7 (5 425) fghijklmnopqrst	0.75 (5.43) efghijklmnop	10
LS 6444 R	6 abcdefgh	2.3 (280) abcdefgh	0.1 (0.28) abc	4	9.4 defghijklmnop	3.4 (3 098) defghijklmnopqrst	0.57 (3.09) abcdefghijklmn	5
LS 6453 R	5.4 abc	2.34 (683) abcdefgh	0.2 (0.68) abcdef	9	9.6 efghijklmnop	3.6 (4 918) efghijklmnopqrst	0.71 (4.92) defghijklmnop	9
LS 6466 R	5.7 abcd	2.6 (8171) abcdefghijklmn	0.21 (0.82) abcdefg	11	11.5 pq	4.1 (13 195 nopqrst)	1.12 (13.2) nopqr	23
NS 5009 R	6.3 abcdefghijkl	3 (1 675) bcdefghijklmnop	0.35 (1.68) abcdefghij	22	10.9 nopq	3.9 (12 513) 3klmnopqrst	1l (12.51) mnopq	22
NS 5909 R	5ab	3.3 (4 238) defghijklmnopqrst	0.53 (4.24) abcdefghijklmn	56	10.2 klmnopq	4.2 (18 305) pqrst	1.25 (18.31) pqrs	32
NS 6448	5.3 ab	2.7 (1 817) bcdefghijklmn	0.38 (1.82) abcdefghij	24	12.2 pq	4.2 (16 503) opqrst	1.18 (16.5) opqrs	29
NS 7211 R	5.9 abcdefg	1.6 (213) ab	0.08 (0.21) ab	3	10.6 mnopq	3 (1 456) bcdefghijklmnopq	0.34 (1.46) abcdefghi	3
PAN 1454 R	6 abcdef	2.8 (905) bcdefghijklmnop	0.25 (0.98) abcdefgh	13	9.4 defghijklmnop	3.9 (11 480) jklmnopqrst	0.98 (11.48) klmnopq	20
PAN 1500 R	4.9 ab	2.3 (1 358) abcdefgh	0.27 (1.36) abcdefgh	18	11.8 pq	3.3 (2 450) defghijklmnopqrst	0.49 (2.45) abcdefghijkl	4
PAN 1513 R	4.7 ab	3.5 (7 158) efghijklmnopqrst	0.79 (7.16) fghijklmnop	95	13.2 pq	4.4 (28 998) qrst	1.43 (28.1) qrs	49
PAN 1521 R	6.3 abcdefghijkl	2.9 (1 050) bcdefghijklmnop	0.29 (1.05) abcdefgh	14	12 pq	3.9 (8 820) jklmnopqrst	0.93 (8.82) jklmnopq	15

Table 2.1 continues								
Genotype name	Experiment 1				Experiment 2			
	Root mass (g)	Eggs and J2/root system	Rf value	% R	Root mass (g)	Eggs and J2/root systems	Rf value	% R
PAN 1583 R	6 abcdefgh	2.8 (2 748) bcdefghijklmno	0.43 (2.75) abcdefghijkl	36	9.9 ijklmnopq	3.6 (4 480) efghijklmnopqrst	0.69 (4.48) cdefghijklmnop	8
PAN 1614 R	6.8 abcdefghijklmn	2.5 (410) abcdefghijk	0.14 (0.41) abcd	5	11 opq	3.3 (2 555) defghijklmnopqrst	0.51 (2.56) abcdefghijklm	4
PAN 1623 R	4.6 ab	2.7 (645) abcdefghijklmno	0.21 (0.65) abcdefg	9	10.5 mnopq	4 (12 075) lmnopqrst	1.01 (12.08) lmnopq	21
PAN 1664 R	4.6 ab	2.4 (951) abcdefgh	0.22 (0.95) abcdefg	13	12.4 pq	3.7 (6 670) hijklmnopqrst	0.83 (6.67) hijklmnop	12
PAN 1666 R	5.4 ab	3.2 (2 784) defghijklmnopqr	0.49 (2.78) abcdefghijkl	37	10.6 mnopq	3.7 (6 335) ghijklmnopqrst	0.79 (6.34) ghijklmnop	11
PAN 1729 R	6.3 abcdefghijklmn	3.3 (4 372) defghijklmnopqrst	0.57 (4.37) abcdefghijklmn	58	12.2 pq	4.7 (55 802) st	1.7 (55.8) rs	98
PHB 94 Y 80 R	4.4 ab	2.6 (515) abcdefghijkl	0.17 (0.52) abcde	7	9.7 efghijklmnopq	3.9 (10 255) jklmnopqrst	0.98 (10.26) klmnopq	18
PHB 95 Y 20	4.3 ab	2.2 (774) abcdef	0.18 (0.77) abcde	10	9.6 fghijklmnopq	3 (1 097) bcdefghijklmnop	0.3 (1.1) abcdefgh	2
PRF-GCI4	4.6 ab	2.3 (221) abcdefgh	0.09 (0.22) abc	3	10.6 nopq	2.9 (1 205) bcdefghijklmnop	0.3 (1.21) abcdefgh	2
PRF-GCI5	6.6 abcdefghijklm	2.4 (900) abcdefgh	0.2 (0.9) abcde	12	10.4 hijklmnopq	3.2 (2 475) defghijklmnopqr	0.47 (2.48) abcdefghijkl	4
PRF-GCI6	5.1 ab	2.3 (330) abcdefg	0.11 (0.33) abc	4	14.2 pq	3 (3 007) bcdefghijklmnopqr	0.4 (3.01) abcdefghijk	5
PRF-GCI7	6.1 abcdefghij	1.2 (42) a	0.02 (0.04) a	1	11.6 pq	2.7 (566) bcdefghijklmn	0.19 (0.57) abcde	1
S 722/6/1E	8.9 cdefghijklmno	2.8 (890) bcdefghijklmnop	0.25 (0.89) abcdefgh	12	15.4 q	4.1 (14 502) mnopqrst	1.1 (14.5) mnopqr	25
SC SORCERER	5.7 abcde	2.5 (379) abcdefghij	0.13 (0.41) abcd	5	13.8 pq	3.7 (6 738) ghijklmnopqrst	0.8 (6.74) ghijklmnop	12

Means in the same column followed by the same letters or numbers do not differ significantly at $P \leq 0.5$ according to the Tukey HSD Test; Log(x+1) transformed data; followed Real means in brackets;
 *Resistant standard, **Susceptible standard.

2.4 Discussion

The host response of the soybean genotypes included in this study to *M. incognita* infection varied from resistant to highly susceptible. Cultivars LS 5995 and PRF-GCI7 consistently had low values for both parameters (R_f and %R) in both experiments. Our study confirms the resistance to *M. incognita* of these two genotypes as previously reported (Fourie *et al.*, 2006; Venter, 2014). Genotypes such as LS 6146 R, DM 6.2i RR, PAN 1583 R, PHB 95 Y 20 and all the PRF-GCI lines had high nematode population levels in both experiments although the R_f observed for these genotypes were still considerably lower compared to the two highly susceptible cultivars (DM 5.1i RR and DM 5953 RSF). Therefore, the aforementioned cultivars are classified as moderately resistant to *M. incognita*. By contrast, Venter (2014) and Fourie *et al.* (2015b) reported all the PRF-GCI lines as being resistant to *M. incognita*. However, variations in host response to root-knot nematode infection of the same soybean genotype in different screening experiments is not uncommon. For example, in Brazil the soybean cultivar BRSGO Raissa was classified as susceptible to *M. javanica* (Embrapa, 2011), however, Teixeira *et al.* (2017) reported the same cultivar as resistant to the same root-knot nematode species.

Despite the uniformity of the experimental conditions, the results from the two experiments differed. Nematode-host plant reactions are known to be influenced by several environmental conditions such as temperature and moisture (Ferris *et al.*, 2013; Vandegehuchte *et al.*, 2015). The differences in results obtained between the two experiments in this study have most probably been caused by the higher mean temperature (range of 2.5 °C) recorded during Experiment 2 compared to Experiment 1. This was the only difference observed between the two experiments since the seedlings were grown in similar containers in soil from the same source and inoculated with the same *M. incognita* population at the same Pi. Also, the water regime (schedule, volume and source) was similar for both experiments. Nardacci and Barker (1978) reported that the R_f of *M. incognita* in soybean roots was profoundly influenced by temperature and that it was lowest at temperatures ranging from 16 to 20 °C and highest at 30 °C. Results of this study are in agreement with Thomason and Lear (1961) who reported that the reproduction factor of four different *M. incognita* populations was higher at 25–32 °C compared to lower temperature

ranges. Taking into account that the optimal temperature range for *M. incognita* development is 22–30 °C (Diez & Duzenbery, 1989), the results of Experiment 2 are considered be more accurate.

The eight and 28 times higher susceptibility levels recorded for cultivars DM 5.1i RR and DM 5953 RSF, respectively, in Experiment 2 compared to Experiment 1 could illustrate the impact of temperature on the reproduction of *M. incognita*. However, in Experiment 2 (during which the higher temperature range was recorded) the Rf value for the resistant standard LS 5995 was still < 1 although it was 4 % higher than that recorded in Experiment 1. Except for LS 5995, only line PRF-GCI7 had an Rf value < 1 in Experiment 2. Although 21 genotypes had Rf values $\leq$ 1 in Experiment 1, all of them except PRF-GCI7 and DM 6.2i RR, were not successful in maintaining the resistance trait under the higher temperature regime that prevailed in Experiment 2. Teixeira *et al.* (2017) evaluated 29 soybean cultivars for their host response to *M. javanica* in two separate experiments conducted in the same greenhouse one after the other. These authors reported that in the first experiment 14 cultivars were classified as resistant whereas only two of these cultivars were also classified as resistant in the second experiment confirming that results like these reported for this study are not uncommon.

The Rf is considered a quantitative parameter that could be compared across studies (Niblack, 1986; Windham & Williams, 1988). The concept of determining the Rf for root-knot nematodes in host response and host range studies is well established (Moura & Régis, 1987; Sharma *et al.*, 2006; Bruinsma & Antoniolli, 2015). Using this parameter, an Rf value of < 1 indicates that a plant is resistant, as no increase occurred in nematode numbers extracted at the end of the experiment compared to the number inoculated at the beginning of the experiment (Oostenbrink, 1966). However, due to the strictness of this system, plant genotypes with Rf values as high as 57 are classified similarly as plant genotypes with Rf values of 1.1 (*viz.* susceptible). This strictness render Rf values insufficient to describe the potential of root-knot nematodes to reproduce on different soybean genotypes. Windham and Williams (1988) also suggested that using Rf values alone may be misleading at times. Thus, in this study Rf was supplemented by the %R of each soybean genotype (Hussey & Janssen, 2002). Using this criterion, a %R value of < 10 %

indicates that a plant is resistant. Consequently, in this study nine genotypes that were susceptible based on their Rf values were resistant to *M. incognita* infection based on their %R values. Discrepancy in terms of Rf and %R values was reported by Venter (2014) who examined the host response of 31 soybean genotypes to *M. incognita* infection. Nine of these genotypes had a %R < 10 % while their Rf values were > 1. Fourie *et al.* (2012) reported a similar tendency when screening tomato cultivars for resistance against *M. javanica*. In this study, for example, LS 6444 R had in Experiment 2 an Rf value of 3, indicating susceptibility, but a %R value of 3 indicating resistance. It is hence crucial to use more than one parameter to evaluate the host response of genotypes to root-knot nematode infection to make more accurate and informed decisions, and recommendations to producers.

Since the soybean cultivar DM 6.2i RR was classified as moderately resistant to *M. incognita* based on its Rf (1) and %R (2) values, this cultivar should be the first choice of producers that experience problems with this root-knot nematode species in their fields. Moreover, this cultivar showed a high yield potential when evaluated in soybean production areas with moderate temperatures such as Cedara (Kwa-Zulu Natal Province) and Glen (Free State Province) (De Beer, 2015) and areas with warm temperatures such as Atlanta and Brits (North-West Province). Unfortunately, since the resistant line PRF-GCI7 is not commercially available it cannot be recommended to producers as a management strategy to reduce *M. incognita* population densities. Other cultivars identified in Experiment 2 with low Rf values (≤ 5) and %R (≤ 10) such as PHB 95 Y 20 (1.1 and 2, respectively) and NS 7211 R (1.46 and 3, respectively) should be the second choice to DM 6.2i RR. This will be applicable where the latter cultivar is not well adapted to the areas where producers are experiencing problems with *M. incognita*. Cultivation of cultivars that showed high susceptibility (Rf > 5) (Windham & Williams, 1988) LS 6161 R, LS6164 R, LS 6261 R, LS 6444 R, LS 6466 R, NS 5009 R, NS 5909 R, NS 6448, PAN 1454 R, PAN 1513 R, PAN 1521 R, PAN 1623 R, PAN 1664 R, PAN 1666 R, PAN 1729 R, PHB 94 Y 80 R, S 722/6/1E and SC SORCERER should, however, be avoided in *M. incognita* infested fields.

The importance of determining the host response of soybean genotypes to root-knot nematodes, particularly the predominant species *M. incognita*, in South Africa cannot

be overstated. Soybean genotypes must be evaluated not only for their yield potential but also for their susceptibility and sensitivity to diseases and pests including nematodes. Although the results of several glasshouse studies in South Africa regarding the host response of soybean genotypes to root-knot nematodes have been reported (Fourie *et al.*, 1999, 2006, 2010, 2015b; Mbatyoti *et al.*, 2013; Venter, 2014), the results of our study provide a larger database.

These results update and broaden knowledge of soybean germplasm which local producers can use to minimise the damage *M. incognita* may cause to their soybean harvest. In conclusion it is accentuated that the host response of local cultivars to *M. incognita* and other *Meloidogyne* spp. that are prevalent in the local soybean-based production areas in South Africa, should be done on a regular bases to provide producers with the most up-to-date information.

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Chapter 3

Plant-parasitic nematode assemblages associated with glyphosate-tolerant soybean cultivars in South African soybean-based agro-ecosystems

Abstract

Nine plant-parasitic nematode genera and 10 species were identified in root and soil samples of glyphosate-tolerant soybean cultivars collected from seven localities in selected production areas of South Africa during the 2014/15 season. No nematode genera/species were added to the official, existing list of plant-parasitic nematodes recorded on soybean in South Africa. The predominant endoparasitic nematode genera present in root samples were *Meloidogyne* and *Pratylenchus*. *Meloidogyne incognita* was the predominant root-knot nematode species, followed by *Meloidogyne javanica*. *Pratylenchus brachyurus* was the predominant lesion nematode in roots, followed by *Pratylenchus zeae* and *Pratylenchus teres*. *Rotylenchulus parvus* was the predominant semi-endoparasitic nematode present in soil samples, followed by *Scutellonema brachyurus* and *Helicotylenchus* sp. Ectoparasitic nematode genera in soil samples were *Criconemoides sphearocephalus*, followed by *Tylenchorhynchus brevilineatus*, *Criconema* sp. and *Nanidorus minor*. Only two cultivars, PAN 1583 R and PAN 1521, maintained less than 10 % of the *Meloidogyne* spp. population densities present in roots of the most susceptible cultivar PAN 1623 R. Hence these two cultivars are suggested to be tolerant to this nematode pest. All cultivars were, however, highly susceptible to the *Pratylenchus* spp.

Key words: *Meloidogyne* spp., *Pratylenchus* spp., soybean, survey.

3.1 Introduction

In South Africa, soybean (*Glycine max* L.) can be grown in all provinces (Dlamini *et al.*, 2014). However, soybean cultivars are bred to be adapted to specific environmental conditions of which day length and temperature are probably the most important that can affect yield potential (Liebenberg, 2012). Thus, growing soybean cultivars without appropriate knowledge of their performance in a specific climatic zone may result in poorer yields than expected (Zilli *et al.*, 2009). Therefore, the agronomic performance and adaptability of cultivars to a specific climatic zone are continuously evaluated (De Beer & Prinsloo, 2013). The inclusion of newly developed genotypes in evaluation processes is especially important to ensure that they are fit to be cultivated in both traditional and new production areas. In South Africa, such an evaluation program organised by the Grain Crops Institute (GCI) of the Agricultural Research Council (ARC) has led to the expansion of the soybean production area and, consequently, in an increase in soybean production (Dlamini *et al.*, 2014).

Despite being optimally adapted to a specific production area, soybean is attacked by diseases and pests wherever the crop is grown (Sinclair & Hartman, 1999). Also plant-parasitic nematodes can cause significant soybean yield losses. Approximately less than 49 plant-parasitic nematode species are known to be associated with soybean in South Africa, of which the root-knot nematodes (*Meloidogyne* spp.) are the most important ones (Fourie *et al.*, 2015a). The two root-knot nematode species that pose a threat to local soybean production are *Meloidogyne incognita* (Kofoed & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. Lesion nematodes (*Pratylenchus* spp.) rank second in terms of economic significance (Fourie *et al.*, 2015a). The soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, that cause major yield losses to soybean production in Brazil, the United States of America (USA) and several Asian countries (Bridge & Starr, 2007) has not been reported in South Africa so far. However, only one nematode survey has been conducted in South African soybean production areas 22 years ago (Fourie *et al.* 2001). It cannot be excluded that additional surveys will increase the number of plant-parasitic nematode species identified in association with soybean in South Africa.

Changes in annual rainfall and temperature, agronomic practices as well as disease and pest management are all factors that may change nematode composition and structure (assemblages) over time in agro-ecosystems (Cadet *et al.*, 2005; Ali *et al.*, 2017). These anthropogenic processes also exist in South Africa where soybean production areas have expanded and where traditional conventional soybean cultivars have often been replaced by high- yielding glyphosate-tolerant cultivars. The increased use of glyphosate to control weeds has raised environmental concerns on its potential effects on non-target soil organisms such as nematodes (Zhao *et al.*, 2013). Information on the effects of glyphosate on nematodes is scarce and the findings often inconclusive or even conflicting. Therefore, periodical monitoring of the terrestrial non-parasitic nematode assemblages (see Chapter 5) associated with soybean is necessary, especially when new generations of genetically-modified (GM) glyphosate-tolerant cultivars are increasingly being cultivated and these cultivars have not been evaluated.

The objectives of this study were to determine: 1) the abundance, diversity and frequency of plant-parasitic nematodes in existing and potential soybean production areas of South Africa, and 2) the host response of 29 glyphosate-tolerant cultivars to the most important nematode pests (*Meloidogyne* spp. and *Pratylenchus* spp.) associated with soybean crops in South Africa.

3.2 Materials and methods

3.2.1 Localities

During the 2014/15 growing season (February-March), rhizosphere soil and root samples of 29 glyphosate-tolerant soybean cultivars (included in the National Soybean Cultivar Trials conducted by the ARC-Grain Crops Institute (ARC-GCI)) were collected at seven localities representative of the major soybean production areas in South Africa (Fig. 3.1). These localities were located in three provinces namely the Free State Province (Bethlehem, Hoopstad and Kroonstad), North West Province (Brits and Potchefstroom) and Mpumalanga Province (Groblersdal and Middelburg).

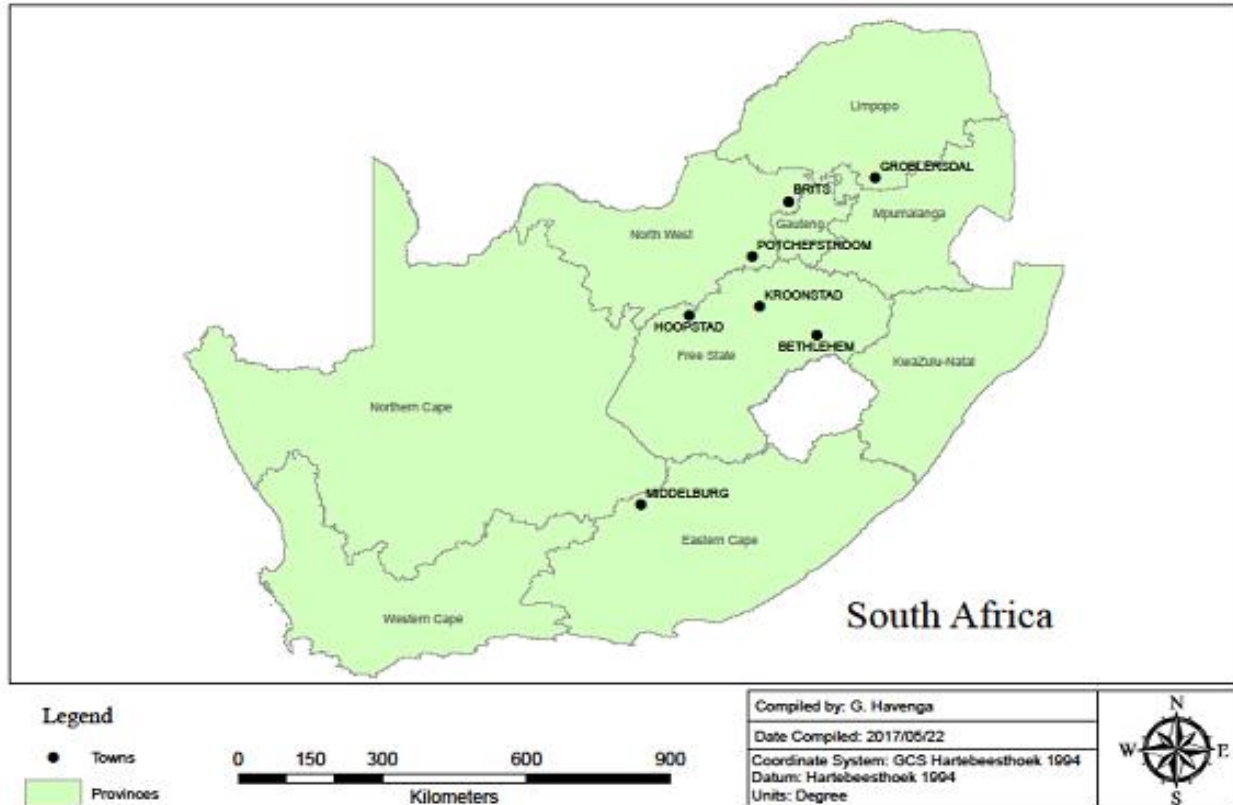


Figure 3.1. The locations of the seven localities included in the National Soybean Cultivar Trials during the 2014/15 growing season where plant-parasitic nematode assemblages in the rhizosphere soil and roots of 29 soybean cultivars were examined (compiled by: Mr G. Havenga, NWU).

The trial layout at each of the seven localities was a randomised block design, comprising of the 29 soybean cultivars that were each replicated three times. Each plot was planted with 170 seeds per row and consisted of four rows (5-m-long) with inter- and intra-row spacings of 90 and 3 cm, respectively. Seeds were inoculated with *Bradyrhizobium japonicum* strain WB74 (Soygro (Pty) Ltd, Potchefstroom) before planting (250 g/50 kg seed; www.soygro.co.za). Physical and chemical soil properties, crop history, rainfall and irrigation of each locality are listed in Table 3.1.

Table 3.1. Soil physical and chemical properties, crop history, rainfall and irrigation figures for the seven localities where the study was conducted.

Locality	Soil physical properties (%)			Soil chemical properties	Crop history (2013/14)	Rainfall	Irrigation
	Sand	Silt	Clay	pH		(mm)	(mm)
Bethlehem	80	4	16	6.73	Soybean	639	0
Brits	66	8	26	7.53	Soybean	429	300
Groblersdal	71	17	12	6.59	Soybean	598	315
Hoopstad	95	0	5	7.35	Maize	403	0
Kroonstad	91	7	2	7.93	Soybean	608	0
Middelburg	81	10	9	6.58	Soybean	784	0
Potchefstroom	66	8	26	7.39	Maize	548	420

3.2.1.1 Nematode sampling and extraction

At each locality, rhizosphere soil and roots were collected during the flowering period from six soybean plants per cultivar (*i.e.* per 5-m-long row, per replicate) chosen at random. Of each sample one sub-sample of 200 g soil and 5 g and 50 g roots was taken for nematode extraction. Root-knot nematode eggs and second stage juveniles (J2) were extracted from the 50 g root sub-samples using the 1 % NaOCl method (Riekert, 1995) and counted. Both root-knot nematode J2 as well as the vermiform stages (juveniles and adults) of a wide range of migratory endoparasitic nematodes were extracted from the 5 g root sub-samples using the centrifugal-flotation method (Hooper *et al.*, 2005). The decanting and sieving method (Hooper *et al.*, 2005) was used to extract terrestrial non-parasitic and ectoparasitic nematodes (juveniles and adults) from the 200 g soil sub-samples for counting and identification purposes.

3.2.1.2 Nematode identification and assessment of nematode reproduction

Nematodes extracted from the 5 and 50 g root sub-samples were transferred to a De Grisse counting dish (De Grisse, 1969) and, using a stereomicroscope (60^x magnification), were identified to genus level and counted. Eggs and J2 of *Meloidogyne* spp. were counted in 50 g root samples, but only J2 were counted in 5 g root samples as well as the vermiform stages of all other plant-parasitic nematode genera. No eggs were counted in either 5 g root or 200 g soil samples since nematodes cannot be

identified to genus/species level using eggs. Nematodes extracted from the 200 g soil sub-samples were transferred to a 1-ml Hawksley slide using a light microscope (1 000 $\times$ magnification). These were identified at genus level and counted (Docaster *et al.*, 1967). For identification at species level, at least 30 individuals of each genus were fished out from the counting dishes after counting was done. These individuals were fixed in a heated formaldehyde-propionic-acid-water (FPG) solution (100 ml of a 40 % formalin solution, 10 ml propionic acid and 890 ml distilled water). The glass dish with the fixed nematodes were placed in an incubator at 40 °C for 72 h and the FPG solution stepwise replaced with glycerin (De Grisse, 1969; Marais *et al.*, 2017). The fixed nematodes were hand-picked from the glycerin using a fine tip needle and permanently mounted in glycerin on slides using the paraffin-ring method (Hooper, 1986). The slides were sent to Drs. Mariette Marais and Antoinette Swart, nematode specialist-taxonomists of the ARC - Plant Health and Protection, Roodeplaat, for identification at species level.

Meloidogyne spp. were identified both on the shape of their perineal and esophagus regions as well as by molecular techniques. The eggs that had been extracted from the 50 g root sub-samples were pooled for each locality and inoculated on the roots of potted (10 L pots) seedlings of the root-knot nematode susceptible tomato cultivar Floradade (Fourie *et al.*, 2012). Thus, seven *Meloidogyne* populations were established from the seven sampling sites, maintained and multiplied in the greenhouse at a $\pm$ 20-26 °C and a 14:10 light:dark photoperiod. Forty days after nematode inoculation, 21 mature females of each of the seven *Meloidogyne* populations were hand-picked, using a fine tip needle from the infected tomato roots and their perineal and esophagus regions cut and prepared for light microscope (1 000 $\times$ magnification) observation and species identification (Hartman & Sasser, 1985; Kleynhans, 1991).

For the molecular identification of the *Meloidogyne* spp., 15 J2 were isolated from the 50 g root samples and transferred to individual 1.5-ml Eppendorf tubes, each containing 25 μ l double distilled water. DNA was extracted from the J2 using the modified Chelex method, followed by the polymerase chain reaction (PCR) (Shokoohi *et al.* 2017). The forward and reverse primers, D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B

(5'-TCGGAAGGAACCAGCTACTA-3') (Subbotin *et al.*, 2006), respectively, were used in the PCR reactions for amplification of the partial 28S ribosomal DNA (rDNA). In addition, the forward NAD5F2 (5'-TATTTTTTGTGTTGAGATATATTAG-3') and the reverse NAD5R1 primers (5'-CGTGAATCTTGATTTTCCATTTTT-3') (Janssen *et al.*, 2016), respectively, were used in the PCR reactions to amplify the mitochondrial DNA (mtDNA) (*NADH5* gene). Sequencing reactions were performed by inqaba biotec™ (Pretoria, South Africa). Primers for the sequencing reaction were those used in the amplification step. Publicly available sequences for other *Meloidogyne* spp. were selected from NCBI GenBank for construction of phylogenetic trees. The outgroups used were *Heterodera avenae* Wollenweber, 1924 (acc. no. GU595443) for the D2-D3 and *Caenorhabditis briggsae* Dougherty & Nigon, 1949 (acc. no. GU452323) for constructing the *NADH5* trees, respectively. Genetic pairwise distance estimates of *Meloidogyne* spp. based on mtDNA (*NADH5*) using maximum composite likelihood were finally done using Mega6 software (Tamura *et al.*, 2013).

3.2.1.3 Data analyses

Plant-parasitic nematode population densities extracted from the 5 and 50 g root, and the 200 g rhizosphere soil sub-samples of each cultivar at the seven localities were pooled and the frequency of occurrence, mean population density (MPD) and prominence value (PV) of each nematode genus calculated (De Waele & Jordaan, 1988):

i) Frequency of occurrence:

$$\frac{\text{number of localities at which the genus occurred in the root and soil sub-samples of each cultivar}}{\text{number of localities sampled (7)}} \times 100$$

ii) Mean population density (MPD) at each locality:

$$\frac{\text{total number of individuals of a genus present in root/soil samples of each cultivar}}{\text{number of localities in which the genus occurred in root/soil samples of each cultivar}}$$

iii) Prominence value (PV):

$$\frac{\text{mean population density of each genus} \times \sqrt{\text{frequency of occurrence}}}{10}$$

To determine the host response of the soybean cultivars, in terms of susceptibility/resistance, to the *Meloidogyne* spp. prevailing at the seven localities, the percentage reproduction reduction (%Rr) was calculated as the reduction in Pf (eggs + J2 extracted from the 50 g root sub-samples) of a given genotype compared with the Pf of the most susceptible genotype (Hussey & Janssen, 2002; Albuquerque *et al.*, 2010). A cultivar was regarded as highly resistant when it had a %Rr value > 10 %. The percentage reproduction reduction (%Rr) was also calculated for the *Pratylenchus* spp. extracted from the 5 g root sub-samples using the sugar-flotation method. This was, however, not done for *Meloidogyne* J2 data obtained for 5 g roots since the extraction method used is not optimal for isolating *Meloidogyne*.

3.3 Results

Nine plant-parasitic nematode genera and 10 plant-parasitic nematodes species were found in the rhizosphere soil and soybean roots at the seven localities. The genera included *Criconema*, *Criconemoides*, *Helicotylenchus*, *Meloidogyne*, *Nanidorus*, *Pratylenchus*, *Rotylenchulus*, *Scutellonema* and *Tylenchorhynchus*. The species identified were: *Criconemoides sphaerocephalus* (Taylor, 1936), *M. incognita*, *M. javanica*, *Nanidorus minor* (Colbran, 1956) Siddiqi, 1974, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus teres* Khan & Singh, 1975, *Pratylenchus zeae* Graham, 1951, *Rotylenchulus parvus* (Williams, 1960) Sher, 1961, *Scutellonema brachyurus* (Steiner, 1938) Andr ssy, 1958 and *Tylenchorhynchus brevilineatus* Williams, 1960. Only immature, unidentifiable stages were found of *Helicotylenchus*.

3.3.1. *Meloidogyne* spp. identification

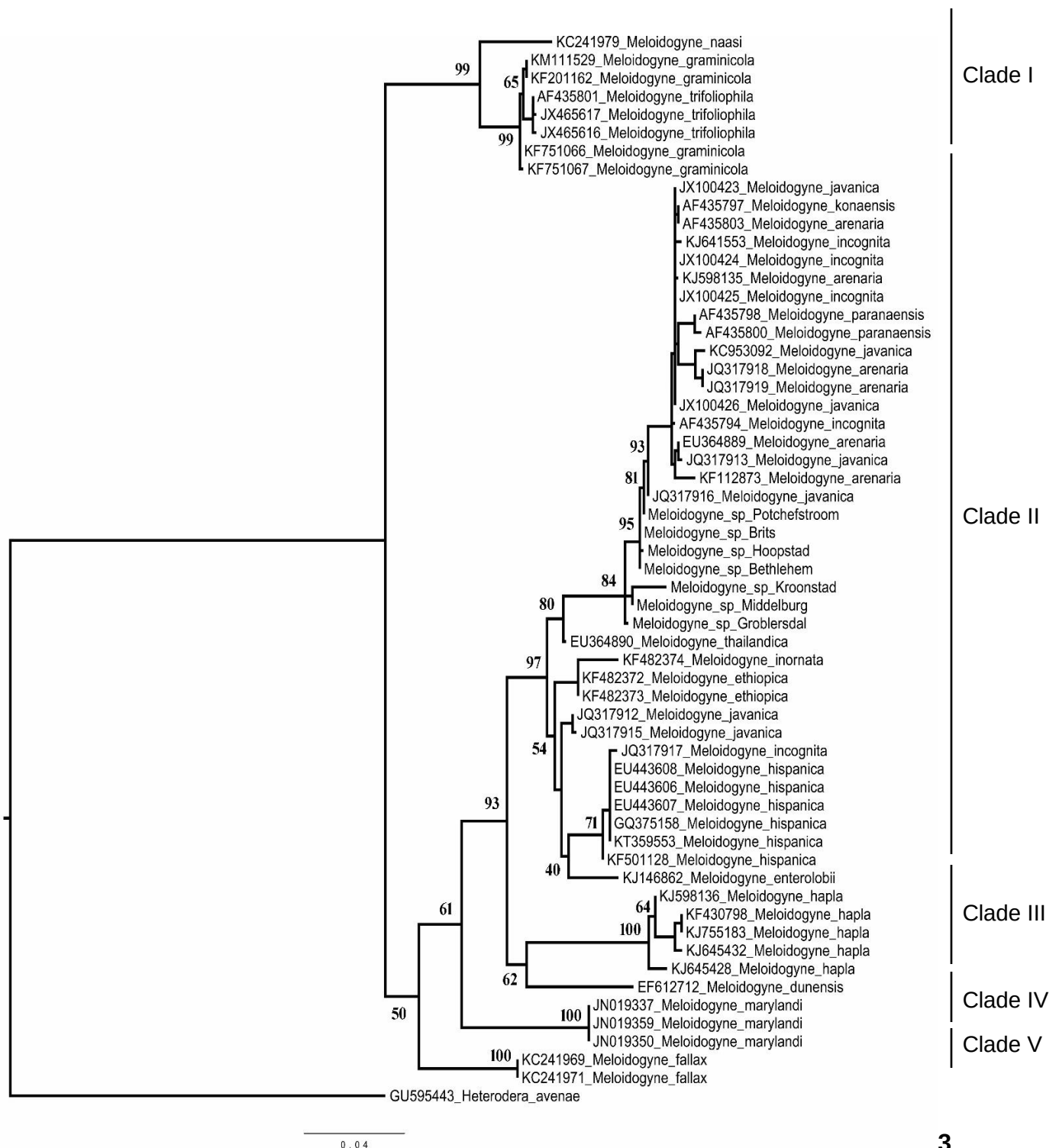
Two *Meloidogyne* spp. were identified using both morphological and molecular techniques. These were *M. incognita* and *M. javanica* (Table 3.2). Based on the morphological identification, a single species population of *M. incognita* was present at six of the seven localities (Bethlehem, Brits, Grobersdal, Hoopstad, Middelburg and Potchefstroom). A single species population of *M. javanica* was present at only one locality (Kroonstad).

Table 3.2. Morphological and molecular identification of the *Meloidogyne* spp. found in the rhizosphere soil and roots of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season.

Locality	Morphological identification	Molecular analysis	
	Shape of perineal pattern	28S rDNA D2/D3 gene	mtDNA NADH5 gene
Bethlehem	<i>M. incognita</i>		
Brits	<i>M. incognita</i>		
Groblersdal	<i>M. incognita</i>	<i>M. arenaria</i> , <i>M. enterolobii</i> , <i>M.</i>	<i>M. arenaria</i> , <i>M.</i>
Hoopstad	<i>M. incognita</i>	<i>ethiopica</i> , <i>M. incognita</i> <i>M. hispanica</i> ,	<i>ethiopica</i> , <i>M. incognita</i> ,
Kroonstad	<i>M. javanica</i>	<i>M. inornata</i> , <i>M. javanica</i> , <i>M. konaensis</i> ,	<i>M. javanica</i> , <i>M. luci</i> ,
Middelburg	<i>M. incognita</i>	<i>M. paranaensis</i> or <i>M. thailandica</i>	
Potchefstroom	<i>M. incognita</i>		

Molecular results using the D2-D3 gene, ribosomal DNA (rDNA) resulted in a phylogenetic tree with five clades. The DNA sequences of these seven populations grouped in the same clade with the following thermophilic species selected from NCBI Genbank: *M. arenaria* (Neal, 1889), Chitwood, 1949, *M. enterolobii* Yang & Eisenback, 1983, *M. ethiopica* Whitehead, 1968, *M. incognita* (Kofoid & White, 1919), Chitwood, 1949, *M. hispanica* Hirschmann, 1986, *M. inornata* Lordello, 1956, *M. javanica* (Treub, 1885) Chitwood, 1949, *M. konaensis* Eisenback, Bernard & Schmitt, 1995, *M. paranaensis* Carneiro, Carneiro, Abrantes, Santos & Almeida, 1996 or *M. thailandica* Handoo, Skantar, Carta & Erbe, 2005 (Fig. 3.2).

The NADH5 analysis (using mitochondrial DNA: mtDNA), resulted in configuration of a phylogenetic tree with two clades (Fig. 3.3). This analyses grouped the seven populations in a clade with *M. arenaria*, *M. ethiopica*, *M. incognita*, *M. javanica*, *M. luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno & Karssen, 2014 of which the sequences were also selected from GenBank. The methods could hence not distinguish these species from one another.



2. Phylogenetic analysis of *Meloidogyne* spp., using the D2-D3 segment of 28S rDNA, identified from rhizosphere samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season. *Heterodera avenae*, selected from NCBI Genbank, was used as the outgroup.

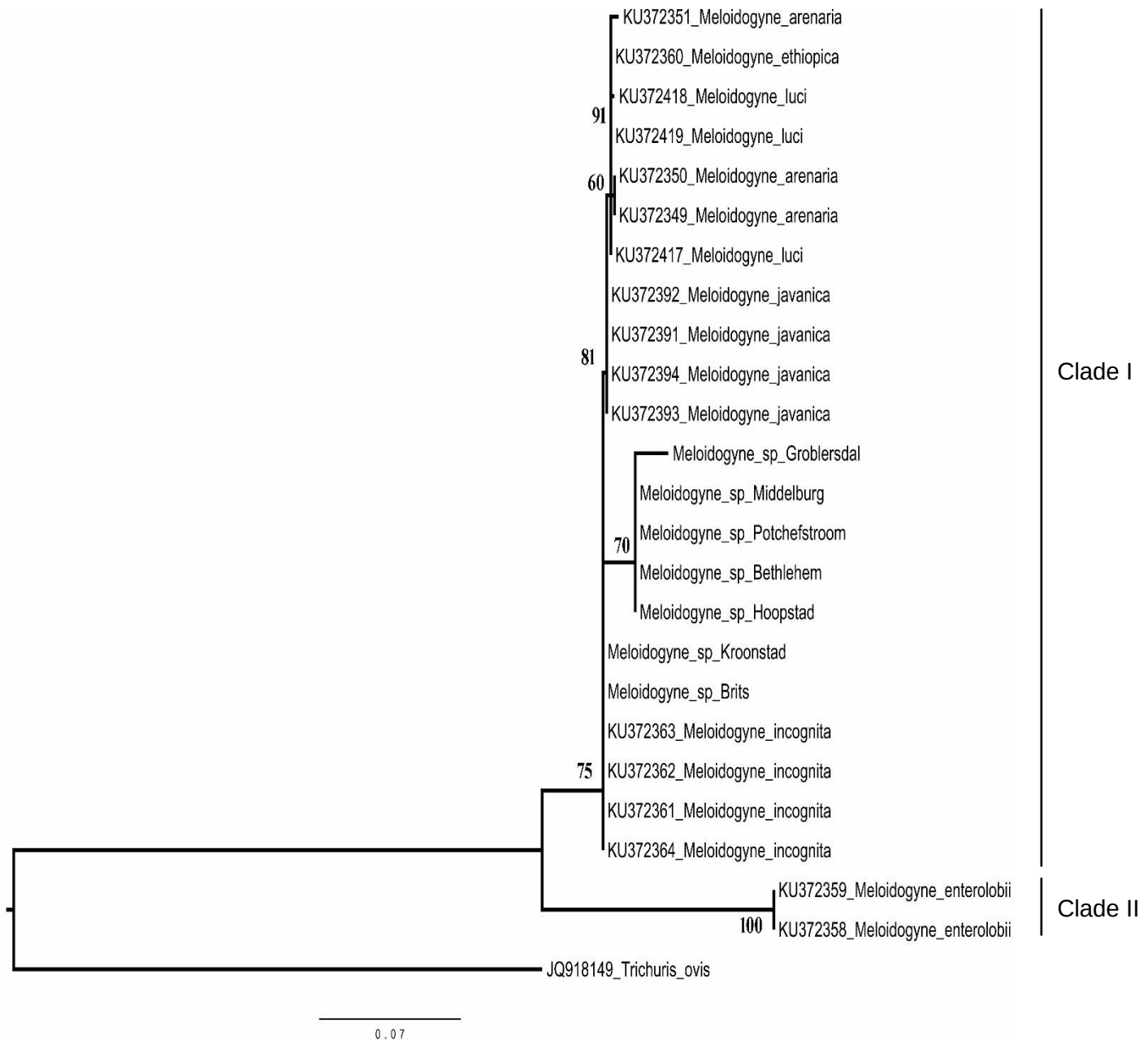


Figure 3.3. Phylogenetic analysis of *Meloidogyne* spp., using the NADH5 gene (mtDNA), that were identified from rhizosphere samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season. *Caenorhabditis briggsae*, selected from NCBI Genbank, was used as the outgroup.

3.3.2 Root-knot nematode population densities per 50 g roots

Meloidogyne incognita was according to PVs, MPDs and frequency of occurrence of the only and therefore predominant species in the roots of the 29 soybean cultivars sampled, followed by *M. javanica* (Table 3.3).

3.3.2.1 Data pooled for the localities and cultivars

Table 3.3. Prominence values, frequencies of occurrence and mean population densities of *Meloidogyne incognita* and *Meloidogyne javanica* in 50 g root samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season.

Nematode	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
<i>Meloidogyne incognita</i>	236 680	86	252 219
<i>Meloidogyne javanica</i>	25 915	14	69 260

*Prominence value (PV): population density of each species $\times \sqrt{\text{frequency of occurrence}/10}$.

3.3.2.2 Data of individual cultivar, but pooled for the localities

Root-knot nematode egg and J2 densities per 50 g roots were high in roots of all 29 soybean cultivars sampled, with PV values ranging from 1 684 (PAN 1583 R) to 24 480 (PAN 1623 R) (Table 3.4). The MPDs of the *Meloidogyne* ranged from 1 816 (PAN 1583 R) to 29 052 (PAN 1623 R). Root-knot nematodes were present in 71 to 100 % of the root samples.

The extent to which *Meloidogyne* spp. numbers were reduced ranged between 6 (PAN 1583 R) to 74 % (LS6164 R) when compared to the most susceptible cultivar PAN 1623 R (Table 3.4). The latter cultivar had the highest mean root-knot nematode population density. Only two cultivars, PAN 1583 R and PAN 1521 R, contained less than 10 % of the *Meloidogyne* spp. numbers in their roots than that recorded for cultivar PAN 1623 R.

Table 3.4. Prominence values, frequencies of occurrence, mean population densities and percentage reproduction reduction of *Meloidogyne incognita* and *Meloidogyne javanica* in 50 g root samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season.

Cultivar	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	% Reproduction reduction (%Rr)**
PAN 1623 R	24 480	71	29 052	100
LS 6164 R	19 912	86	21 472	74
LS 6261 R	19 837	86	21 391	74
NS 5009 R	17 309	86	18 665	64
LS 6146 R	15 081	71	17 898	62
PAN 1729 R	13 696	100	13 696	47
LS 6161 R	12 692	100	12 692	44
DM 5.1i RR	11 868	86	12 797	44
NS 7211 R	11 644	100	11 644	40
LS 6248 R	11 622	100	11 622	40
PHB 95 Y 20	11 609	100	11 609	40
LS 6466 R	10 994	86	11 855	41
LS 6240 R	10 913	100	10 913	38
LS 6444 R	10 644	86	11 478	40
LS 6453 R	9 192	100	9 192	32
PHB 94 Y 80 R	8 602	100	8 602	30
PAN 1664 R	7 930	86	8 551	29
PAN 1513 R	7 348	86	7 924	27
NS 5909 R	6 940	86	7 484	26
PAN 1500 R	6 937	86	7 480	26
PAN 1454 R	6 394	71	7 588	26
DM 6.2i RR	6 031	86	6 503	22
PAN 1614 R	5 667	86	6 111	21
DM 6.8i RR	5 420	100	5 420	19
DM 5953 RSF	5 003	100	5 003	17
NS 6448	4 706	86	5 075	18
PAN 1666 R	2 900	86	3 128	11
PAN 1521 R	2 462	100	2 462	9
PAN 1583 R	1 684	86	1 816	6

*Prominence value (PV) = final population density (Pf) of each species $\times \sqrt{\text{frequency of occurrence}/10}$;

**Reproductive percentage reduction = Pf in the roots of a genotype/Pf in the roots of the genotype with the highest Pf.

3.3.2 Plant-parasitic nematode population densities per 5 g roots

Meloidogyne, followed by *Pratylenchus* were the predominant genera present in the 5 g root samples of the 29 soybean cultivars according to PVs (Table 3.5). Other plant-parasitic nematode species found in 5 g root samples were, in descending order of predominance, *Pratylenchus brachyurus* (Godfrey, 1919) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus zeae* Graham, 1951, *Pratylenchus teres* Khan & Singh, 1975, *Rotylenchulus* sp., *Scutellonema brachyurus* (Steiner, 1938) Andr  ssy, 1958 and *Helicotylenchus* sp.

Meloidogyne incognita was the most dominant species, followed by *M. javanica*. The predominant root-lesion nematode species was *P. brachyurus*, followed by *Pratylenchus zeae* and *P. teres*.

Table 3.5. Prominence values, frequencies of occurrence and mean population densities of plant-parasitic nematodes in 5 g root samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season.

Nematode	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
<i>Meloidogyne incognita</i>	3 294	29	6 117
<i>Meloidogyne javanica</i>	1 873	14	5 005
<i>Pratylenchus brachyurus</i>	1 198	14	2 996
<i>Pratylenchus zeae</i>	992	29	1 983
<i>Pratylenchus teres</i>	503	43	718
<i>Rotylenchulus</i> sp.	436	29	809
<i>Scutellonema brachyurus</i>	153	14	382
<i>Helicotylenchus</i> sp.	115	14	2 87

*Prominence value (PV): Final population density of each species $\times \sqrt{\text{frequency of occurrence}/10}$.

3.3.2.1 *Meloidogyne*

The PVs, frequency of occurrence and MPD of *Meloidogyne* spp. per 5 g roots ranged between 5 (PAN 1513 R) to 3 902 (PAN 1623 R), 14 to 43 %, and 9 (PAN 1513 R) to 5 951 (PAN 1623 R), respectively (Table 3.6).

Table 3.6. Prominence values, frequencies of occurrence and mean population densities of *Meloidogyne* spp. in 5 g root samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season.

Cultivar	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
PAN 1623 R	3 902	43	5 951
DM 5.1i RR	3 379	29	6 275
PAN 1729 R	2 969	29	5 513
PAN 1666 R	2 875	43	4 384
LS 6261 R	1 361	43	2 075
LS 6164 R	1 122	29	2 083
LS 6240 R	844	29	1 567
DM 6.2i RR	751	43	1 146
PAN 1454 R	570	29	1 059
DM 6.8i RR	565	29	1 050
LS 6146 R	551	29	1 024
LS 6466 R	483	43	736
DM 5953 RSF	453	29	841
LS 6453 R	444	29	824
PAN 1583 R	326	29	605
PHB 94 Y 80 R	217	29	403
NS 5009 R	169	29	313
NS 5909 R	132	29	246
PAN 1614 R	132	29	246
PAN 1500 R	116	43	177
PHB 95 Y 20	104	29	193
LS 6161 R	90	29	168
LS 6248 R	80	29	149
PAN 1521 R	80	29	149
NS 6448	76	29	141
PAN 1664 R	33	14	88
LS 6444 R	29	29	54
Standard	24	29	44
NS 7211 R	20	14	53
PAN 1513 R	5	29	9

*Prominence value (PV): Final population density of each genus $\times \sqrt{\text{frequency of occurrence}/10}$.

3.3.2.2 *Pratylenchus*

The PV, frequency of occurrence and MPD of *Pratylenchus* per 5 g roots ranged between 1 367 (PAN 1664) R to 5 315 (DM 6.2i RR), 71 to 100 % and 1 622 (PAN 1664 R) to 5 731 (DM 6.2i RR) (Table 3.7). The extent to which reproduction of *Meloidogyne*

spp. population densities were reduced ranged between 26 to 86 % (when compared to cultivar DM 6.2i RR which had the highest mean population densities).

Table 3.7. Prominence values, frequencies of occurrence and mean population densities of *Pratylenchus* spp. in 5 g root sub-samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season.

Cultivar	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	% Reduction in reproduction (%Rr)**
DM 6.2i RR	5 315	86	5 731	100
PAN 1666 R	4 571	86	4 929	86
PAN 1513 R	4 132	86	4 456	78
PAN 1623 R	3 944	100	3 944	69
LS 6444 R	3 853	86	4 155	73
LS 6240 R	3 768	71	4 472	78
LS 6146 R	3 725	86	4 017	70
DM 5.1i RR	3 594	100	3 594	63
LS 6261 R	3 466	86	3 738	65
PAN 1729 R	3 379	71	4 010	70
PAN 1454 R	3 375	86	3 639	64
PAN 1614 R	3 331	86	3 592	63
NS 7211 R	3 115	86	3 359	59
LS 6466 R	3 071	86	3 312	58
DM 6.8i RR	2 978	71	3 534	62
NS 5009 R	2 908	71	3 451	60
LS 6164 R	2 891	86	3 118	54
PHB 94 Y 80 R	2 822	86	3 043	53
PAN 1521 R	2 817	86	3 038	53
LS 6453 R	2 772	86	2 989	52
PHB 95 Y 20	2 631	71	3 122	55
LS 6248 R	2 610	71	3 098	54
NS 5909 R	2 567	86	2 768	48
PAN 1500 R	2 542	86	2 741	48
PAN 1583 R	2 503	71	2 971	52
DM 5953 RSF	2 036	71	2 416	42
NS 6448	1 550	86	1 671	29
LS 6161 R	1 398	86	1 508	26
PAN 1664 R	1 367	71	1 622	28

*Prominence value (PV): Final population density of each genus $\times \sqrt{\text{frequency of occurrence}/10}$; ** Reproductive percentage reduction = Pf contained in roots of an individual genotype / Pf contained in roots of the most susceptible genotype

3.3.3 Plant-parasitic nematode population densities per 200 g rhizosphere soil

Rotylenchulus parvus Colbran, 1962, followed by *S. brachyurus* were the predominant plant-parasitic nematode species present in rhizosphere soil samples according to PVs (Table 3.8). Other nematode genera/species in decreasing order of predominance were: *Helicotylenchus* sp., *C. sphaerocephalus* Taylor, 1936, *P. teres*, *T. brevilineatus* Williams, 1960, *P. zaeae*, *Criconema* sp. and *Nanidorus minor* (Colbran, 1956) Siddiqi, 1974.

Table 3.8. Prominence values, frequencies of occurrence and mean population densities of plant-parasitic nematodes in 200 g rhizosphere soil samples of 29 soybean cultivars grown at seven localities in South Africa during the 2014/15 growing season.

Nematode	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
<i>Rotylenchulus parvus</i>	2 145	43	3 271
<i>Scutellonema brachyurus</i>	1 217	29	2 260
<i>Helicotylenchus</i> sp.	765	29	1 421
<i>Criconemoides sphaerocephalus</i>	18	14	49
<i>Pratylenchus teres</i>	17	29	31
<i>Tylenchorhynchus brevilineatus</i>	8	29	15
<i>Pratylenchus zaeae</i>	7	43	10
<i>Criconema</i> sp.	5	29	9
<i>Nanidorus minor</i>	2	14	4

*Prominence value (PV): Final population density of each genus $\times \sqrt{\text{frequency of occurrence}/10}$

²3.4 Discussion

The endoparasitic nematode genera *Meloidogyne* (*M. incognita*, followed by *M. javanica*) and *Pratylenchus*. (*P. brachyurus*, followed by *P. zeae*) were identified as the predominant nematode genera in the soybean roots. This observation is in agreement with Fourie *et al.* (2001) who listed the same genera and species as the most abundant genera and species in local soybean production areas resulting from a survey done in 1995/96. Also the predominancy of the ectoparasitic species identified in the rhizosphere soil, viz. *S. brachyurus* and *Helicotylenchus* sp., is in agreement with numbers in the survey reported on by Fourie *et al.* (2001). An interesting result from our study, however, is the high population representation of the semi-endoparasitic species (*R. parvus*) in the rhizosphere soil which was reported in low numbers by Fourie *et al.* (2001). This study did not reveal the presence of new genera and species other than the ones reported by Fourie *et al.* (2015a) and listed in the South African Plant-Parasitic Nematode Database (SAPPNS¹) (Marais *et al.*, 2017). Moreover, the 1995/96 survey was conducted at 17 localities, compared to seven for this study, which reduces the possibility of finding more *Meloidogyne* spp. associated with soybean. Furthermore, the presence of *Meloidogyne ethiopica* Whitehead (1968) and *Meloidogyne hapla* Chitwood (1949) recorded during the 1995/96 survey was not confirmed by current survey. This can be partially explained since the KwaZulu-Natal production areas where the latter species were found in the first survey, was not included in this study.

The MPDs and PVs of the plant-parasitic nematode taxa recorded during this study were substantially higher compared to those of the 1995/96 survey. Although *M. incognita* and *M. javanica* were also reported as predominant in the latter survey (Fourie *et al.*, 2001), their MPDs (252 219 and 69 260, respectively) and PVs (236 680 and 25 915, respectively) recorded in the current survey were substantially higher than that of all *Meloidogyne* spp. recorded in combination for the 1995/96 survey (MPD = 999 and PV = 953/5 g roots).

¹Dr Mariette Marais of the Nematology Unit, Biosystematics Division, Agricultural Research Council – Plant Health and Protection is thanked for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database; E-mail: maraism@arc.agric.za

The two predominant root-knot nematode species identified during this survey occur as either single or mixed populations in traditional maize production areas (Riekert, 1996; Riekert & Henshaw, 1998). Soybean production has been expanding to these areas during the last two decades. Rotation crops used in such areas, viz. groundnut (*Arachis hypogaea* L.), dry bean (*Phaseolus vulgaris* L.), sunflower (*Helianthus annuus* L.) (Fourie *et al.*, 2017), potato (*Solanum tuberosum* L.) (Jones *et al.*, 2017) and maize (*Zea mays* L.) (Mc Donald *et al.*, 2017) in particular are also susceptible to *M. incognita* and *M. javanica*. The root-knot nematode problem in grain production areas are hence aggravated by such crop rotation systems and ultimately limit sustainable crop production.

Another interesting phenomenon emanating from this survey is the substantially higher abundance of *Pratylenchus* spp. in soybean roots compared to that recorded for the 1995/96 survey (Fourie *et al.*, 2001). *Pratylenchus brachyurus*, *P. teres* and *P. zae* identified in the current study is in agreement with results by Fourie *et al.* (2001), but *Pratylenchus crenatus* Loof, 1960, *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurman Stekhoven, 1941, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurman Stekhoven, 1941 and *Pratylenchus thornei* Sher & Allen, 1953 (Fourie *et al.*, 2015) have not been found during this survey. During the 1995/96 survey, *P. zae* was the predominant lesion nematode in soybean fields, followed by *P. brachyurus*. Contrary to this, the opposite order in terms of dominance was recorded for these two species during the current study. Although *Pratylenchus* spp. were previously regarded of lesser importance to soybean globally (Bridge and Starr, 2007) and as a secondary nematode pest of the crop in Brazil (Ribeiro *et al.*, 2010), the high abundance of lesion nematodes in this study suggests that it should be considered as a major pest of soybean crops. Interestingly, PVs for *P. brachyurus* and *P. zae*, respectively, were 18 and nine times higher than those reported for the 1995/96 survey. This is in agreement with reports that *P. brachyurus* is a very important nematode pest of soybean in Brazil (Ribeiro *et al.*, 2010; Machado, 2014; Lima *et al.*, 2015). This lesion nematode species adversely impact on plant growth and yield of soybean (Koenning *et al.*, 1985) with soybean yield losses reported to range between 30 and 85 % (Lima *et al.*, 2015). The mean

population density of *P. brachyurus* of 2 996/5 g roots was well within the range as reported for Brazilian soybean fields (24 – 5 482/10 g roots). This species has become a major pest of soybean in especially the Cerrado region of Brazil (Ribeiro *et al.*, 2010; Lima *et al.*, 2015) and may have the same potential under South African environmental conditions. Also concerning is that Lima *et al.* (2015) showed that rotation of soybean with maize or sorghum in Brazilian production areas, favoured reproduction of *P. brachyurus*. These authors also reported the susceptibility of *Crotalaria juncea* (Braz *et al.*, 2016) to *P. brachyurus*, suggesting these crops should not be included in soybean rotation systems, but that fallow or non-host cover crops are used to manage this lesion nematode in Brazil. A similar situation is applicable for South Africa, since *P. brachyurus*, and also *P. zaeae*, reproduce well on crops that are commonly used in rotation with local soybean crops. This includes maize (Mc Donald *et al.*, 2017) and grain legumes such as groundnut and sunflower (Fourie *et al.*, 2017), suggesting that high population densities of such lesion nematodes may occur. The presence of *P. teres* in soybean roots was also 126 times higher than that recorded for the species in the 1995/96 survey (Fourie *et al.*, 2001). This species has also been reported from Vaalharts (Northern Cape Province, South Africa) where it occurred in higher numbers in cotton-based rotations (Van Biljon *et al.*, 2015) where soybean is also grown. Its damage potential to soybean is, however, unknown. Lesion nematodes is endemic to South Africa, has a wide host range and is distributed widely in local grain crop production areas (Van den Berg *et al.*, 2017). The occurrence of *Pratylenchus* at all localities sampled, and at high population densities suggests that more research should be aimed at studying the impact of this genus on soybean growth and yield.

Concerning the semi-endo/ectoparasitic and ectoparasitic nematodes identified from soil samples, *R. parvus* was predominant in soil samples, followed by *S. brachyurus* while *H. dihystra* followed by *S. brachyurus* were predominant in the 1995/96 survey (Fourie *et al.*, 2001). *Scutellonema brachyurus* occurred at approximately 63 % of the localities compared to that of the 1995/96 survey, and was nearly 10 times more abundant with a six times higher PV in this study than recorded for the first survey. Such data are useful since in Brazil, *H. dihystra* and *S. brachyurus* are listed as emerging

species with the potential to become major pests of soybean due to an increase in their prevalence in soybean growing areas (Maceda *et al.*, 2009; Lima *et al.*, 2009). Higher abundance and occurrence values for other soil nematodes listed for this study, compared to those for the 1995/96 survey, are also interesting. The 48 and 16 times higher PV and MPD, respectively, of *R. parvus* and its three times higher frequency for this study compared to those for the first survey may be attributed to the constant expansion of soybean production in maize production areas. Recently, Bekker *et al.* (2016) reported that *R. parvus* has been frequently encountered at high population levels in local maize production areas since 2011. Although this species has in the past been listed to occur in local soils where grain and legume crops were produced, the impact of this nematode species on grain and legume crops is unknown (Bekker *et al.*, 2016). The occurrence of species of the ectoparasitic *Criconema*, *Criconemoides*, *Nanidorus* and *Tylenchorhynchus* are not considered to be important on soybean locally (Fourie *et al.*, 2015a). Nonetheless, results from this study confirmed that soybean is a host to them and therefore potentially exist that they can become major pests of the crop as has been found with *H. dihystra* and *S. brachyurus* in Brazil (Maceda *et al.*, 2009; Lima *et al.*, 2009)

The presence of mixed root-knot nematodes species in soybean-based cropping systems (Fourie *et al.*, 2017) and the possibility that new species may be found necessitated the use of more than one identification method to ensure accurate identification (Devran & Söğüt, 2009). Identification of *M. incognita* (present at 86 % of the localities) and *M. javanica* (occurring at 14 % of the localities) was obtained by means of morphological identification based on perineal-pattern morphology. However identification attempted using molecular techniques were disappointing since the use of neither the D2-D3 nor NADH5 molecular techniques could distinguish among the *Meloidogyne* spp. present at the seven localities. These two techniques were only able to confirm that the species present at the seven localities sampled were thermophiles. For example, for the D2-D3 analysis, the seven local *Meloidogyne* populations grouped together with *M. arenaria*, *M. enterolobii*, *M. ethiopica*, *M. incognita*, *M. hispanica*, *M. inornata*, *M. javanica*, *M. konaensis*, *M. paranaensis* and *M. thailandica* selected from

NCBI Genbank. For NADH5 the range was narrower since the seven local species grouped together with only five species selected from Genbank, viz. *M. arenaria*, *M. ethiopica*, *M. incognita*, *M. javanica* and *M. luci*. A similar phenomenon was reported by Tao *et al.* (2017) who showed that for the D2-D3 was not able to distinguish among various thermophilic, but also cryophilic *Meloidogyne* spp. they selected from NCBI Genbank. Hence, the use of the sequence characterised amplified region-polymer chain reaction (SCAR-PCR) method that make use of species-specific primers of *Meloidogyne* should be the preferred method to distinguish *Meloidogyne* spp. for local studies together with morphological and morphometric analyses.

Except for reassessing the identity, abundance and occurrence of nematode pests of soybean, valuable information about the host status of soybean cultivars to the predominant endoparasitic plant-parasitic nematode genera (*Meloidogyne* and *Pratylenchus*) identified has also been generated. Although the MPDs of *Meloidogyne* spp., for both 5 and 50 g roots varied considerably among the cultivars, only data for the latter parameter will be discussed since the extraction technique (NaOCl method) used in this case was specifically developed to extract root-knot nematode eggs (Riekert, 1995). All cultivars, except two (PAN 1583 R and PAN 1521 R) had high susceptibility levels for *Meloidogyne* spp. when data were pooled across the seven localities. Only cultivars PAN 1583 R and PAN 1521 R maintained less than 10% of the population density that was recorded for the most susceptible cultivar PAN 1623 R. According to the host status protocol of Hussey & Janssen (2002), these two cultivars only can be regarded as resistant to the *Meloidogyne* spp. identified. This data imply that the majority of cultivars sampled in this study were susceptible to the two *Meloidogyne* spp. identified. Results hence do not correspond with the results in the greenhouse evaluations, which indicated DM 6.2i RR as a poor host of *M. incognita* (see Chapter 2) and *M. javanica* (Fourie *et al.*, 2015b). The latter cultivar hosted intermediate root-knot nematode population numbers (PV = 6 031) during this study for pooled data but maintained less than 10 % of the populations of *M. incognita* at Groblersdal (1 %), Hoopstad (0 %) and Potchefstroom (1 %) (data not shown), rendering it as resistant. Also at Kroonstad where *M. javanica* occurred, DM 6.2i RR maintained only 7 % of the

population density of the most susceptible cultivar (data not shown). These data are in agreement with glasshouse screenings that listed cultivar DM 6.2i RR as resistant to *M. incognita* (see Chapter 2; Paragraph 2.1.3.3) and that use of this cultivar will reduce *M. incognita* population densities substantially compared to a highly susceptible cultivar. Inconsistent findings for glasshouse and field experiments where DM 6.2i RR was susceptible to *M. incognita* (at localities Bethlehem, Brits and Middelburg), however, is not unexpected since such anomalies has been reported from other studies and other resistant cultivars (Fourie *et al.*, 2006; Sharma, *et al.*, 2006). For example, such contrasting results may be due to the occurrence of different races or pathotypes or virile populations of the target root-knot nematode species, as well as abiotic or biotic factors that occur in different climatic zones (Hussey & Jansen, 2002) where soybean is grown.

Pooled data for *Pratylenchus* spp. population densities per 5 g roots were high for all cultivars but varied markedly between the cultivars. PAN 1664 R maintained the lowest lesion nematode numbers and will be the best cultivar to plant to limit population growth of lesion nematodes. The opposite is applicable for cultivar DM 6.2i RR, resistant to *M. incognita*, which was the most susceptible and will generally support high numbers of lesion nematodes. In Brazil where *P. brachyurus* is also widespread, also no resistant cultivar against this nematode pest has also been identified (Rios *et al.*, 2016).

Dominance of *Meloidogyne* and *Pratylenchus* spp. that now parasitise soybean roots at notably higher population densities compared to those recorded 22 years earlier when the first soybean survey has been done, is concerning. Although initial population density, rotation history and various other abiotic and biotic factors impact on the build-up of nematode pest populations in specific fields, it is concluded that an increase in nematode pest numbers since the 1995/96 survey must be taken cognizance of. This trend re-emphasizes that *Meloidogyne* and *Pratylenchus* spp. in particular are of economic importance in local soybean production, but that other nematode species such as *R. parvus*, *S. brachyurus* and *H. dihystra* must also be considered when diagnostic analyses are done for producers.

This study also revealed the susceptibility of the majority of local, commercially-available glyphosate-tolerant soybean cultivars to *Meloidogyne* and *Pratylenchus* spp. The conclusion is that these two nematode genera especially pose a challenge to producers since no nematicide is registered for use on soybean in South Africa. Moreover, only a few cultivars with resistance to *Meloidogyne incognita* is available, which makes the local industry vulnerable to these pests.

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Chapter 4

Plant-parasitic nematode assemblages associated with glyphosate-tolerant and conventional soybean in South Africa

Abstract

The identity, abundance and frequency of occurrence of 13 plant-parasitic nematode genera and 25 species associated with glyphosate-tolerant (genetically modified; GM) and conventional soybean cultivars were recorded during two subsequent growing seasons. However, a total of 11 genera and 22 species were listed when the plant-parasitic nematodes associated with natural vegetation adjacent to the soybean fields were added to this list. *Meloidogyne incognita*, followed by *Meloidogyne javanica* and *Pratylenchus zeae* generally dominated in the roots of both soybean crops and natural vegetation. In rhizosphere soil samples, *Meloidogyne* followed by either *Helicotylenchus* or *Scutellonema* were the predominant genera. Non-GM soybean cultivars hosted the highest nematode diversity (11 genera and 22 species) followed by the natural vegetation (nine genera and 22 species) and the glyphosate-tolerant soybean cultivars (10 genera and 17 species). *Pratylenchus* was the most diverse genus (nine species identified) with *Criconea*, *Hemicycliophora*, *Nanidorus* and *Rotylenchus* the least (one species each identified). Seven species, viz. *Pratylenchus flakkensis*, *Pratylenchus scribneri*, *Pratylenchus vulnus*, *Rotylenchulus brevicaudatus*, *Telotylenchus avaricus*, *Tylenchorhynchus brevicaudatus* and *Quinisulcius capitatus* are first reports for soybean in South Africa.

Keywords: *Glycine max*, glyphosate-tolerant, *Meloidogyne*, *Pratylenchus*.

4.1 Introduction

Soybean (*Glycine max* L.) is an important oilseed and protein crop cultivated in South Africa (Dlamini *et al.*, 2014). The commercialisation of genetically-modified (GM), glyphosate-tolerant soybean cultivars commenced in South Africa during the 2001/2002 growing season (Wolson, 2007). This resulted in a substantial increase in local soybean production (Dlamini *et al.*, 2014). Moreover, growing soybean proved to be advantageous to producers due to nitrogen (N) residues becoming available in soils after cultivation of the crop. This resulted in a reduction of the application of fertilizers, particularly N, and lower input costs for follow-up crop (Torres *et al.*, 2015).

Plant-parasitic nematodes remain a major challenge in crop production, especially in sub-Saharan African countries such as South Africa (Coyne *et al.*, 2009; Fourie *et al.*, 2017). Keetch (1989) reported up to 9 % yield losses to local soybean crops due to parasitism by various nematode pests. Approximately 10 years later, yield losses ranging from 25 to 100 % were recorded as a result of root-knot nematode (*Meloidogyne*) parasitism (Riekert & Henshaw, 1998; De Beer *et al.*, 2014). These figures show that damage to soybean caused by nematode pests is larger than anticipated since symptoms are usually non-specific due to these microscopic organisms often not being detected (Bridge & Starr, 2007).

About 50 plant-parasitic nematode species have been listed on soybean in South Africa (Fourie *et al.*, 2015), with *Meloidogyne* being the predominant genus (Fourie *et al.*, 2015). This genus is also reported as the economically most important of soybean in other parts of the world (Bridge & Starr, 2007; Machado *et al.*, 2014). The predominant *Meloidogyne* spp. occurring in the soybean production areas in South Africa are *Meloidogyne incognita* (Kofoed & White, 1919) Chitwood, 1949, followed by *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. The presence of *Meloidogyne hapla* (Chitwood, 1949) and *Meloidogyne ethiopica* (Whitehead, 1968) in local production areas has also been reported (Fourie *et al.*, 2001). The predominance of root-knot nematodes raises concern because only a limited number of commercial cultivars with resistance to these pests are available. These, for example, include the

conventional cultivar Egret (Fourie *et al.*, 2015) and the glyphosate-tolerant cultivar DM 6 2i RR that exhibit resistance to *M. incognita* (See Chapter 2, Paragraph 4.1.3.2).

Another economically important plant-parasitic nematode genus that damages soybean crops worldwide is lesion nematodes (*Pratylenchus*) (Bridge & Starr, 2007). In South Africa, *Pratylenchus zeae* Graham, 1951 and *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 were recorded as the predominant species during a survey conducted in 1995/96 (Fourie *et al.*, 2001). *Pratylenchus crenatus* Loof, 1960, *Pratylenchus neglectus* (Rensch, 1924), Filipjev & Schuurmans Stekhoven, 1949, *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus teres* Khan & Singh, 1975 were also recorded during this survey. A major concern is that in contrast with root-knot nematodes no systematic screening for resistance to lesion nematodes is being done in locally commercially available soybean cultivars. Semi-ecto/endoparasitic nematode species also found in association with local soybean are *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961, *Scutellonema brachyurus* (Steiner, 1938) Andr  ssy, 1958, and a variety of other plant-parasitic nematodes of lesser importance (Fourie *et al.*, 2001; Fourie *et al.*, 2015).

The soybean nematode survey conducted in 1995/96 was done during a time when only conventional soybean cultivars were grown in South Africa. At present, however, the soybean market is dominated by glyphosate-tolerant cultivars (Dlamini *et al.*, 2014). For these cultivars, no information on their host response to plant-parasitic nematode infection is available. Furthermore, the cyst nematode *Heterodera glycines* (Ichinohe, 1952) that can cause devastating yield losses of soybean in North and South America, and in Asia, has not been found in local soybean production areas to date (M. Marais, 2017, Agricultural Research Council-Plant Protection Research Institute; ARC - Plant Health and Protection, Roodeplaat). The search for plant-parasitic nematodes that are known to be threats to a certain crop and that could be expected to occur in the production areas of that crop is important to allow for development of a timely management strategy and minimise yield losses (Palomarez-Rius & Kikuchi, 2013; Jones *et al.*, 2013).

The substantial increase in soybean production in South Africa (about 11-fold from 68 000 to 720 000 ha during the past 20 years) (GrainSA, 2017) warranted a follow-up nematode survey. In South Africa, soybean production has been expanded into areas where either maize (*Zea mays* L.) was traditionally grown or where natural vegetation has been removed to plant the crop. Insight into the nematode population composition (assemblages) in these new soybean production areas can be considered crucial to ensure optimal production of the crop. Also, in contrast with maize, which has been and still is by many producers cultivated as a monocrop, soybean is cultivated in South Africa in rotation with groundnut (*Arachis hypogaea* L.), potato (*Solanum tuberosum* L.) and sunflower (*Helianthus annuus* L.) (Nel, 2005; Duarte *et al.*, 2011). *Meloidogyne incognita* and *M. javanica*, as well as various *Pratylenchus* spp. are also abundant on these crops (De Waele *et al.*, 1988; Bolton *et al.*, 1989; Venter *et al.*, 1992; Riekert, 1996; Riekert & Henshaw, 1998; Engelbrecht, 2012).

In view of the continuous expansion outside the traditional soybean production areas locally, this study was undertaken to examine the current abundance and diversity of plant-parasitic nematodes in fields planted with glyphosate-tolerant and conventional soybean cultivars as well as in adjacent natural vegetation.

4.2 Methodology

4.2.1 Description of sampled localities and nematode sampling

During the 2011/12 growing season, soybean root systems and rhizosphere soil were collected from eight soybean fields of which four were planted with genetically-modified glyphosate-tolerant and four with conventional soybean cultivars (Fig. 4.1). Concurrently, root and soil samples were also collected from natural vegetation either adjacent to, or within 50 to 100 m from the soybean fields. At each sampling locality, 80 rhizosphere soil and 80 root samples were collected, which were pooled and 20 sub-samples of each examined. Glyphosate had been applied annually for a minimum of five years prior to our study in the fields with the glyphosate-tolerant soybean cultivars. However, in the fields planted with conventional soybean cultivars, no glyphosate-tolerant cultivars were grown and no glyphosate was applied for at least 5 years prior

this study. No crop cultivation has taken place for at least 10 years prior to this study in the areas where the natural vegetation was sampled.

During the 2012/13 growing season, the same soybean fields and natural vegetation sites sampled during the preceding season were sampled again, as well as nine additional fields including natural vegetation (Fig. 4.1). Five of these additional fields were planted with glyphosate-tolerant and four with conventional soybean cultivars. In total, 18 soybean fields were sampled during the latter season.

Information about the soybean cultivar, crop history and soil properties for each field sampled are listed in Table 4.1. The names of the soybean cultivars planted and the history of each field for two years prior to our study were also obtained from the farmers. Soil properties were determined by the EcoAnalitica Laboratory of North-West University (NWU, Potchefstroom) using internationally-accredited protocols (Walkey & Black, 1947; Bouyoucos, 1962; Beretta *et al.*, 2014). Total rainfall and mean temperature data for each locality, from planting of the soybean crops until nematode sampling, are also listed in Table 4.2. The data were obtained from Ms Irene Joubert, Agricultural Research Council - Infruitec (ARC - Infruitec), Stellenbosch. Rip and till was the soil cultivation practices used in all soybean fields.

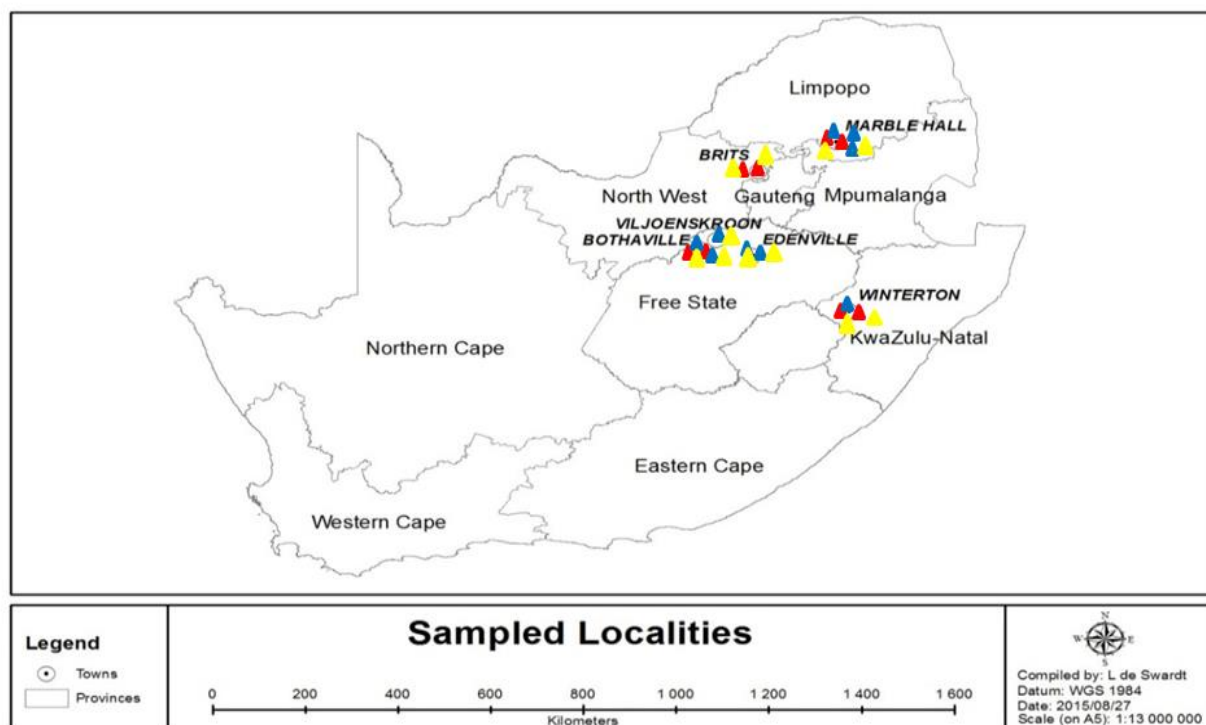


Figure 4.1. Location of the six localities included in the plant-parasitic nematode survey carried out during the 2011/12 and 2012/13 growing seasons. Fields sampled during 2011/12 are indicated by red triangles and those sampled during 2012/13 by blue triangles while adjacent natural vegetation sites are indicated by yellow triangles for both seasons. (Compiled by: Ms L. de Swart, NWU).

4.2.2 Nematode extraction

Nematodes were extracted from 5 and 50 g root sub-samples, and 200 g rhizosphere soil sub-samples using the same, respective protocols as described in Chapter 3, Paragraph 3.2.1.1.

4.2.3 Nematode identification

Nematode genera and species identifications were done as described in Chapter 3, Paragraph 3.2.1.2.

4.2.4 Data analyses

The frequency of occurrence, mean population density (MPD) and prominence value (PV) of each nematode genus/species were calculated as described in Chapter 3, Paragraph 3.2.1.3.

Plant-parasitic nematode data were $\log_{10}(x+1)$ transformed in Microsoft Excel (Version 2013) and subjected to the Student's t-test using Statistica Version 13.2 (www.statsoft.com). This was done to determine whether significant differences existed in the occurrence of the predominant genera (*Meloidogyne* in 50 g roots, *Pratylenchus* in 5 g roots, *Helicotylenchus* in 200 g rhizosphere soil) among the localities as well as ecosystems (glyphosate-tolerant and conventional soybean, conventional soybean and natural vegetation, and glyphosate-resistant and natural vegetation).

Table 4.1. Soybean cultivar planted at each soybean field, crop history, and selected soil chemical and physical properties of each field where plant-parasitic nematodes from roots and rhizosphere soil samples were collected during the 2011/12 and 2012/13 growing seasons.

Sampling season	Locality	Ecosystem and cultivar	Crop history	Soil chemical properties					Soil physical properties				
				pH (H ₂ O)	Ca	Mg	K	Na	P	% Sand	% Silt	% Clay	% total C
2011/12 & 2012/13	Bothaville	Glyphosate-tolerant soybean (PAN1664R)	Maize/Sunflower	6.48	381	107	205	0.5	204	94.7	0.7	4.6	0.21
2012/13		Glyphosate-tolerant soybean (PAN1664R)	Maize/Sunflower	6.48	437	81	170	0.5	170	94.5	0.7	4.8	0.10
2011/12 & 2012/13		Conventional soybean (Egret)	Maize/Sunflower	6.89	581	81	246	1	166	92.6	0.7	6.7	0.23
2012/13		Conventional soybean (Egret)	Maize/Sunflower	6.43	374	96	203	0.5	203	94.6	0.7	4.7	0.22
2011/12 & 2012/13		Natural vegetation (grass)		6.77	446	78	194	0.5	169	94.5	0.7	4.7	0.21
2011/12 & 2012/13		Natural vegetation (grass)		6.06	574	165	400	5	252	89.6	3.4	7	1.5
2011/12 & 2012/13	Brits	Glyphosate-tolerant soybean (PAN1583R)	Maize/Sunflower	7.28	1434	304	497	2	500	79.9	5.5	14.6	1.36
2011/12 & 2012/13		Conventional soybean (Egret)	Soybean/Wheat	7.49	1699	346	291	58.5	399.	81.4	7.6	11	0.76
2011/12 & 2012/13		Natural vegetation (grass)		7.11	2840	559	417	91.5	509	58.3	12.4	29.2	1.51
2011/12 & 2012/13		Natural vegetation (grass)		7.6	3206	636	342	98	53	74.1	9.1	16.8	3.95
2012/13	Edenville	Glyphosate-tolerant soybean (PAN1664R)	Soybean	6.15	1486	401	419	60.5	40	59.3	19.1	21.6	0.20

Table 4.1. Continued

Sampling season	Locality	Ecosystem and cultivar	Crop history	Soil chemical properties					Soil physical properties				
				pH (H ₂ O)	Ca	Mg	K	Na	P	% Sand	% Silt	% Clay	% total C
2012/13	Marble Hall	Conventional soybean (Superboon)	Soybean	4.97	512	213	292	9.5	118	70.7	9.3	20	0.49
2012/13		Natural vegetation (grass)		5.92	396	67	268	3	93	85.9	3.4	10.7	3.89
2012/13		Natural vegetation (grass)		5.64	222	52	271	1.5	86	86.6	3.2	10.1	0.34
2011/12 & 2012/13		Glyphosate- tolerant soybean (PAN1454R)	Maize/ Soybean	6.09	666	228	390	20	402	84.9	6.7	8.4	0.56
2012/13		Glyphosate- tolerant soybean (LS6164R)	Maize/ Soybean	6.64	541	149	146	23	118	91	3.7	5.1	0.84
2012/13		Glyphosate- tolerant soybean (LS6164R)	Maize/ Soybean	6.07	826	212	291	176	336	88.7	3.7	7.6	0.40
2011/12 & 2012/13		Conventional soybean (Egret)	Soybean	7.05	1001	402	390	34.5	67	78.6	8.7	12.7	0.58
2012/13		Conventional soybean (MC555)	Soybean	6.62	1012	244	509	14.5	156	91.2	3.7	5.1	1.76
2011/12 & 2012/13		Natural vegetation (grass)		6.83	968	238	346	35.5	151	69.2	9.3	21.5	1.8
2011/12 & 2012/13		Natural vegetation (grass)		6.52	455	104	192	24.5	164	86.1	1.4	12.6	0.54
2011/12 & 2012/13	Viljoens-kroon	Natural vegetation (grass)		5.93	810	121	375	25	419	83.7	3.8	12.5	2.54
2012/13		Glyphosate- soybean tolerant (PAN1583R)	Maize/ Soybean	6.82	381	96	330	5	295	94.1	1.2	4.7	0.2

Table 4.1. Continued

Sampling season	Locality	Ecosystem and cultivar	Crop history	Soil chemical properties					Soil physical properties				
				pH (H ₂ O)	Ca	Mg	K	Na	P	% Sand	% Silt	% Clay	% total C
2012/13	Winterton	Natural vegetation (grass)		6.14	433	195	370	9.5	227	84.4	5.9	9.7	1.14
2011/12 & 2012/13		Glyphosate-tolerant soybean (PAN6164R)	Soybean	5.67	1782	400.5	381	20.5	85	81.2	10.4	8.5	2.49
2012/13		Conventional soybean (Mukwa)	Maize/ Soybean	6.33	1565	198	182	2	390	78.8	12.9	8.4	1.3
2011/12 & 2012/13		Conventional soybean (Mukwa)	Maize/ Soybean	5.92	1827	139	551	5.5	532	44.8	38.4	16.7	2.77
2011/12 & 2012/13		Natural vegetation (grass)		6.85	751	120	86	0.5	335	61.4	23.3	15.3	1.62
2011/12 & 2012/13		Natural vegetation (grass)		5.65	887	208	295	4.5	358	45.9	31.4	22.8	2.59

Table 4.2. Mean temperature and total rainfall figures for the six localities where a nematode survey was conducted during the 2011/12 and 2012/13 growing seasons.

Locality and province	Growing season	Temperature (°C)		Rainfall (mm)
		Min.	Max.	
Bothaville (Free State)	2011/12	13	26	272
	2012/13	14	30	255
Brits (North-West)	2011/12	16	33	414
	2012/13	16	32	365
Edenville (Free State)	2012/13	16	32	303
Marble Hall (Mpumalanga)	2011/12	18	31	402
	2012/13	18	35	353
Viljoenskroon (Free State)	2012/13	14	31	373
Winterton (Kwa-Zulu Natal)	2011/12	14	30	409
	2012/13	14	30	417

4.3 Results

Eleven genera and 31 plant-parasitic nematode species were identified in the roots and rhizosphere soil of the soybean cultivars and natural vegetation (Table 4.3). Of these, 10 genera and 25 species were associated with the soybean cultivars (both glyphosate-tolerant and conventional).

Table 4.3. Plant-parasitic nematodes associated with soybean and natural vegetation at 28 sites in the soybean production areas of South Africa during the 2011/12 and 2012/13 growing seasons (✓ indicates the presence of a genus/species).

Genus and species	Glyphosate-tolerant soybean	Conventional soybean	Natural vegetation
<i>Criconema mutabile</i> (Taylor, 1936) Raski & Luc, 1985	✓	✓	
<i>Criconemoides ferniae</i> Luc, 1959			✓
<i>Criconemoides obtusicaudatus</i> Heyns, 1962			✓
<i>Criconemoides parvus</i> Raski, 1952		✓	✓
<i>Criconemoides sphaerocephalus</i> Taylor, 1936	✓	✓	✓
<i>Helicotylenchus dihystra</i> (Cobb, 1893) Sher, 1961	✓	✓	✓
<i>Helicotylenchus martini</i> Sher, 1966			✓
<i>Helicotylenchus parapatyurus</i> Siddiqi, 1972			✓
<i>Helicotylenchus pseudorosbustus</i> (Steiner, 1914) Golden, 1956		✓	
<i>Helicotylenchus</i> spp. Steiner, 1945	✓	✓	✓
<i>Hemicycliophora typica</i> de Man, 1921		✓	
<i>Meloidogyne incognita</i> (Kofoid & White, 1919) Chitwood, 1949	✓	✓	✓
<i>Meloidogyne javanica</i> (Treub, 1885) Chitwood, 1949	✓	✓	✓
<i>Nanidorus minor</i> (Colbran, 1956), Siddiqi, 1974	✓		
<i>Pratylenchus brachyurus</i> (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941	✓		✓
<i>Pratylenchus crenatus</i> Loof, 1960		✓	
<i>Pratylenchus flakkensis</i> Seinhorst, 1968		✓	
<i>Pratylenchus neglectus</i> (Rensch, 1924), Filipjev & Schuurmans Stekhoven, 1941	✓	✓	✓
<i>Pratylenchus scribneri</i> Steiner, 1943		✓	✓
<i>Pratylenchus teres</i> Khan & Singh, 1975	✓		✓
<i>Pratylenchus thornei</i> Sher & Allen, 1953	✓	✓	
<i>Pratylenchus vulnus</i> Allen & Jensen, 1951	✓	✓	
<i>Pratylenchus zaeae</i> Graham, 1951	✓	✓	✓
<i>Quinisulcius capitatus</i> (Allen, 1955) Siddiqi, 1971		✓	
<i>Rotylenchus brevicaudatus</i> (Williams, 1960) Sher, 1961			✓
<i>Rotylenchulus parvus</i> Colbran, 1962	✓	✓	✓
<i>Rotylenchus unisex</i> Sher, 1965	✓	✓	✓
<i>Scutellonema brachyurus</i> (Steiner, 1938) Andr�ssy, 1958	✓	✓	✓
<i>Scutellonema truncatum</i> Sher, 1964		✓	✓
<i>Telotylenchus avaricus</i> Kleynhans, 1975			✓
<i>Tylenchorhynchus brevilineatus</i> Williams, 1960	✓	✓	✓

4.3.1 Plant-parasitic nematodes per 50 g roots

Two root-knot nematode species were identified from 50 g root sub-samples of soybean and the natural vegetation using both perineal-pattern morphology and the molecular Sequenced Characterized Amplified Region – Polymerase Chain Reaction (SCAR-PCR) technique (data not shown). *Meloidogyne incognita* was present in 25 % and *M. javanica* in 11 % of all samples. These species occurred in either single or mixed populations at all the localities, except for Edenville where the prevailing *Meloidogyne* sp. could not be identified to date using both morphological and molecular techniques.

The PVs and MPDs of *Meloidogyne* were the highest for the glyphosate-tolerant soybean cultivars during the 2011/12 season followed by the natural vegetation and the conventional soybean cultivars (Table 4.4). The MPDs of *Meloidogyne* were significantly ($P \leq 0.05$) higher for the glyphosate-tolerant cultivars compared to the conventional soybean cultivars, and the natural vegetation during this season (Table 4.5). By contrast, conventional soybean cultivars had the highest PV and MPD for *Meloidogyne*, followed by the glyphosate-tolerant soybean cultivars and natural vegetation during the 2012/13 season (Table 4.4). The MPDs of *Meloidogyne* were significantly ($P \leq 0.05$) higher in the natural vegetation compared to both glyphosate-tolerant and conventional soybean cultivars during this season (Table 4.5).

The frequency of occurrence of *Meloidogyne* ranged from 75 to 88 % and from 50 to 88 % for glyphosate-tolerant and conventional soybean cultivars, respectively, during the two seasons (Table 4.4). In the natural vegetation, *Meloidogyne* occurred in 75 and 83 % of the samples, respectively for the two seasons.

Table 4.4. Prominence values, frequencies of occurrence and mean population densities of *Meloidogyne* in 50 g root samples of glyphosate-tolerant and conventional soybean cultivars, as well as natural vegetation at 28 sites in South Africa during the 2011/12 and 2012/2013 growing seasons.

Ecosystem	Prominence value(PV)*	Frequency of occurrence	Mean population density (MPD)	Ecosystem	Prominence value(PV)*	Frequency of occurrence	Mean population density (MPD)
2011/12							
Glyphosate-tolerant soybean	402 276	75	464 522	Glyphosate-tolerant soybean	244 479	88	260 639
Conventional soybean	2 333	50	3 300	Conventional soybean	2 085 251	88	2 223 083
Natural vegetation	20 753	75	23 964	Natural vegetation	140 754	83	154 506

*Prominence value (PV) = final population density (Pf) of each species $\times \sqrt{\text{frequency of occurrence}/10}$.

Table 4.5. Comparison of the population densities of *Meloidogyne* per 50 g root sub-samples of glyphosate-tolerant and conventional soybean cultivars, as well of natural vegetation at 28 sites in South Africa during the 2011/12 and 2012/2013 growing seasons.

Growing seasons					
2011/12			2012/13		
Ecosystems	t-value	P	Ecosystems	t-value	P
Glyphosate-tolerant soybean:1.13* (12 213**±5 880***) Conventional soybean: 0.48 (155±41)	2.88	0.005	Glyphosate-tolerant soybean:1.88 (1 448±2 165) Conventional soybean: 1.92 (12 351±35 088)	0.22	0.83
Glyphosate-tolerant soybea: 1.13 (12 213±5 880) Natural vegetation: 0.72 (128±310)	2.13	0.03	Glyphosate-tolerant soybean:1.88 (1 448±2 165) Natural vegetation: 0.91 (594±2194)	6.83	0.001
Conventional soybean: 0.48 (155±41) Natural vegetation: 0.72 (155±41)	-1.71	0.09	Conventional soybean: 1.92* (12 351±35 088) Natural vegetation: 0.91 (594±2194)	6.81	0.001

*log₁₀ (x+1) transformed data; **real means; ***standard deviation (Student's t-test where $P \leq 0.05$).

4.3.2 Plant-parasitic nematodes per 5 g roots

For both seasons combined, 10 plant-parasitic nematode genera and 19 species were identified from the roots of the conventional soybean cultivars, seven genera and 15 species from those of glyphosate-tolerant soybean cultivars, and nine genera and 13 species from roots of natural vegetation (Table 4.6). The PVs and MPDs of the majority of the plant-parasitic nematode species were substantially higher during the 2012/13 compared to the 2011/12 season, except for *Meloidogyne* and *Pratylenchus*.

Lesion nematodes dominated in all three ecosystems, except for glyphosate-tolerant soybean during the 2011/12 season when root-knot nematodes dominated, and in natural vegetation during the 2012/13 season when *R. unisexus* dominated (Table 4.6). The highest diversity of lesion nematodes, viz. seven species, was observed in the conventional soybean ecosystem opposed to six for the glyphosate-tolerant soybean ecosystem and three for the natural vegetation ecosystem. *Pratylenchus zeae* dominated in all three ecosystems during both seasons. This species had the highest PVs and MPDs compared to the other lesion nematode species. No significant differences were observed for the MPDs of lesion nematodes for glyphosate-tolerant vs conventional soybean, glyphosate-tolerant vs natural vegetation and conventional soybean vs natural vegetation for both seasons (Table 4.6). *Pratylenchus* individuals were present in 20 to 75 % of conventional soybean, in 17 to 83 % of glyphosate-tolerant soybean, and in 13 to 50 % of natural vegetation samples.

Other genera (and species: see Table 4.6) identified from 5 g roots of the three ecosystems included: *Criconema* (only on conventional soybean cultivars), *Criconemoides* (all three ecosystems), *Helicotylenchus* (all three ecosystems), *Hemicyclophora* (only on conventional soybean cultivars), *Nanidorus* (only on glyphosate-tolerant soybean cultivars), *Quinisulcius* (only on conventional soybean cultivars), *Rotylenchus* (only on natural vegetation), *Rotylenchulus* (all three ecosystems), *Scutellonema* (all three ecosystems) and *Tylenchorhynchus* (all three ecosystems) (Table 4.6).

Table 4.6. Prominence values, frequencies of occurrence and mean population densities of plant-parasitic nematodes in 5 g root sub-samples of glyphosate-tolerant and conventional soybean cultivars, as well as natural vegetation at 28 sites in South Africa during the 2011/12 and 2012/2013 growing seasons.

Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
Glyphosate-tolerant soybean				Conventional soybean				Natural vegetation			
2011/12 season											
Meloidogyne spp.	54 406	25	108 812	Pratylenchus zeae	5 468	75	6 076	Pratylenchus zeae	240	38	400
Pratylenchus zeae	3 531	50	7 061	Pratylenchus thornei	798	25	1 596	Meloidogyne spp.	180	25	360
Criconemoides sphaerocephalus	335	25	670	Rotylenchulus parvus	121	25	241	Scutellonema brachyurus	73	25	146
Pratylenchus thornei	233	50	466	Pratylenchus scribneri	102	25	203	Helicotylenchus dihystra	54	25	108
Scutellonema brachyurus	176	25	352	Scutellonema brachyurus	73	25	146	Pratylenchus teres	23	13	57
Pratylenchuhs neglectus	104	25	207	Pratylenchus neglectus	70	25	140	Rotylenchus unisexus	2	13	5
Pratylenchus vulnus	78	25	155	Helicotylenchus pseudorosbustus	29	25	57	Tylenchorhynchus brevilineatus	2	15	4
Rotylenchus unisexus	59	25	117	Criconema mutabile	27	25	53	-	-	-	-
Tylenchorhynchus brevilineatus	18	25	35	Criconemoides sphaerocephalus	25	25	50	-	-	-	-
Pratylenchus teres	5	25	9	Quinisulcius capitatus	24	25	47	-	-	-	-
Helicotylenchus dihystra	2	25	3	Criconemoides parvus	20	25	40	-	-	-	-
-	-	-	-	Meloidogyne spp.	15	50	22	-	-	-	-
-	-	-	-	Helicotylenchus dihystra	13	25	26	-	-	-	-
-	-	-	-	Pratylenchus vulnus	11	25	21	-	-	-	-

*Prominence value (PV) = final population density (Pf) of each species $\times \sqrt{\text{frequency of occurrence}/10}$

Table 4.6 continues											
Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
Glyphosate-tolerant soybean				Conventional soybean				Natural vegetation			
2012/13 season											
<i>Pratylenchus zeae</i>	26 469	83	29 410	<i>Pratylenchus zeae</i>	27 839	60	5 034	<i>Rotylenchus unisexus</i>	994	17	2 484
<i>Pratylenchus brachyurus</i>	1 574	17	3 935	<i>Meloidogyne</i> spp.	6 887	40	3 720	<i>Helicotylenchus paraplatus</i>	635	17	1 587
<i>Meloidogyne</i> spp.	1 109	33	1 849	<i>Pratylenchus crenatus</i>	2 517	20	3 550	<i>Pratylenchus zeae</i>	377	50	538
<i>Pratylenchus neglectus</i>	772	17	1 930	<i>Pratylenchus flakkensis</i>	1 860	20	2 843	<i>Pratylenchus brachyurus</i>	48	33	80
<i>Rotylenchulus parvus</i>	178	17	446	<i>Scutellonema brachyurus</i>	1 775	40	2 303	<i>Criconemoides freniae</i>	7	33	18
<i>Tylenchorhynchus brevilineatus</i>	157	17	393	<i>Pratylenchus thornei</i>	1 706	40	1 037	<i>Telotylenchus avaricus</i>	3	17	7
-	-	-	-	<i>Rotylenchus unisexus</i>	1 156	20	1 033	<i>Meloidogyne</i> spp.	3	17	6
-	-	-	-	<i>Pratylenchus neglectus</i>	519	20	536	-	-	-	-
-	-	-	-	<i>Rotylenchulus parvus</i>	517	20	482	-	-	-	-
-	-	-	-	<i>Helicotylenchus dihystra</i>	268	20	270	-	-	-	-
-	-	-	-	<i>Scutellonema truncatum</i>	241	20		-	-	-	-
-	-	-	-	<i>Pratylenchus scribneri</i>	121	20		-	-	-	-

No significant differences were observed among the different ecosystems with regard to the MPDs of *Pratylenchus* spp. during both seasons (Table 4.7).

Table 4.7. Comparison of *Pratylenchus* spp. population densities per 5 g root sub-samples of glyphosate-tolerant and conventional soybean cultivars, as well natural vegetation at 28 sites in South Africa during the 2011/12 and 2012/2013 growing seasons.

Growing seasons					
2011/12			2012/13		
Ecosystems	t-value	P	Ecosystems	t-value	P
Glyphosate-tolerant soybean: 0.59* (658**±1 611***)	0.17	0.87	Glyphosate-tolerant soybean: 0.74(658±1611)	0.01	0.99
Conventional soybean: 0.76 (1 290±2 884)			Conventional soybean: 0.76 (1 290±2 884)		
Glyphosate-tolerant soybean: 0.59 (658±1 611)	0.44	0.67	Glyphosate-tolerant soybean: 0.74 (658±1 611)	0.36	0.73
Natural vegetation:0.48(44±107)			Natural vegetation: 0.44 (44±107)		
Conventional soybean: 0.76 (1 290±2 884)	0.15	0.89	Conventional soybean: 0.7 (1 290±2 884)	0.35	0.73
Natural vegetation: 0.48 (44±107)			Natural vegetation: 0.44 (44±107)		

*log₁₀ (x+1) transformed data; **real means; ***standard deviation (Student's t-test where $P \leq 0.05$)

4.3.1 Plant-parasitic nematodes per 200 g rhizosphere soil

Twenty-three plant-parasitic nematode species belonging to twelve genera were identified from the three ecosystems, viz. *Criconema*, *Criconemoides*, *Helicotylenchus*, *Hemicyclophora*, *Meloidogyne*, *Nanidorus*, *Pratylenchus*, *Quinisulcius*, *Rotylenchus*, *Scutellonema*, *Telotylenchus* and *Tylenchorhynchus* (Table 4.8).

For both seasons combined, nine genera and 19 plant-parasitic nematode species were identified from rhizosphere soil of the conventional soybean cultivars, eight genera and 19 species for the natural vegetation and eight genera and 14 species for the glyphosate-tolerant soybean cultivars (Table 4.8). Based on the population densities of plant-parasitic nematodes per 200 g rhizosphere soil samples, the PVs and MPDs of the majority of the species were substantially higher during the 2012/13 compared to the 2011/12 season. For the 2011/12 and 2012/13 seasons the highest diversity of plant-parasitic nematodes was recorded for conventional soybean and natural vegetation ecosystems, respectively.

Meloidogyne dominated in rhizosphere soil of the glyphosate-tolerant soybean cultivars and the natural vegetation, while *Rotylenchulus* were most abundant in soil samples of conventional soybean cultivars during the 2011/12 season (Table 4.8). During 2012/13 lesion nematodes dominated in soil of glyphosate-tolerant cultivars and *Helicotylenchus* in soil of conventional soybean cultivars, and natural vegetation. The highest diversity of lesion nematodes was observed in rhizosphere soil of the conventional soybean cultivars. In the latter ecosystem, six lesion nematode species were identified opposed to five species for glyphosate-tolerant soybean cultivars and four for natural vegetation. Not one specific lesion nematode species, but different ones, dominated in the different ecosystems during the two seasons.

During the 2012/13 season, the highest PVs and MPDs of *Helicotylenchus* were observed in the rhizosphere soil of conventional soybean cultivars and natural vegetation (Table 4.8). The frequency of occurrence of *Helicotylenchus* over the two

seasons ranged from 25 to 60 % for soil of the conventional soybean cultivars, 33 to 50 % for glyphosate-tolerant soybean cultivars and 13 to 83 % for natural vegetation.

Other genera and species (see Table 4.6) identified from the soil rhizosphere samples of the three ecosystems included: *Criconema* (only on conventional soybean cultivars), *Criconemoides* (all three ecosystems), *Helicotylenchus* (all three ecosystems), *Hemicycliophora* (only on conventional soybean cultivars), *Nanidorus* (only on glyphosate-tolerant soybean cultivars), *Quinisulcius*, (only on conventional soybean cultivars), *Rotylenchus* (only on natural vegetation), *Rotylenchulus* (all three ecosystems), *Scutellonema* (all three ecosystems), *Telotylenchus* (only on natural vegetation) and *Tylenchorhynchus* (all three ecosystems) (Table 4.8).

Table 4.8. Prominence values, frequencies of occurrence and mean population densities of plant-parasitic nematodes in 200 g soil rhizosphere sub-samples of glyphosate-tolerant and conventional soybean cultivars, as well as natural vegetation at 28 sites in South Africa during the 2011/12 and 2012/2013 growing seasons.

Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
Glyphosate-tolerant soybean				Conventional soybean				Natural vegetation			
2011/12 growing season											
<i>Meloidogyne spp.</i>	963	25	1 925	<i>Rotylenchulus parvus</i>	1 850	25	3 703	<i>Meloidogyne spp.</i>	4 020	38	6 700
<i>Helicotylenchus dihystra</i>	741	50	1 059	<i>Scutellonema brachyurus</i>	1 125	25	2 250	<i>Rotylenchulus parvus</i>	2 829	25	5 658
<i>Scutellonema brachyurus</i>	340	25	679	<i>Pratylenchus zeae</i>	353	50	479	<i>Helicotylenchus dihystra</i>	2 239	25	4 477
<i>Criconemoides sphearocephalus</i>	188	25	375	<i>Helicotylenchus pseudorosbutus</i>	295	25	589	<i>Rotylenchus unisexus</i>	1 719	25	3 437
<i>Rotylenchus unisexus</i>	115	25	229	<i>Helicotylenchus dihystra</i>	200	25	400	<i>Scutellonema brachyurus</i>	1 659	25	3 317
<i>Pratylenchus thornei</i>	178	25	355	<i>Meloidogyne spp.</i>	175	25	350	<i>Helicotylenchus paraplatyurus</i>	1 500	13	3 749
<i>Pratylenchus neglectus</i>	88	25	175	<i>Pratylenchus scribneri</i>	133	25	266	<i>Rotylenchus brevicaudatus</i>	786	13	1 965
<i>Pratylenchus zeae</i>	44	50	63	<i>Pratylenchus neglectus</i>	85	25	170	<i>Pratylenchus zeae</i>	538	25	1 075
<i>Tylenchorhynchus brevilineatus</i>	1.2	25	6	<i>Criconemoides sphaerocephalus</i>	55	25	109	<i>Criconemoides sphaerocephalus</i>	310	25	620
-	-	-	-	<i>Criconemoides parvus</i>	26	25	51	<i>Criconemoides obtusicaudatus</i>	13	13	32
-	-	-	-	<i>Criconema mutabile</i>	26	25	51	<i>Tylenchorhynchus brevicaudatus</i>	1.2	13	3
-	-	-	-	<i>Quinisulcius capitatus</i>	25	25	50	-	-	-	-
-	-	-	-	<i>Pratylenchus vulnus</i>	17	25	34	-	-	-	-
2012/13 growing season											
<i>Pratylenchus zeae</i>	12 579	67	14 680	<i>Helicotylenchus spp.</i>	13 253	60	24 508	<i>Helicotylenchus spp.</i>	31 835	83	34 318
<i>Helicotylenchus spp.</i>	5 088	33	8 430	<i>Scutellonema brachyurus</i>	3 947	40	6 579	<i>Rotylenchulus parvus</i>	6 193	33	10 322
<i>Scutellonema brachyurus</i>	3 524	33	5 874	<i>Pratylenchus thornei</i>	3 475	40	5 791	<i>Rotylenchus unisexus</i>	2 858	17	7 146

Table 4.8 continues											
Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Species/genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
Glyphosate-tolerant soybean				Conventional soybean				Natural vegetation			
<i>Pratylenchus scribneri</i>	1 333	17	3 332	<i>Pratylenchus zeae</i>	2 582	60	3 227	<i>Criconeoide ferniae</i>	1 775	17	4 438
<i>Pratylenchus vulnus</i>	514	17	1 286	<i>Meloidogyne</i> spp.	1 820	60	2 275	<i>Scutellonema trancatum</i>	1 332	17	3 330
<i>Rotylenchulus parvus</i>	447	17	1 118	<i>Pratylenchus crenatus</i>	1 350	40	2 250	<i>Scutellonema brachyurus</i>	1 196	17	2 990
<i>Meloidogyne</i> spp.	179	50	256	<i>Rotylenchulus parvus</i>	655	20	1 310	<i>Criconeoides parvus</i>	1 005	17	2 512
<i>Pratylenchus neglectus</i>	90	17	225	<i>Pratylenchus vulnus</i>	313	20	625	<i>Meloidogyne</i> spp.	300	17	750
<i>Tylenchorhynchus brevilineatus</i>	78	17	195	<i>Rotylenchus unisexus</i>	213	20	426	<i>Pratylenchus teres</i>	229	17	573
<i>Nanidorus minor</i>	17	17	42	<i>Hemicycliophora typica</i>	189	20	377	<i>Telotylenchus avaricus</i>	201	17	503
<i>Pratylenchus thornei</i>	2.4	17	6	<i>Pratylenchus scribneri</i>	63	20	126	<i>Pratylenchus zeae</i>	100	17	250
-	-	-	-	-	-	-	-	<i>Pratylenchus neglectus</i>	58	17	146
-	-	-	-	-	-	-	-	<i>Pratylenchus scribneri</i>	29	17	73

*Prominence value (PV) = final population density (Pf) of each species $\times \sqrt{\text{frequency of occurrence}/10}$.

Significant differences were observed among the different ecosystems with regard to the MPDs of *Helicotylenchus* during 2012/13 only (Table 4.9). During this season, the rhizosphere soil of the glyphosate-tolerant soybean cultivars contained significantly ($P \leq 0.05$) higher population densities compared to the conventional soybean cultivars as well as the natural vegetation.

Table 4.9. Comparison of *Helicotylenchus* spp. population densities per 200 g soil rhizosphere sub-samples of glyphosate-tolerant and conventional soybean cultivars, as well natural vegetation at six localities in South Africa during the 2011/12 and 2012/2013 growing seasons.

Growing seasons					
2011/12			2012/13		
Ecosystems	t-value	P	Ecosystems	t-value	P
Glyphosate-tolerant soybean: 0.61* (13**±28***)	0.3	0.76	Glyphosate-tolerant soybean: 0.48 (31±89)	7.79	0.001
Conventional soybean: 0.65 (12±18)			Conventional soybean: 1.18 (88±170)		
Glyphosate-tolerant soybean: 0.61 (13±28)	1.04	0.3	Glyphosate-tolerant soybean: 0.48 (31±89)	8.22	0.001
Natural vegetation: 0.74 (51±89)			Natural vegetation: 1.21 (99±177)		
Conventional soybean: 0.65 (12±18)	-0.77	0.44	Conventional soybean: 1.18(88±120)	-0.33	0.74
Natural vegetation: 0.74 (51±89)			Natural vegetation: 1.21 (99±177)		

*log₁₀ (x+1) transformed data; **real means; ***standard deviation (Student's t-test where $P \leq 0.05$).

4.4 Discussion

Novel findings from this two-year survey are: i) seven plant-parasitic nematode species, viz. *Pratylenchus flakkensis*, *Pratylenchus scribneri*, *Pratylenchus vulnus*, *Rotylenchulus brevicaudatus*, *Telotylenchus avaricus*, *Tylenchorhynchus brevicaudatus* and *Quinisulcius capitatus* have been for the first time associated with soybean in South Africa (SAPPNS¹; Fourie *et al.*, 2001; 2015); ii) the high diversity of plant-parasitic nematode species associated with both glyphosate-tolerant and conventional soybean cultivars and iii) the identification and general high abundance of plant-parasitic nematode species associated with natural vegetation adjacent to soybean fields. Especially high *Meloidogyne* population densities were observed in the roots and rhizosphere soil of natural vegetation during both seasons.

Another main finding is that *Meloidogyne* dominated in 50 g roots of both glyphosate-tolerant and conventional soybean cultivars due to a combination of high population densities and a high frequency of occurrence. This is in agreement with reports by Fourie *et al.* (2001; 2015) that root-knot nematodes are the most abundant plant-parasitic nematodes in local soybean production areas. Also, this finding agrees with results presented in Chapter 3 of this thesis, indicating that root-knot nematodes were the most abundant and prevalent group of plant-parasitic nematodes in roots of glyphosate-tolerant soybean cultivars included in the National Cultivar Trials of the Agricultural Research Council's Grain Crops Institute, Potchefstroom, sampled during the 2014/15 season. Of similar importance is the predominancy of *Pratylenchus* in 5 g roots of both glyphosate-resistant and conventional soybean cultivars as well as the natural vegetation. This observation is also in agreement with Fourie *et al.* (2001) and results from the 2014/15 survey (see Chapter 3). *Meloidogyne* spp. are generally regarded as the economically most important group of nematodes on soybean in many other countries (Bridge & Starr, 2007; Ibiam *et al.*, 2014; Machado, 2014; Andersen *et al.*, 2016). *Pratylenchus*, on the other hand, has been reported as a secondary pest of soybean worldwide (Bridge & Starr, 2007), but nowadays is regarded as a potential threat to soybean production in Brazil where it severely damages this crop (Machado, 2014; Lima *et al.*, 2015).

³Only two of the economically most important *Meloidogyne* species, viz. *M. incognita* followed by *M. javanica*, that also occur in the local grain-based production areas (Mc Donald *et al.*, 2017), including soybean (Fourie *et al.*, 2017), were identified during this study. Nonetheless, the *Meloidogyne* sp. from Edenville could not be identified using morphology, morphometrics and/or molecular techniques to date. The field from which it were sampled will thus be visited again to obtain more individuals to elucidate its identity. *Meloidogyne arenaria* (Neal, 1889) Cobb, 1890, *M. ethiopica* and *M. hapla* that had been found on local soybean (SAPPNS¹; Fourie *et al.*, 2001; Fourie *et al.*, 2015) were not found during our study. The presence of *M. incognita* and *M. javanica*, in either single or in mixed populations, in glyphosate-tolerant and conventional soybean fields in this study, is in agreement with reports by Fourie *et al.* (2001), Riekert (1996) and Mc Donald *et al.* (2017) for maize-based production areas. In countries such as Brazil (Machando, 2014), Nigeria (Ibiam *et al.*, 2014) and the United States of America (Andersen *et al.*, 2016), *M. incognita* is also considered as the most damaging and predominant root-knot nematode species of soybean. Furthermore, both *M. incognita* and *M. javanica* can interact with soilborne fungal pathogens such as *Fusarium virguliforme*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*, which can result in a highly damaging disease complex causing even higher soybean yield losses (Back *et al.*, 2002; Akinsanmi & Adekunle, 2003; Xing & Westphal 2013). Several *Fusarium* spp. complexes are present in local soybean-based production areas (Lamprecht & Tewoldemedhin 2011; Ncube *et al.*, 2011; Tewoldemedhin & Lamprecht, 2014), but interactions between these pathogens and root-knot nematodes has not been studied so far.

A possible explanation for the variation in population densities of root-knot nematodes recorded in the roots of conventional soybean cultivars during the two seasons of our study, is that the low population densities observed during the 2011/12 season could be ascribed to cultivation of the root-knot nematode-resistant cultivar Egret (Fourie *et al.*, 2006) at Bothaville. However, this was not the case in 2012/13 since the fields planted with conventional soybean cultivars were then planted with the root-knot nematode-susceptible cultivars Superboon (Edenville) and

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⁴Marula (Marble Hall) (Fourie *et al.*, 2006; Mbatyoti *et al.*, 2013). By contrast, glyphosate-tolerant soybean cultivars sampled during this study are all listed as susceptible to both *M. incognita* and *M. javanica* (Mbatyoti *et al.*, 2013; Venter, 2014) and can, hence, support high population densities of these species. It can be concluded that these two root-knot nematode species have a high reproduction potential on both glyphosate-tolerant and conventional soybean cultivars grown. Therefore, no significant differences should be expected between the two soybean ecosystems. An additional explanation for the observation that root-knot nematode population densities were substantially higher during the 2012/13 compared to the 2011/12 season could also have been due to the impact of various other abiotic and biotic environmental factors that were not recorded.

The genus *Pratylenchus* had the highest diversity, with nine species identified, compared to the other plant-parasitic nematodes. For the conventional soybean cultivars eight species, and for the glyphosate-tolerant soybean cultivars, six species were identified. This number is higher than the seven species that are known to be associated with soybean locally, viz. *P. brachyurus*, *P. crenatus*, *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 *P. teres*, *P. thornei*, and *P. zaeae* (SAPPNS¹; Keetch & Buckley, 1989; Fourie *et al.* 2015). *Pratylenchus zaeae*, the predominant species identified during this study was also reported as the predominant species during the first official soybean survey of 1995/1996 (Fourie *et al.*, 2001). This finding is, however, in contrast with the results reported in Chapter 3 where *P. brachyurus* was recorded as the most abundant lesion nematode species in roots of glyphosate-tolerant soybean cultivars sampled in the National Cultivar Trials. *Pratylenchus brachyurus* is considered the economically most important species that can cause major losses worldwide to soybean production, especially in Brazil (Bellé *et al.*, 2017). Although *P. brachyurus* occurred in relatively low numbers compared to *P. zaeae* in this study, the occurrence of this lesion nematode species should be of great concern to local soybean producers since in South Africa soybean are increasingly cultivated in rotation with maize. The damage potential of *P. brachyurus* has been demonstrated in Brazil where a 30 to 80 % reduction in

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soybean yield was observed (Lima *et al.*, 2015). Three other lesion nematode species, viz. *P. flakkensis*, *P. scribneri* and *P. vulnus* are for the first time reported on soybean in South Africa as a result of our study (SAPPNS¹).

High population densities of the genus *Helicotylenchus* were found in the rhizosphere soil of the glyphosate-tolerant and conventional soybean cultivars. Four species belonging to this genus were identified: *Helicotylenchus dihystra*, *Helicotylenchus martini*, *Helicotylenchus paraplatus* and *Helicotylenchus pseudorobustus*, of which the first and the last were generally predominant in the rhizospheres of both the glyphosate-tolerant and conventional soybean cultivars. *Helicotylenchus martini* and *H. paraplatus*, however, occurred only in the rhizospheres of natural vegetation. Although several *Helicotylenchus* spp. have been found associated with soybean worldwide, no yield loss caused by these plant-parasitic nematodes has been reported so far. However, in Brazil, *H. dihystra* and another spiral nematode (*S. brachyurus*) are considered potential pests of soybean due to the increase in their population densities and spread in the soybean production areas (Lima *et al.*, 2009; Machado *et al.*, 2014).

Except for *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* that had high population densities in both soybean ecosystems sampled, species of other nematode genera of supposedly lesser importance were also identified. These include species of *Criconema*, *Criconemoides*, *Hemicycliophora*, *Nanidorus*, *Quinisulcius*, *Rotylenchus*, *Rotylenchulus*, *Scutellonema*, *Telotylenchus* and *Tylenchorhynchus* spp. Although these plant-parasitic nematodes occur in low population densities it cannot be excluded that their combined damage to plant roots may result in poor crop growth and yield loss. Although *N. minor* only occurred in rhizosphere soil in a field near Bothaville where glyphosate-tolerant soybean cultivars were grown, and in very low population densities, this trichodorid nematode is known to vector plant-pathogenic viruses (e.g. Tobacco Rattle Virus) (Decraemer & Robbins, 2007; Marais *et al.*, 2015). The virus can hence be damaging to crop production systems including groundnut, maize, potato and soybean (Duarte *et al.*, 2011) that are grown in local grain-based production areas (Nel, 2009).

A wide diversity of plant-parasitic nematodes was found associated with natural vegetation adjacent to soybean fields during this study. This shows that natural vegetation sites can host high population densities of economically important nematode pests and can act as the primary source of pathogen inoculum. Planting soybean or other annual crops on a cleared natural field may thus have an adverse impact on crop production. Local soybean producers face a major challenge to control nematode pests and ensure sustainable soybean production. The lack of nematicides registered on soybean in South Africa (Van Zyl, 2013) and the availability of only a few root-knot nematode resistant cultivars aggravates the nematode problem. Studies such as this survey are hence crucial to continuously update the status of plant-parasitic nematodes associated with soybean in South Africa.

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Chapter 5

Terrestrial, non-parasitic, nematode assemblages associated with glyphosate-tolerant vs. conventional soybean in South Africa

Abstract

Non-parasitic nematodes have never been used as bio-indicators of soil quality in South African soybean fields. In this study, information about their abundance, identity and occurrence was hence generated for soils where glyphosate-tolerant and conventional soybean were grown. The study was conducted during two consecutive growing seasons. Twenty-seven non-parasitic nematode genera were collectively identified from soils of glyphosate-tolerant and conventional soybean fields (21 in glyphosate-tolerant and 23 in conventional soybean) and 28 from natural vegetation sites. A total of 32 genera were listed for the three ecosystems. Bacterivores dominated in soils of all three ecosystems, followed by fungivores, predators and omnivores. The predominant genera were *Acrobeles* (bacterivores), *Acrobeloides* (bacterivores), *Aphelenchus* (fungivores), *Eucephalobus* (bacterivores) and *Panagrolaimus* (bacterivores). Principal Component Analysis showed no correlations between these genera and any of the three ecosystems. According to soil food web analysis, soils from all sites of the three ecosystems were disturbed and degraded.

Key words: faunal analyses, glyphosate-tolerant soybean, nematodes, trophic groups.

5.1 Introduction

Soybean (*Glycine max* L.) is an important oil and protein seed crop worldwide, with production increasing substantially in South Africa since the past two decades (Hartman *et al.*, 2011; Dlamini *et al.*, 2014; PRF, 2017). Various agrochemicals (e.g. herbicides and pesticides) are applied on soybean and other crops used in rotation with it (Bhandari, 2014). The commercialization of genetically-modified (GM) glyphosate-tolerant soybean more than two decades ago led to widespread and intensive application of glyphosate worldwide, including South Africa (Fernandez-Cirnejo *et al.*, 2012; Dlamini *et al.*, 2014). Controversy, however, exists about the effect of continued application of this herbicide on nematodes (both pathogenic and non-parasitic) and other organisms (e.g. microbes) that occur in agricultural soils (Zhao *et al.*, 2013, Newman *et al.*, 2016).

Soil biotic communities perform key soil services such as nutrient cycling, regulation of pathogenic organisms and detoxification of toxic chemicals (Neher, 2001). Nematodes are regarded as good indicators of soil quality, because they are widespread in agricultural soils and are characterised by diverse trophic groups within communities (Yeates *et al.*, 1993; Ferris & Bongers, 2006). Soil quality can be monitored by determining the occurrence, abundance and diversity of non-parasitic nematodes using a tailor-made tool known as the Faunal Analysis. This tool was originally developed by Bongers (1990), improved later (Bongers & Bongers, 1998) and refined by Ferris *et al.* (2001) using coloniser-persister (c-p) values and guilds allocated to non-parasitic and plant-parasitic nematodes. Their efforts culminated into the development of the online application 'An automated calculation system for nematode-based biological monitoring (NINJA)' (Sieriebriennikov *et al.*, 2014) that is easy to use. This application allows nematologists worldwide to easily group soils into one of four quadrants, depending on enrichment and structure indices, by assigning c-p values to the non-parasitic nematode genera present (see Chapter 1, Section 3.1, Paragraph 5). Conventionally, all nematodes that are not animal parasites are called free-living or non-parasitic, including the herbivores or plant-parasitic nematodes (Poinar 1983). However, in this chapter the term non-parasitic will be used in a more restricted sense to refer only to non-plant-parasitic, terrestrial nematodes. Faunal analysis is an excellent and appropriate tool, has a wide application and can be used in agriculture to establish the effect of agronomic

practices, the application of agrochemicals or other interventions on soils using non-parasitic nematode community structures.

Concerning the non-target effects of glyphosate on nematodes (non-parasitic and plant-parasitic), limited and fragmented information is available. Moreover, controversial literature exists in this regard. Liphadzi *et al.* (2005), for example, observed no significant differences between the nematode community structures of glyphosate-tolerant and conventional soybean crops grown in rotation with maize over three growing seasons. In contrast, a decrease in population densities of nematode predators and fungivores, and an increase in bacterivores and omnivores was reported by Zhao *et al.* (2013) in soils where glyphosate-tolerant soybean was grown in the United States of America. For South Africa, no significant differences were, however, recorded between the structure of non-parasitic nematode communities in glyphosate-treated vs. conventional soybean fields during a pilot study (Jansen, 2014).

The main objectives of this study were i) to identify the non-parasitic nematode genera present in rhizosphere soils in glyphosate-tolerant and conventional soybean fields in South Africa and ii) to determine whether differences in the abundance and diversity of non-parasitic nematodes exist between these crops and adjacent natural vegetation.

5.2 Methodology

5.2.1 Nematode sampling and sites description

Sampling dates and protocols were the same as described for the study of plant-parasitic nematodes (Chapter 4, Paragraph 4.2.1). Nematode sampling was done in the same soybean fields and natural vegetation sites as listed in Chapter 4, Table 4.1, Fig. 4.1.

5.2.2 Nematode extraction and identification

Nematodes were extracted from 200 g soil samples using the same protocols as described in Chapter 3, Paragraph 3.2.1.1. Preparation of permanent slides, containing individuals of the different nematode genera present in the soil samples,

were prepared and nematode identification done to genus level as described in Chapter 3, Paragraph 3.2.1.2.

5.2.3 Data analyses

Data obtained for non-parasitic nematodes were $\log_{10}(x+1)$ transformed using Microsoft Excel, Version 2013. Prominence values (PV) for non-parasitic nematodes per 200 g rhizosphere soil were calculated for each nematode genus using the protocol of De Waele and Jordaen (1988) as referred to in Chapter 3, Paragraph 3.2.1.3

$\log_{10}(x+1)$ transformed nematode data were also subjected to Student's t-test using Statistica Version 13.2 (www.statsoft.com). This was done to determine whether any significant differences existed between the predominant genera identified at each of the sampling sites with regard to the three ecosystems (*viz.* glyphosate-tolerant vs conventional soybean, conventional soybean vs. natural vegetation and glyphosate-resistant vs natural vegetation). In addition, nematode population densities were also submitted to principal component analysis (PCA) (Hotelling, 1933; Thioulouse *et al.*, 1997). This way it could be determined whether correlations existed for all the genera found in a specific ecosystem(s). Finally, to assess soil quality as expressed by the enrichment and structure values according to colonizer-persister (c-p) values of nematode genera, the data were submitted to the faunal analyses (Ferris *et al.*, 2001). This was done using the NINJA tool (Sieriebriennikov *et al.*, 2014) for graphical expression of the data on a faunal plot.

5.3 Results

Twenty-seven non-parasitic nematode genera were identified from rhizosphere soil of glyphosate-tolerant and conventional soybean cultivars, representing 21 in glyphosate-tolerant and 23 in conventional soybean. In natural vegetation sites, 28 genera were identified. Ultimately, a total of 32 genera were listed for the three ecosystems. The genera identified were represented by different feeding groups and functional guilds, and included bacterivores, fungivores, predators and omnivores. The natural vegetation ecosystem had the highest diversity of non-parasitic nematode genera (Table 5.1).

Table 5.1. Non-parasitic nematodes associated with soybean and natural vegetation at 28 sites in the soybean production areas of South Africa during the 2011/12 and 2012/13 growing seasons (✓ indicates the presence of a genus).

Genus	Functional guild, followed by c-p value	Glyphosate-tolerant soybean	Conventional soybean	Natural vegetation
<i>Mesorhabditis</i> (Osche, 1952), Dougherty, 1953	Ba***1	✓	✓	✓
<i>Panagrolaimus</i> Fuchs, 1930	Ba1	✓	✓	✓
<i>Rhabditis</i> Dujardin, 1845	Ba1	✓	✓	✓
<i>Acrobeles</i> Linstow, 1877	Ba2	✓	✓	✓
<i>Acrobeloides</i> (Cobb, 1924), Thorne, 1937	Ba2	✓	✓	✓
<i>Cephalobus</i> Steiner, 1929	Ba2	✓	✓	✓
<i>Chiloplacus</i> Thorne, 1937	Ba2	✓	✓	✓
<i>Eucephalobus</i> Steiner, 1936	Ba2	✓	✓	✓
<i>Monhystera</i> Bastian, 1865	Ba2	✓	✓	✓
<i>Plectus</i> Bastian, 1865	Ba2		✓	✓
<i>Seleborca</i> Andrásy, 1985	Ba2			✓
<i>Wilsonema</i> Cobb, 1913	Ba2			✓
<i>Zeldia</i> Thorne, 1937	Ba2	✓		✓
<i>Teratocephalus</i> de Man, 1876	Ba4			✓
<i>Alaimus</i> de Man, 1880	Ba4		✓	
<i>Aphelenchoides</i> Fischer, 1894	Fu2	✓	✓	✓
<i>Aphelenchus</i> (Bastian, 1865), Cobb, 1927	Fu2	✓	✓	✓
<i>Ditylenchus</i> Filipjev, 1936	Fu2	✓	✓	✓
<i>Psilenchus</i> de Man, 1921	Fu2	✓	✓	✓
<i>Tylenchus</i> Bastian, 1865	Fu2	✓	✓	✓
<i>Coslenchus</i> Siddiqi, 1978	Fu3			✓
<i>Leptonchus</i> Cobb, 1920	Fu4		✓	✓
<i>Tylencholaimellus</i> (Cobb, 1915), de Man 1921	Fu4		✓	✓
<i>Tylencholaimus</i> de Man, 1880	Fu4	✓		
<i>Dorylaimus</i> Thorne, 1939	Om4		✓	✓
<i>Eudorylaimus</i> Andrásy, 1959	Om4			✓
<i>Thornenema</i> Andrásy, 1959	Om4	✓	✓	✓
<i>Mononchus</i> Chitwood & Allen, 1959	Pr4		✓	
<i>Paraxonchium</i> Krall, 1958	Pr4	✓	✓	
<i>Aporcelaimellus</i> Heyns, 1965	Pr5	✓		✓
<i>Discolaimium</i> Thorne, 1939	Pr5	✓		✓
<i>Discolaimoides</i> Heyns, 1963	Pr5	✓	✓	✓

*Functional guilds (Ferris *et al.*, 2001); **Colonizer-persister (c-p) values (Bongers, 1990); ***Trophic group with Ba = Bacterivores; Fu = Fungivores; Om = Omnivores and Pr = Predators

During the 2011/12 season, the predominant non-parasitic nematode genera in the glyphosate-tolerant soybean ecosystem in descending order were *Aphelenchus*, *Acrobeles* and *Acrobeloides* since they had the highest PVs (Table 5.2A). *Aphelenchus* occurred in soils at all sites, while *Acrobeles* and *Acrobeloides* occurred in 50 % of soil samples from glyphosate-tolerant soybean ecosystems. In conventional soybean ecosystems, the predominant genera in descending order were *Panagrolaimus*, *Acrobeloides* and *Aphelenchus* since they had the highest PVs. *Panagrolaimus* occurred in 75 % of the soil samples, while *Acrobeloides* and *Aphelenchus* occurred in 50 % of the samples from conventional soybean ecosystems. In the natural vegetation ecosystem, the predominant genera in descending order were *Acrobeles*, *Aphelenchus* and *Acrobeloides*. *Acrobeles* occurred in all soil samples, while *Aphelenchus* and *Acrobeloides* were found in 75 % of the samples from natural vegetation ecosystems.

During the 2012/13 season, the predominant non-parasitic nematodes in the glyphosate-tolerant soybean ecosystem in descending order were *Aphelenchus*, *Acrobeles* and *Eucephalobus* (Table 5.2B). *Aphelenchus* occurred in all sampling sites, *Acrobeles* and *Eucephalobus* in 89 and 78 % of the soil samples, respectively. In the conventional soybean ecosystem, the predominant genera in descending order were *Aphelenchus*, *Eucephalobus* and *Acrobeloides*. *Aphelenchus* occurred in 100 %, and *Eucephalobus*, and *Acrobeloides* in 44 and 67 % of the samples, respectively. In the natural vegetation ecosystem, the predominant genera were *Aphelenchus*, *Eucephalobus* and *Panagrolaimus*. *Aphelenchus* and *Panagrolaimus* occurred in all soil samples and *Eucephalobus* in 87 % of the samples.

No significant differences were apparent among the ecosystems regarding the abundance of the predominant genera (*Acrobeles*, *Acrobeloides*, *Aphelenchus*, *Eucephalobus* and *Panagrolaimus*) (Tables 5.3A & B).

Table 5.2A. Prominence values, frequencies of occurrence and mean population densities of non-parasitic nematodes in 200 g soil rhizosphere of glyphosate-tolerant and conventional soybean cultivars, as well as natural vegetation for 28 sites in South Africa during the 2011/12 growing season.

Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
Glyphosate-tolerant soybean				Conventional soybean				Natural vegetation			
<i>Aphelenchus</i>	3 646	100	3 646	<i>Panagrolaimus</i>	2 723	75	3 144	<i>Acrobeles</i>	3 885	100	3 885
<i>Acrobeles</i>	2 131	50	3 014	<i>Acrobelloides</i>	1 963	50	2 804	<i>Aphelenchus</i>	3 741	75	4 320
<i>Acrobelloides</i>	982	50	1 403	<i>Aphelenchus</i>	1 683	50	2 380	<i>Acrobelloides</i>	2 758	75	3 185
<i>Eucephalobus</i>	668	25	1 335	<i>Aphelenchoides</i>	840	25	1 680	<i>Panagrolaimus</i>	1 310	50	1 853
<i>Aphelenchoides</i>	572	25	1 144	<i>Acrobeles</i>	444	50	634	<i>Eucephalobus</i>	907	50	1 281
<i>Psilenchus</i>	452	25	905	<i>Plectus</i>	338	25	675	<i>Rhabditis</i>	648	50	915
<i>Panagrolaimus</i>	253	25	505	<i>Cephalobus</i>	216	25	431	<i>Cephalobus</i>	570	25	1 139
<i>Rhabditis</i>	197	25	393	<i>Eucephalobus</i>	171	25	342	<i>Tylenchus</i>	534	75	615
<i>Mesorhabditis</i>	168	25	335	<i>Monhystera</i>	158	25	315	<i>Psilechus</i>	184	50	262
<i>Tylenchus</i>	17	25	34	<i>Mesorhabditis</i>	130	25	259	<i>Zeldia</i>	71	25	142
<i>Discolaimoides</i>	3	25	5	<i>Tylenchus</i>	40	25	80	<i>Dorylaimus</i>	5	25	9
<i>Thornenema</i>	3	25	5	<i>Psilenchus</i>	20	25	40	<i>Aporcellaimellus</i>	4	25	8
<i>Paraxonchium</i>	2	25	4	<i>Dorylaimus</i>	4	25	7	<i>Discolaimium</i>	3	25	5
-	-	-	-	<i>Discolaimoides</i>	3	25	5	<i>Discolaimoides</i>	2	25	3
-	-	-	-	<i>Paraxonchium</i>	2	25	3	-	-	-	-
-	-	-	-	<i>Thornenema</i>	2	25	3	-	-	-	-

*Prominence value (PV) = final population density (Pf) of each species $\times \sqrt{\text{frequency of occurrence}/10}$.

Table 5.2B. Prominence values, frequencies of occurrence and mean population densities of non-parasitic nematodes in 200 g soil rhizosphere of glyphosate-tolerant and conventional soybean cultivars, as well as natural vegetation for 28 sites in South Africa during the 2012/13 growing season.

Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
Glyphosate-tolerant soybean				Conventional soybean				Natural vegetation			
<i>Aphelenchus</i>	2 275	100	2 275	<i>Aphelenchus</i>	1 448	100	1 448	<i>Aphelenchus</i>	2 590	100	2 590
<i>Acrobeles</i>	1 230	89	1 304	<i>Eucephalobus</i>	988	44	1 490	<i>Eucephalobus</i>	2 425	83	2 662
<i>Eucephalobus</i>	941	78	1 065	<i>Acrobeloides</i>	821	67	1 003	<i>Panagrolaimus</i>	1 615	100	1 615
<i>Acrobeloides</i>	827	67	1 010	<i>Panagrolaimus</i>	553	33	962	<i>Cephalobus</i>	1 414	67	1 728
<i>Rhabditis</i>	824	67	1 007	<i>Acrobeles</i>	365	33	635	<i>Acrobeloides</i>	1 156	83	1 269
<i>Aphelenchoides</i>	781	56	1 043	<i>Aphelenchoides</i>	334	22	711	<i>Acrobeles</i>	849	56	1 134
<i>Panagrolaimus</i>	576	56	770	<i>Seleborca</i>	286	22	610	<i>Aphelenchoides</i>	817	67	998
<i>Zeldia</i>	298	33	519	<i>Cephalobus</i>	587	56	784	<i>Ditylenchus</i>	323	33	562
<i>Cephalobus</i>	148	22	315	<i>Plectus</i>	241	22	513	<i>Plectus</i>	183	33	319
<i>Chiloplacus</i>	63	11	190	<i>Ditylenchus</i>	212	22	451	<i>Mesorhabditis</i>	151	17	365
<i>Ditylenchus</i>	53	11	161	<i>Mesorhabditis</i>	206	33	439	<i>Seleborca</i>	77	17	186
<i>Mesorhabditis</i>	42	11	126	<i>Plectus</i>	201	22	429	<i>Rhabditis</i>	68	17	165
<i>Plectus</i>	34	11	108	<i>Zeldia</i>	98	11	295	<i>Zeldia</i>	66	17	159
<i>Tylencholaimus</i>	7	11	22	<i>Chiloplacus</i>	78	11	235	<i>Wilsonema</i>	65	17	157
<i>Discolaimium</i>	3	22	7	<i>Dorylaimus</i>	8	55	11	<i>Chilopacus</i>	63	17	154
<i>Aporcelaimellus</i>	3	22	6	<i>Leptonchus</i>	4	11	11	<i>Teratocephalus</i>	7	17	16
<i>Eudorylaimus</i>	2	11	7	<i>Tylencholaimus</i>	3	11	10	<i>Eudorylaimus</i>	7	33	12
<i>Discolaimoides</i>	2	11	6	<i>Alaimus</i>	3	11	9	<i>Leptonchus</i>	5	17	13
-	-	-	-	<i>Monochus</i>	2	11	5	<i>Coslenchus</i>	4	17	10

Table 5.2A continues											
Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)	Genus	Prominence value (PV)*	Frequency of occurrence	Mean population density (MPD)
Glyphosate-tolerant soybean				Conventional soybean				Natural vegetation			
-	-	-	-	-	-	-	-	<i>Tylencholaimellus</i>	4	17	9
-	-	-	-	-	-	-	-	<i>Discolaimoides</i>	3	17	8

*Prominence value (PV) = final population density (Pf) of each species $\times \sqrt{\text{frequency of occurrence}/10}$.

Table 5.3A. Comparison of non-parasitic nematodes population densities per 200 g rhizosphere soil of glyphosate-tolerant and conventional soybean cultivars, as well natural vegetation for 28 sites in South Africa during the 2011/12 growing season.

Genus	Ecosystems	t-value	P
<i>Acrobeles</i>			
	Glyphosate-tolerant soybean: 0.7* (603**±1 348***) vs. Conventional soybean: 0.56*(127**±284***)	0.15	0.88
	Glyphosate-tolerant: 0.7* (603**±1 348***) vs. Natural vegetation: 0.72* (777**±1 737***)	-0.02	0.98
	Conventional soybean: 0.56*(127**±284***) vs. Natural vegetation: 0.72* (777**±1 737***)	-0.17	0.87
<i>Acrobeloides</i>			
	Glyphosate-tolerant soybean: 0.63* (281**±627***) vs. Conventional soybean: 0.69*(560**±1 254***)	0.06	0.96
	Glyphosate-tolerant: 0.63* (281**±627***) vs. Natural vegetation: 0.7* (637**±1 424***)	0.08	0.94
	Conventional soybean: 0.69*(560**±1 254***) vs Natural vegetation: 0.7* (637**±1 424***)	-0.01	0.99
<i>Aphelenchus</i>			
	Glyphosate-tolerant soybean: 0.71* (729**±1 631***) vs. Conventional soybean: 0.68*(476**±1 064***)	-0.04	0.97
	Glyphosate-tolerant: 0.71* (729**±1 631***) vs. Natural vegetation: 0.73* (864**±1 932***)	-0.12	0.91
	Conventional soybean: 0.68*(476**±1 064***) vs. Natural vegetation: 0.73* (864**±1 932***)	-0.05	0.96
<i>Eucephalobus</i>			
	Glyphosate-tolerant soybean: 0.63* (267**±597***) vs. Conventional soybean: 0.51*(68**±153***)	-0.15	0.89
	Glyphosate-tolerant: 0.63* (267**±597***) vs. Natural vegetation: 0.62* (256**±573***)	-0.004	1
	Conventional soybean: 0.51*(68**±153***) vs. Natural vegetation: 0.62* (256**±573***)	-0.14	0.89
<i>Panagrolaimus</i>			
	Glyphosate-tolerant soybean: 0.54* (101**±226***) vs. Conventional soybean: 0.7*(629**±1 406***)	0.18	0.86
	Glyphosate-tolerant: 0.54* (101**±226***) vs. Natural vegetation: 0.65* (371**±829***)	0.13	0.9
	Conventional soybean: 0.7*(629**±1 406***) vs. Natural vegetation: 0.65* (371**±829***)	0.05	0.96

*log₁₀ (x+1) transformed data; **real means; ***standard deviation (Student's t-test where $P \leq 0.05$)

Table 5.3B. Comparison of non-parasitic nematodes population densities per 200 g rhizosphere soil of glyphosate-tolerant and conventional soybean cultivars, as well natural vegetation for 28 sites in South Africa during the during the 2012/13 growing season.

Genus	Ecosystems	t-value	P
<i>Acrobeles</i>	Glyphosate-tolerant soybean: 0.62* (261**±583***) vs. Conventional soybean: 0.58* (150**±335***)	0.06	0.97
	Glyphosate-tolerant soybean: 0.62* (261**±583***) vs. Natural vegetation: 0.61* (227**±507***)	0.01	0.99
	Conventional soybean: 0.58* (150**±335***) vs. Natural vegetation: 0.61* (227**±507***)	-0.04	0.97
<i>Acrobeloides</i>	Glyphosate-tolerant soybean: 0.6* (202**±452***) vs. Conventional soybean: 0.6*(201**±449***)	0.001	1
	Glyphosate-tolerant: 0.6* (202**±452***) vs. Natural vegetation: 0.62* (254**±568***)	0.02	0.98
	Conventional soybean: 0.6*(201**±449***) vs. Natural vegetation: 0.62* (254**±568***)	0.02	0.98
<i>Aphelenchus</i>	Glyphosate-tolerant soybean: 0.67* (455**±1 017***) vs. Conventional soybean: 0.63*(290*±648***)	0.04	0.97
	Glyphosate-tolerant: 0.67* (455**±1 017***) vs. Natural vegetation: 0.68* (518**±1 158***)	0.01	0.99
	Conventional soybean: 0.63*(290*±648***) vs. Natural vegetation: 0.68* (518**±1 158***)	0.05	0.96
<i>Eucephalobus</i>	Glyphosate-tolerant soybean: 0.61* (213**±476***) vs. Conventional soybean: 0.64*(298**±666***)	-0.03	0.97
	Glyphosate-tolerant: 0.61* (213**±476***) vs. Natural vegetation: 0.69* (532**±1 191***)	0.09	0.93
	Conventional soybean: 0.64*(298**±666***) vs. Natural vegetation: 0.69* (532**±1 191***)	0.05	0.96
<i>Panagrolaimus</i>	Glyphosate-tolerant soybean: 0.58* (154**±344***) vs. Conventional soybean: 0.6*(192**±430***)	-0.02	0.98
	Glyphosate-tolerant: 0.58* (154**±344***) vs. Natural vegetation: 0.64* (323**±722***)	0.07	0.94
	Conventional soybean: 0.6*(192**±430***) vs. Natural vegetation: 0.64* (323**±722***)	0.05	0.96

*log₁₀ (x+1) transformed data; **real means; ***standard deviation (Student's t-test where $P \leq 0.05$)

According to PCA analysis, no differences were observed among the nematode communities from the three ecosystems when nematode data for the sites were combined. It was therefore decided to investigate the variation of nematode communities among the three ecosystems per site. Distinct variations among the nematode communities from each ecosystem were observed. The nematode communities in the glyphosate-tolerant soybean ecosystem differed in the majority of the sites from those in the conventional soybean and natural vegetation ecosystems. Since these observations were made at most of the sampling sites, only one example (Edenville) is given (Fig. 5.4). The difference between the three ecosystems at Edenville is explained by the glyphosate-tolerant ecosystem having the highest population densities of the genera *Acrobeles*, *Aphelenchoides*, *Zeldia*, *Cephalobus*, *Mesorhabditis*, *Eucephalobus* and *Aphelenchus*. The conventional soybean ecosystem in turn had higher population densities of *Acrobelloides* and *Ditylenchus* compared to the two other ecosystems. The natural vegetation ecosystem had the lowest nematode population densities and was positioned opposite the other two ecosystems. For the other localities, similar differences between the nematode communities for the three ecosystems were observed except for Marble Hall in 2013 where the three ecosystems were grouped together (Fig. 5.5).

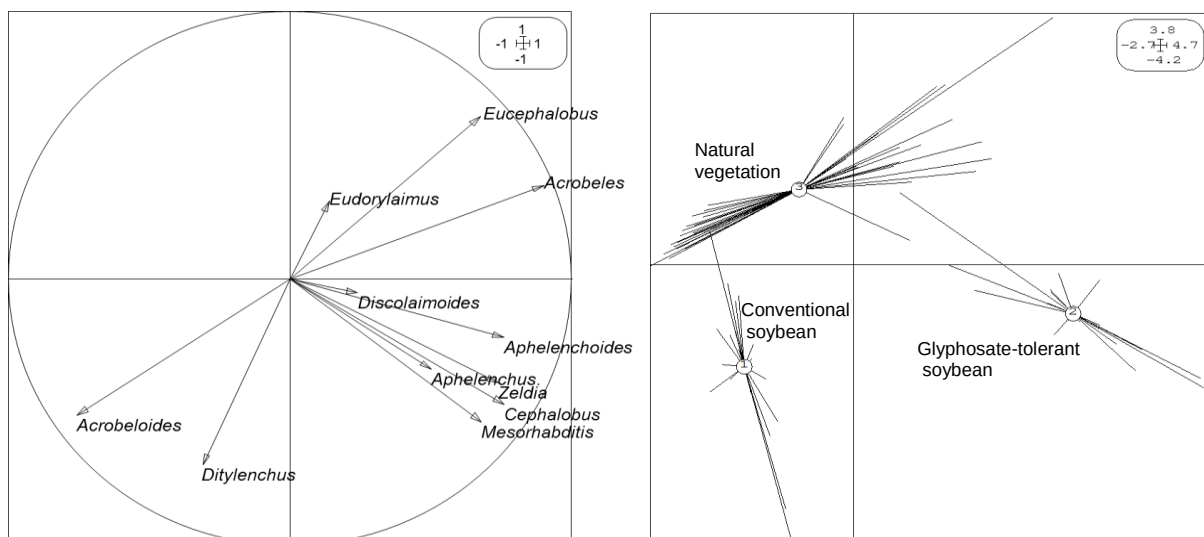


Figure 5.4. Principal component analysis (PCA) of population densities of terrestrial, non-parasitic nematode genera identified from 200 g soil samples for three ecosystems, viz. glyphosate-tolerant soybean fields, conventional soybean fields and natural vegetation at Edenville during the 2012/13 season.

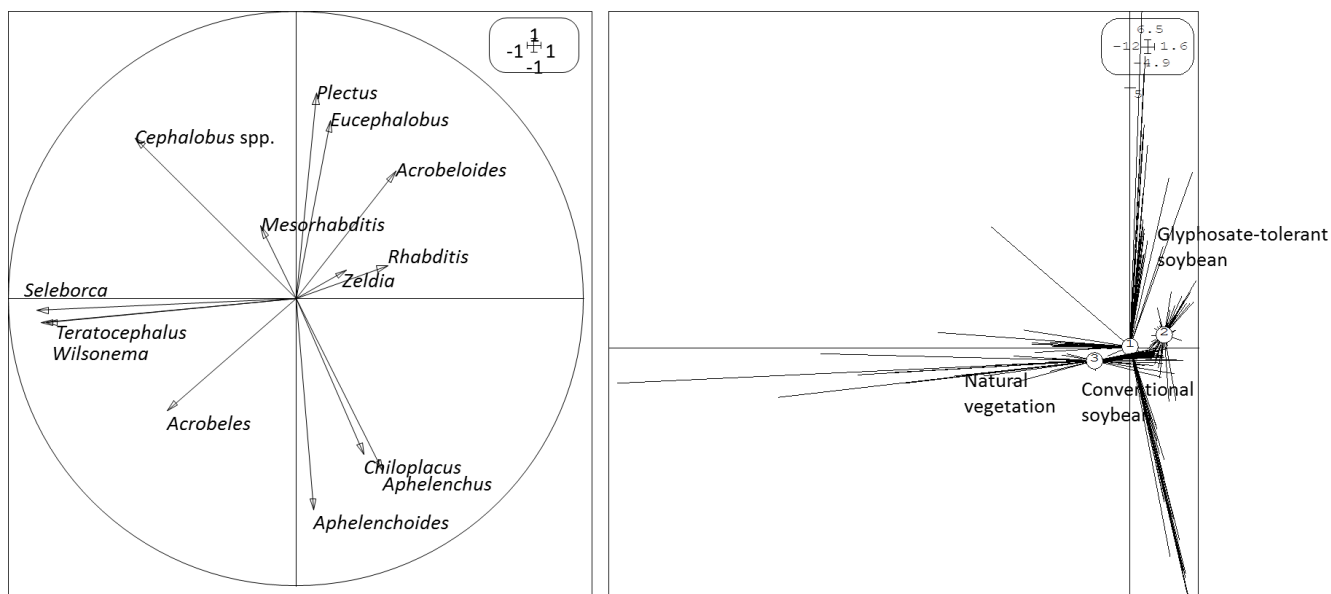


Figure 5.5. Principal component analysis (PCA) of population densities of terrestrial, non-parasitic nematode genera identified from 200 g soil samples for three ecosystems, viz. glyphosate-tolerant soybean fields, conventional soybean fields and natural vegetation at Marble Hall during the 2012/13 season.

According to faunal analysis of the soil food web, soils from the majority of the sites (54 %) sampled during both seasons from the three ecosystems were disturbed and/or degraded. These sites plotted in Quadrant D due to their Enrichment Index (EI) and Structural Index (SI) values being <50 % (Fig. 5.6a). Forty-six percent of the sites plotted in Quadrant A due to their EI being >50 % and SI being <50 %. None of the sampling sites plotted in the maturing (Quadrant B) and matured (Quadrant C) plots.

The metabolic footprints (data pooled for sites from each ecosystem for each season), for the three ecosystems were small (Fig. 5.6b). The EI for the three ecosystems ranged from intermediate (38 %) to moderately high (68 %) for both seasons, while the SI was very low (<10 %) for both seasons.

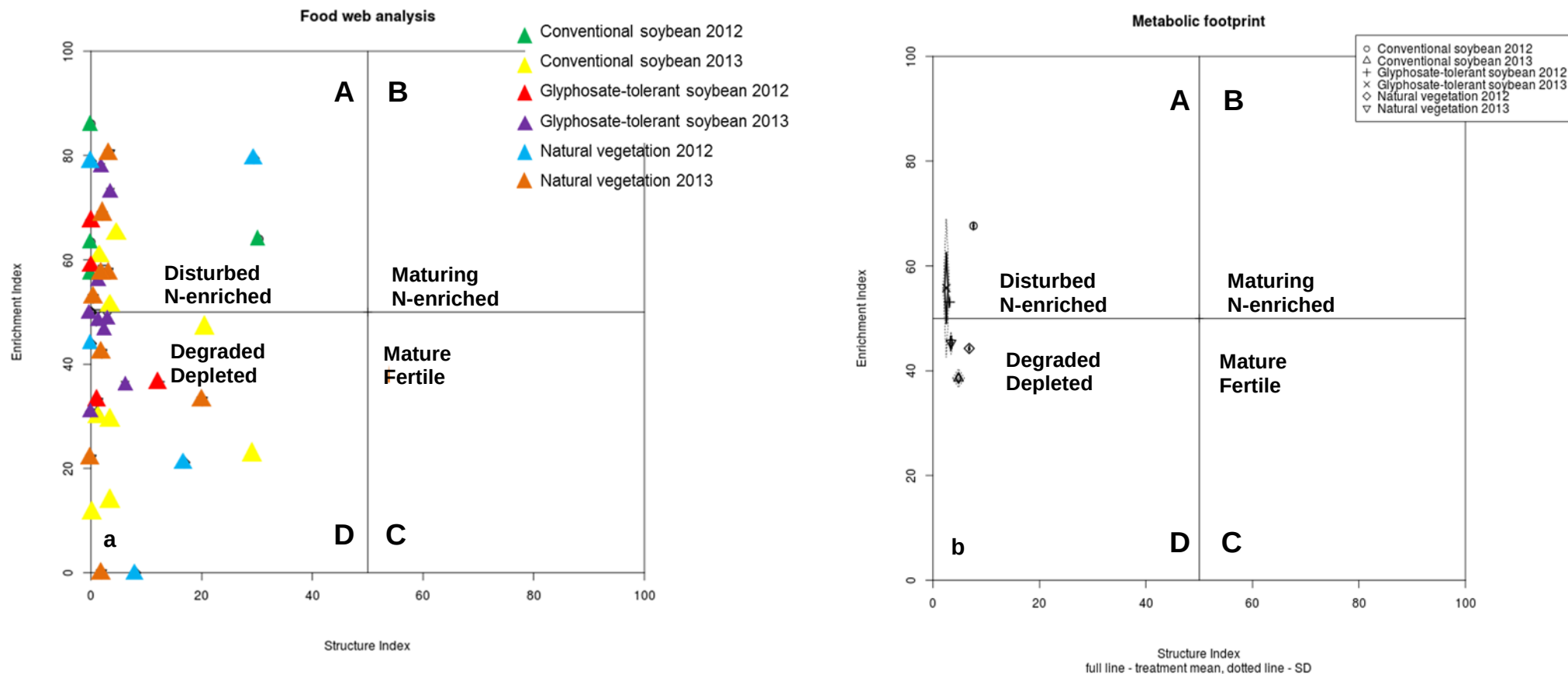


Figure 5.6a & b Faunal profiles (Sieriebriennikov *et al.*, 2014) representing the enrichment and structural conditions of soil food webs based on the abundance and diversity of non-parasitic nematode genera identified from soils of glyphosate-tolerant and conventional soybean fields, as well as adjacent natural vegetation sites (sampled during 2011/12 and 2012/13 seasons (a) and data for such sites pooled for the two seasons (b) in South African soybean production areas.

5.4. Discussion

Thirty-two non-parasitic nematode genera were identified from the rhizosphere soil from local soybean and adjacent natural vegetation ecosystems. All 14 genera identified and those experimental site where a soybean-maize rotation was done. The natural vegetation ecosystem in general had more genera (27) compared to the conventional (23) and glyphosate-tolerant (21) soybean ecosystems. These results are in agreement with that of Bekker (2016) that natural vegetation ecosystems had a higher diversity for non-parasitic nematodes than adjacent conventional and conservation maize ecosystems in South Africa. All genera associated with the soybean ecosystems are first reports for South Africa. The abundance and dominance of the non-parasitic nematode genera varied among the three ecosystems. However, all three ecosystems generally maintained high PVs for genera from the families Acrobelidae, Aphelenchidae, Aphelenchoididae, Cephalobidae and Panagrolaimidae.

The dominance of bacterivores in soils from all three ecosystems recorded for the current study is in agreement with reports by Djigal *et al.* (2004) and Xu *et al.* (2015). These authors suggested that bacterial feeding nematodes are the most abundant metazoans in soil substrates. Fungivores were the second most prevalent group according to results from the current study, followed by predators and omnivores. The lower abundance and occurrence of predators and omnivores are not surprising since these two groups are regarded as being very sensitive to soil disturbances (Ferris *et al.*, 2001). Worthwhile mentioning, however, is that predators (Pr5) were slightly more abundant in the glyphosate-treated soybean, than the other two ecosystems. This is difficult to explain because one would expect that this guild would be more abundant in natural vegetation ecosystems. However, it must be remembered that the predators were generally present in very low numbers in all ecosystems.

Nematode communities can differ and fluctuate substantially in terms of abundance, diversity and occurrence among different locations (Franco-Navarro & Godinez-Vidal, 2017). This tendency was also apparent for the current study and was confirmed using Student's t-test statistics for pooled data of each of the three ecosystems. However, when the three ecosystems were analysed per site, the

glyphosate-treated soybean ecosystem generally (except for Marble Hall) had different nematode communities and was separated from the conventional soybean and natural vegetation ecosystems. Although the two latter ecosystems differed in terms of their nematode communities, there was no trend regarding specific nematode genera being characteristic for each of these two specific ecosystems. It was therefore not possible to deduct the impact of each treatment on the nematode communities.

According to Faunal Analysis (Ferris *et al.*, 2001), none of the soil from the different sites were stable, enriched and fertile (Quadrants B and C) based on the abundance and diversity of non-parasitic nematodes. This is an indication that the quality of these soils is poor for crop production (Ferris *et al.*, 2001) and that all soybean sites sampled were disturbed (Quadrant A) and degraded (Quadrant D) based on the abundance and diversity of non-parasitic nematodes. This situation is often associated with management practices such as repeated tillage (Berkelmans *et al.*, 2003) and pesticide application (Carrascosa *et al.*, 2014) which are typical practices in local soybean production areas (Liebenberg, 2012). Opposed to crop fields, natural vegetation ecosystems are usually regarded as stable and structured due to either no or minimal disturbances (Ferris *et al.*, 2001). However, in the current study all natural vegetation sites also plotted in quadrants A and D indicating degraded or disturbed soils. This might be explained by the vegetation type that was represented by mainly grasses and/or the severe drought season experienced during 2012/13. Although there were no agricultural crops in these areas for the last 10 years, they were covered with grasses. Often natural vegetation consists of woody, perennial vegetation which could be considered less disturbed than grassland vegetation (Cullman *et al.*, 2010). The latter vegetation probably experiences periods with high organic content compared to periods when substantially less organic material and soil cover occurred (Shaw *et al.*, 2016). The dry season of 2012/13 also may have had an effect on grass density and soil cover. These factors could have impacted adversely on the abundance of non-parasitic nematodes (Eisenhauer *et al.*, 2011).

This study added valuable information about non-parasitic nematodes in glyphosate-tolerant versus conventional soybean and natural vegetation ecosystems. However, more studies need to be conducted to elucidate whether glyphosate impacts on

nematode communities. Therefore, the next chapter (Chapter 6) deals with the effects of application of glyphosate on soybean and maize (*Zea mays* L.) tolerant cultivars to nematodes assemblages in the rhizosphere.

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Chapter 6

Effect of glyphosate application on nematode assemblages in the rhizosphere and roots of a soybean-maize rotation sequence

Abstract

Herbicides containing glyphosate as the active substance are used extensively worldwide. However, limited and often contradictory reports exist about the effects of glyphosate on nematodes. A field experiment was conducted to determine the response of nematode communities to glyphosate for a soybean (cultivar LS 6164 R) and maize (cultivar DKC 80-30 RR) cropping system during two consecutive growing seasons. One half of the experimental plot was treated with glyphosate, twice during each growing season at the registered dosage rate, while the other half was not treated. Ten to 20 days after each glyphosate application and at crop maturity, rhizosphere soil and root samples were collected and the nematode population composition analysed. A mixed *Meloidogyne incognita* and *Meloidogyne javanica* population dominated both crops during both seasons. Five other plant-parasitic nematode genera (*Criconea*, *Helicotylenchus*, *Nanidorus*, *Pratylenchus* and *Tylenchorhynchus*) were also identified. No significant differences were observed between the glyphosate-treated and non-treated plots for *Meloidogyne* population densities per 50 g roots for both soybean and maize. Significant ($P \leq 0.05$) differences recorded between the two treatments for some of the other genera, however, were due to their low population densities or absence during some sampling dates. Fourteen non-parasitic nematode genera were identified in the rhizosphere soil samples, viz. *Acrobeles*, *Acrobeloides*, *Aphelenchoides*, *Aphelenchus*, *Aporcelaimellus*, *Cephalobus*, *Discolaimium*, *Ditylenchus*, *Eucephalobus*, *Teratocephalus*, *Leptonchus*, *Panagrolaimus*, *Tylencholaimus* and *Tylenchus*. Population densities of some of these genera differed significantly between the two treatments for both crops. Faunal analyses showed that soils from glyphosate-treated plots were degraded, less enriched and fungal-mediated. Conversely, soils from non-treated plots were disturbed and enriched, and bacterial-mediated.

Key words: beneficial nematodes, glyphosate, nematode pests.

6.1 Introduction

Glyphosate became commercially available in South Africa more than a decade ago and is now widely used in particular in soybean- and maize-based cropping systems (Dlamini *et al.*, 2014). Nematodes are often used as indicators of soil health and environmental changes (Neher, 2001; Ferris, 2010; Cesarz *et al.*, 2015). These multicellular organisms play a crucial role in important ecosystem services such as nutrient decomposition and recycling, suppression of pathogenic micro-organisms and biodegradation of harmful compounds (Bongers & Bongers, 1998; Ferris *et al.*, 1998; Neher, 2001; Wardle *et al.*, 2005). Changes in nematode community composition (assemblage) may have a substantial impact on their ecosystem functioning (Wada *et al.*, 2011; Frascchetti *et al.*, 2016). This is demonstrated by analyses of terrestrial non-parasitic nematode assemblages using faunal analyses that were originally developed by Ferris *et al.* (2001). According to the coloniser-persister (c-p) values and guilds, terrestrial non-parasitic nematodes are categorised and placed in one of the four quadrants of a faunal profile (see Chapter 1, Section 3.1, Paragraph 5).

Agricultural practices can not only affect nematodes directly. They also may indirectly modify nematode assemblages through changes in the availability of resources and this, in turn, may reduce the functional sustainability of soils (Griffiths *et al.*, 2006; Karuri *et al.*, 2013; Neher *et al.*, 2014; Allegrini *et al.*, 2015). The active substance of the herbicide Roundup®, viz. glyphosate or N-phosphonomethyl, is used extensively worldwide in numerous registered products to control weeds (Duke & Powles, 2008; Duke *et al.*, 2012; Allegrini *et al.*, 2015; Newman *et al.*, 2016). Glyphosate is often regarded as less toxic to the environment, because it exhibits little or no activity in the soil due to the rapid adsorption of its active substance to soil inorganic and organic particles (Duke & Powles, 2008; Cerdeira & Duke, 2010). However, the frequent application of glyphosate has raised environmental concerns. The potential non-target effects of this herbicide on biological processes in the soil and micro-organisms such as nematodes (Zhao *et al.*, 2013; Allegrini *et al.*, 2015; Newman *et al.*, 2016) are considered the main reason for such concerns.

Information about the non-target effects of glyphosate on soil microbial communities and nematodes is scarce and as a result these effects are not well documented

(Osman & Viglierchio, 1981; Vega *et al.*, 1993; Yang *et al.*, 2002; Cerdeira *et al.*, 2007; Noel & Wax, 2009; Zobiolo *et al.*, 2010; Partoazar *et al.*, 2011; Lane *et al.*, 2012; Nye *et al.*, 2014). More important, often inconclusive or even conflicting effects are reported. Only six scientific reports, referred to below, could be found about the effects of glyphosate on nematodes. The majority of these reports dealt with root-knot nematodes (*Meloidogyne* spp.) and the soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952). A Brazilian study reported that glyphosate had no effect on *Meloidogyne arenaria* (Neal, 1889) Cobb, 1890, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 reproduction on soybean (Cerdeira *et al.*, 2007). Liphadzi *et al.* (2005) also observed no differences in soil nematode population densities and diversity in soybean- and maize-based cropping systems in the United States of America (USA) treated with glyphosate opposed to systems where other conventional herbicides were applied. Similar results were obtained in a study by Jansen (2014) in soybean fields in South Africa. In contrast with the above mentioned studies, reproduction of *M. incognita* on soybean was significantly reduced in fields treated with glyphosate compared to untreated fields in another USA study (Osman & Viglierchio, 1981), while in Portugal, glyphosate application significantly reduced root galling, egg mass numbers and population densities of *M. incognita* in soybean fields compared with untreated fields (Vega *et al.*, 1993). Noel and Wax (2009), however, reported the opposite effect with *H. glycines* in a field study in the USA. Roots of the susceptible soybean cultivar DSR 320 treated with glyphosate contained higher egg numbers of this cyst nematode compared to untreated roots of the same soybean cultivar. Furthermore, also in the USA, Yang *et al.* (2002) reported more damage caused by *H. glycines* on a susceptible soybean cultivar (Corsoy 79) after glyphosate treatment but not on a resistant soybean cultivar (Countrymark 316).

With the exception of the study by Jansen (2014), no information on the effects of glyphosate on either plant-parasitic or terrestrial non-parasitic nematodes in South Africa is to our knowledge available. Therefore, the main aim of our study was to examine whether glyphosate application can affect nematode assemblages in general, and assemblages that are dominated by *M. incognita* and *M. javanica*.

6.2.1 Materials and methods

A small-field experiment (0.028 ha plot) was conducted over two consecutive growing seasons at the Agricultural Research Council's Grain Crops Institute (ARC-GCI; GPS co-ordinates: 26° 43' 44.8" S, 27° 06' 36 E"), Potchefstroom. The soil of the plot contained 94 % sand and 6 % clay. The organic material content of the soil was 0.18 and 0.23 %, respectively, and the soil pH (H₂O) during the 2013/14 and 2014/15 growing seasons 8 and 7.8, respectively. The crop history, fertilisers and herbicides applied, glyphosate application dosages and dates, nematode sampling dates, rainfall and temperature data are listed in Table 6.1.

Table 6.1. Crop history, agricultural practices, fertilisers applied, glyphosate application dates and dosage rate, nematode sampling dates, rainfall, and minimum and maximum temperatures recorded of the experimental plot during the 2013/14 and 2014/15 growing seasons.

Crop history: growing season, crop and herbicides applied	Growing season, crop and cultivar cultivated	Agricultural practice implemented	Inorganic fertiliser applied and dosage	Glyphosate application dates and dosage rates	Nematode sampling dates	Rainfall (mm)	Minimum temperature (°C)	Maximum temperature (°C)
2011/12, Sunflower, no herbicide used (hand-hoeing of weeds)	2013/14 (1 st year), Soybean (cv. LS 6164 R)	Rip and plough before planting	None	10/01/2014 and 03/02/2014 @ 2 L/ha	1 st = 30/01/2014 2 nd = 14/02/2014 3 rd = 22/04/2014	603	7.9	30.5
2012/13, Maize, Gramoxone® (active substance bipyridyl 200 g/L as dichloride salt 276 g/L) dosage rate 3 L/ha	2014/15 (2 nd year), Maize (cv. DKC 80-30 RR)	Rip and plough before planting	2:3:2 (26) at planting @ 300 kg/ha Ureum @ 50 kg/ha 4 weeks after planting	17/12/2014 and 13/01/2015 @ 2 L/ha	1 st = 08/01/2015 2 nd = 27/01/2015 3 rd = 11/03/2015	510	10.0	30.2

The plot was split into two halves (0.013 ha each) divided by a 2-m-buffer strip. Before planting of the soybean, weeds that grew in the experimental plot were removed using a hand hoe. The layout of the experiment was a split-plot design with 12 replicates (a row represented a replicate) per treatment.

Soybean was planted first, during the 2013/14 season, followed by maize during the 2014/15 season. On 18/11/2013, at the beginning of the 2013/14 growing season, seeds of the glyphosate-resistant soybean cultivar LS 6164 R were planted. This cultivar is susceptible to *M. incognita* and *M. javanica* infection (Mbatyoti *et al.*, 2013; Venter, 2014). The soybean seeds (170 per row) were planted in 5-m-long rows with an intra-row spacing of 3 cm and an inter-row spacing of 0.9 m. Each seed was coated with *Bradyrhizobium japonicum* race WB74 at the recommended dosage rate (Soygro (Pty) Ltd; <http://www.soygro.co.za/index.php/akkerbou-gewasse/sojabone>). At planting, each seed was inoculated with ca. 1 700 eggs and second-stage juveniles (J2) of a mixed *M. incognita* and *M. javanica* (70:30 ratio) population by pipetting the inoculum (20 µl) in holes around each seed. This mixed *Meloidogyne* population was reared in soil on the roots of the susceptible tomato (*Lycopersicon esculentum* L.) cultivar Rodade in a greenhouse of the Nematology Unit of North-West University, Potchefstroom according to the protocol of Fourie *et al.*, (2012). The population was originally isolated from maize roots in the Vryburg area in the North West Province (H. Fourie, NWU, personal communication).

After germination, soybean seedlings were irrigated with approximately 25 mm water three times a week using a sprinkler irrigation system, except when it had rained sufficiently. When naturally occurring weeds were 10-20 cm tall, one half of the experimental plot was treated with glyphosate (Roundup®, Monsanto; active substance 360 g/L glyphosate present as 441 g/L of the potassium salt) at a dosage rate of 2 L/ha) using a knapsack sprayer. The application, spraying on leaves, was done early in the morning to avoid wind and possible drift of the product. Weeds in the untreated plot were removed using a hand hoe but left on the soil. A second glyphosate application was done about three weeks after the first application (Monsanto, 2004; Liphadzi *et al.*, 2005). The other half of the experimental plot was not treated with glyphosate or any other herbicide and represented the control. Ten to 20 days after each glyphosate application and also at 120-140 days after planting

(i.e. at crop maturity), rhizosphere soil and the root system of each replicate were collected, thoroughly mixed and one sub-sample per replicate taken. To extract the nematodes from the soil and root sub-samples the following techniques were used: the adapted 1 % NaOCl method for extraction of *Meloidogyne* eggs and J2 from 50 g roots (Riekert, 1995); the centrifugal-flotation method for extraction of plant-parasitic nematodes from 5 g roots (Hooper *et al.*, 2005), and decanting and sieving followed by the centrifugal-flotation method for the extraction of plant-parasitic and terrestrial non-parasitic nematodes from 200 g soil samples (Coolen, 1979; Hooper *et al.*, 2005).

During the winter of 2014, no crop was grown and both halves of the experimental plot left fallow without any weed control. Before planting the seeds of the glyphosate-resistant maize cultivar DKC 80-30 RR on 18/11/2014, glyphosate was applied on the same plot half where glyphosate had been applied during the previous season. Again, the other half of the experimental plot was not treated and the weeds hand hoed. The maize cultivar DKC 80-30 RR is also susceptible to *M. incognita* and *M. javanica* infection (S. Steenkamp, ARC-GCI, personal communication). Weeds were removed from both plot halves just before planting the maize seeds. Maize seeds were not inoculated with rhizobia as was done with soybean seeds during the preceding season. The maize seeds (25 per row) were planted in 5-m-long rows with an intra-row spacing of 20 cm and 0.9 m inter-spacing. Two glyphosate applications, soil and root (sub) sampling, nematode extraction and counting were carried out as described above for the soybean growing season.

Nematode individuals extracted from 5 and 50 g roots were counted and identified to genus level in a De Grisse counting dish (De Grisse, 1963) using a stereo microscope (60× magnification). Nematodes extracted from 200 g soil samples were counted and identified to genus level in a 1 ml capacity Hawksley slide using a compound microscope (40-1 000× magnification). For species identification, at least 30 individuals of each genus were fixed in a heated formaldehyde-propionic-acid-water (FPG) solution (100 ml of a 40 % formalin solution, 10 ml propionic acid and 890 ml distilled water) (Marais *et al.*, 2017) in an incubator at 40 °C for 72 h and then transferred to a glycerin solution (De Grisse, 1965). The nematodes were removed from the glycerin solution using a fishing needle and permanently mounted on slides

by means of the paraffin-ring method (Hooper, 1986). These slides with the mounted specimens were then sent to the nematode taxonomists of the Agricultural Research Council-Plant Health and Protection, Roodeplaat (Pretoria), for identification to species level.

Meloidogyne spp. identification was done by means of morphological and molecular techniques. The eggs that were counted from the 50 g root samples were inoculated on roots of potted seedlings of the root-knot nematode-susceptible tomato cultivar Floradade (Fourie *et al.*, 2012). Thus, the *Meloidogyne* populations were reared *in vivo* in separate 10 l capacity plastic pots in a glasshouse (max. temperature ± 26 °C, min. temperature of ± 20 °C; 10D:14L photoperiod) to increase their population levels. Forty days after inoculation, 21 mature females were removed from the tomato roots. A scalpel and fine needle were used to isolate the females in order to cut perineal patterns for morphological species identification (Hartman & Sasser, 1985). The identification of the *Meloidogyne* populations to species level were conducted by the root-knot nematode taxonomist Dr Mariette Marais (ARC-PPRI), Pretoria.

6.2.2 Data analyses

Student's t-test (Statistica, Version 13.2; www.statsoft.com) was carried out to determine whether significant ($P \leq 0.05$) differences existed during both seasons between the two treatments (glyphosate-treated and non-treated plot halves) for the nematode population densities. To determine whether significant ($P \leq 0.05$) differences existed for nematode population densities among the three sampling dates for both crops, data were subjected to One Way Analyses of Variance (ANOVA) (Statistica Version 13.2). Sampling dates were used as the treatments and means separated with Tukey's HSD test where $P \leq 0.05$). In addition, terrestrial non-parasitic nematode data were subjected to faunal analyses using the program NINJA, referred to as 'an automated calculation system for nematode-based biological monitoring' (Sieriebriennikov *et al.*, 2014). This way a graphical scheme of the soil food web was obtained using enrichment and structural indices (EI and SI, respectively) (Ferris *et al.*, 2001; Ferris, 2010).

6.3 Results

6.3.1. Plant-parasitic nematode assemblage and trophic status

In addition to the predominant sedentary endoparasites *M. incognita* and *M. javanica*, five other plant-parasitic nematode genera (viz. *Criconema*, *Helicotylenchus*, *Nanidorus*, *Pratylenchus* and *Tylenchorhynchus*) were identified in the rhizosphere soil and root samples. Migratory endoparasitic nematode species identified were *Pratylenchus zaeae* (Graham, 1951), *Pratylenchus vulnus* (Allen & Jensen, 1951) and *Pratylenchus teres* (Khan & Singh, 1974). Ectoparasitic nematode species identified were *Criconema mutabile* (Taylor, 1936) Raski and Luc, 1985, *Nanidorus minor* (Colbran, 1956) Siddiqi, 1974 and *Tylenchorhynchus brevilineatus* Williams, 1960.

6.3.1.1 Roots

6.3.1.1.1 *Meloidogyne* spp. numbers per 50 g roots

High root-knot nematode population densities, not differing significantly between the glyphosate-treated and non-treated plot halves, were recorded for all three sampling dates for both crops (Table 6.2).

Final root-knot nematode population densities (Pf) recorded at the 3rd sampling date were 58 and 99 times higher in the glyphosate-treated and non-treated soybean plot halves, respectively, compared to the 1st sampling date (Table 6.2). For maize, these figures were 95 and 97, respectively.

Root-knot nematode population densities in the soybean roots were significantly ($P \leq 0.05$) higher in the non-treated soybean plot halves at the 3rd sampling date compared to the 1st and 2nd sampling dates (Table 6.2). For the glyphosate-treated soybean plot half, no significant differences were observed in *Meloidogyne* egg and J2 numbers among the three sampling dates.

Root-knot nematode population densities in the maize roots were significantly ($P \leq 0.05$) higher in both glyphosate-treated and non-treated maize plot halves at the 3rd sampling date compared to the 1st and 2nd sampling dates (Table 6.2).

The number of root-knot nematode eggs and J2 were substantially higher at the 2nd and 3rd sampling dates in both the glyphosate-treated and non-treated maize plot halves compared to soybean grown during the first season of the experiment (Table 6.2).

Table 6.2. Number of *Meloidogyne* eggs and second-stage juveniles in 50 g roots of soybean cv. LS 6164 R and maize cv. DKC 80-30 RR in glyphosate-treated and non-treated small-field plot halves at three sampling dates during the 2013/14 and 2014/15 growing seasons.

Soybean					Maize				
Nematode sampling date	Glyphosate-treated	Non-treated	t-value	P	Nematode sampling date	Glyphosate-treated	Non-treated	t-value	P
1 st : 13/01/2014	4.20* aA (41 903**±7 871)	3.74* aA (17 177**±4 203)	0.3	0.75	1 st : 08/01/2015	4.25* aA (25 542**±5 058)	4.07* aA (16 303**±3 748)	1.0	0.33
2 nd : 03/02/2014	4.32* aA (35 956**±7 830)	4.18* aA (23 081**±5 138)	0.6	0.55	2 nd : 27/01/2015	4.32* aA (74 275**±12 446)	4.18* aA (120 486**±32 753)	0.3	0.79
3 rd : 22/04/2014	4.66* aA (100 091**±25 242)	5.09* aB (142 824**±17 979)	-1.8	0.08	3 rd : 11/03/2015	5.55* aB (477 799**±91 957)	5.75* aB (626 979**±79 774)	-1.6	0.11
ANOVA for the three nematode sampling dates									
F value	0.75	8.85				24.71	70.2		
P value	0.48	0.001				0.001	0.001		

*Log₁₀ (x+1) transformed nematode mean data; **Real means in parenthesis followed by the Standard Deviation; Small letters in the same row (for each crop and each sampling date) that differ indicate a significant difference at $P \leq 0.05$ between the two treatments according to Student's t-test, while the same applies for capital letters in the same column indicating significant differences ($P \leq 0.05$) among the three dates for each crop and each treatment according to One-Way ANOVA.

6.3.1.1.2 Plant-parasitic nematode numbers per 5 g roots

In the roots, low to high plant-parasitic nematode population densities were present at the three sampling dates (Table 6.3). From the lowest to the highest, population densities ranged from 0 to 4 nematodes per 5 g roots for *Criconea*; 0 to 83 for *Helicotylenchus*; 0 to 87 for *Tylenchorhynchus*; 0 to 214 for *Pratylenchus*; 0 to 2 264 for *Meloidogyne* (irrespective of glyphosate application or not).

Meloidogyne and *Pratylenchus* population densities differed significantly ($P \leq 0.05$) at the three sampling dates in the glyphosate-treated soybean plants (Table 6.3). In the non-treated soybean plants, *Meloidogyne*, *Pratylenchus*, *Tylenchorhynchus* and *Helicotylenchus* population densities were also significantly ($P \leq 0.05$) different at the three sampling dates. In the glyphosate-treated maize plants, population densities of *Meloidogyne*, *Pratylenchus* and *Tylenchorhynchus* differed significantly ($P \leq 0.05$) at the three sampling dates. In contrast, in the non-treated maize plants, *Meloidogyne*, *Tylenchorhynchus* and *Helicotylenchus* population densities were significantly ($P \leq 0.05$) different at the three sampling dates.

Table 6.3. Number of plant-parasitic nematodes per 5 g roots of soybean cv. LS 6164 R and maize cv. DKC 80-30 RR plants grown in glyphosate-treated and non-treated small-field plot halves at three sampling dates during the 2013/14 and 2014/15 growing seasons.

	Soybean				Maize			
	Glyphosate-Treated	Non-treated	t-value	P	Glyphosate-treated	Non-treated	t-value	P
<i>Meloidogyne</i>								
Sampling date 1	0 A	0 A			1.89* (543**±809) aA	2.56* (736**±486) aB	-1.5	0.15
Sampling date 2	2.25* (617**±763) aB	2.84* (782**±417) bB	-2.06	0.05	0.91* (198**±350) aA	0.79* (122**±280) aA	0.21	0.84
Sampling date 3	2.92* (1 610**±1 390) aB	2.45* (513**±305) aB	1.54	0.14	3.45* (3 523**±2 464) aB	3.52* (3 950**±2 359) aC	-0.56	0.58
F value	62	115			17	37		
P	0.001	0.001			0.001	0.001		
<i>Pratylenchus</i>								
Sampling date 1	0.06* (0.33**±1.2) aA	0.35* (4**±5) aA	-1.81	0.08	1.41* (86**±124) aB	1.23* (57**±57) aA	0.47	0.64
Sampling date 2	0.42* (7**±16) aB	0.17* (2**±4) aA	1.16	0.26	0.73* (54**±91) aB	0.79* (103**±190) aA	-0.16	0.87
Sampling date 3	0.8* (17**±20) aB	0.94* (50**±73) aB	-0.39	0.69	0 aA	0.87* (163**±214) bA	-2.34	0.03
F value	4.86	4.09			10	0.48		
P	0.01	0.03			0.001	0.62		
<i>Tylenchorhynchus</i>								
Sampling date 1	0.08* (1**±2) aA	0 aA	1	0.33	1.1* (40±48) aB	1.02* (41**±50) aB	0.22	0.83
Sampling date 2	0 aA	0 aA	-	-	0 aA	0.36* (8**±18) aA	-1.88	0.07
Sampling date 3	0.08* (1**±2) aA	0.4* (6**±9) aB	-1.67	0.11	0.38* (35**±87) aA	0 aA	1.47	0.15
F value	0.5	5.24			7.32	7.39		
P	0.61	0.01			0.002	0.002		
<i>Criconema</i>								
Sampling date 1	0 a	0.1* (1**±4.) aA	-1	0.33	-	-	-	-
Sampling date 2	0 a	0.08* (1**±2) aA	-1	0.32	-	-	-	-
Sampling date 3	0 a	0 aA	-	-	-	-	-	-
F value		0.52						
P		0.59						
<i>Helicotylenchus</i>								
Sampling date 1	0 a	0.55* (7**±10) bB	-3.2	0.004	0 a	1.36* (50**±59) bB	-6.63	0.001
Sampling date 2	0 a	0 aA	-	-	0 a	0 A	-	-
Sampling date 3	0 a	0 aA	-	-	0 a	0.37* (32**±83) aA	-1.47	0.15
F value		10				14		
P		0.001				0.001		

*Log₁₀ (x+1) transformed nematode mean data; **Real means in parentheses followed by the Standard Deviation; Small letters in the same row (for each crop and each sampling date) that differ indicate a significant difference at $P \leq 0.05$ between the two treatments according to Student's t-test, while the same applies for capital letters in the same column indicating significant differences ($P \leq 0.05$) among the three dates for each crop and each treatment according to One-Way ANOVA; - No nematodes recovered.

6.3.2.2 Soil

6.3.2.2.1 Plant-parasitic nematode numbers per 200 g soil

In the rhizosphere soil, low plant-parasitic nematode population densities were present at the three sampling dates (Table 6.4). From the lowest to the highest, population densities ranged from 0 to 4 nematodes per 200 g soil for *Nanidorus*; 0 to 8 for *Criconea*; 0 to 31 for *Pratylenchus*; 0 to 57 for *Tylenchorhynchus*; 0 to 67 for *Helicotylenchus* and 0 to 74 for *Meloidogyne* (irrespective of glyphosate application or not).

At the three sampling dates, *Meloidogyne* and *Pratylenchus* population densities differed significantly ($P \leq 0.05$) in the glyphosate-treated soybean plot half only (Table 6.4). *Meloidogyne*, *Pratylenchus* and *Tylenchorhynchus* population densities in the glyphosate-treated maize plot half differed significantly ($P \leq 0.05$) at the three sampling dates. In contrast, *Meloidogyne* and *Helicotylenchus* population densities differed significantly ($P \leq 0.05$) in the non-treated maize plot half at the three sampling dates.

Table 6.4. Number of plant-parasitic nematodes per 200 g rhizosphere soil of soybean cv. LS 6164 R and maize cv. DKC 80-30 RR plants grown in glyphosate-treated and non-treated small-field plot halves at the three sampling date during the 2013/14 and 2014/15 growing seasons.

	Soybean				Maize			
	Glyphosate-Treated	Non-treated	t-value	P	Glyphosate-treated	Non-treated	t-value	P
<i>Pratylenchus</i>								
Sampling date 1	0 aA	0.22* (3**±8) aA	-1.48	0.15	0.65* (31**±50) bB	0 aA	2.34	0.03
Sampling date 2	0.11* (2**±6) aA	0.22* (3**±8) aA	-0.59	0.56	0 aA	0 aA	-	-
Sampling interval 3	0.55* (16**±27) bB	0 aA	2.32	0.03	0.33* (5**±9) aA	0.11* (2**±6) aA	1.06	0.29
F value	3.7	1.1			3.29	1		
P	0.004	0.35			0.05	0.38		
<i>Meloidogyne</i>								
Sampling date 1	0.11* (2**±6) aA	0 aA	1	0.33	0 aA	0 aA	-	-
Sampling date 2	0aA	0.38* (10**±23) aA	-1.87	0.07	0 aA	0 aA	-	-
Sampling date 3	0.97* (70**±110) aB	0.58* (22**±41) aA	0.96	0.35	1.11* (74**±108) bB	0.58* (22**±41) aB	1.34	0.19
F value	7.79	2.51			9.78	50		
P	0.002	0.09			0.001	0.009		
<i>Helicotylenchus</i>								
Sampling date 1	0.11* (2**±6) aA	0.33* (5**±9) aA	-1.07	0.29	0.84* (18**±23) aA	0.28* (8**±20) aA	1.92	0.07
Sampling date 2	0.39* (10**±20) aA	0.47* (8**±13) aA	-0.25	0.81	1.26* (67**±80) aA	1.36* (60**±57) aB	-0.27	0.79
Sampling date 3	0 aA	0.33* (5**±9) aA	-1.91	0.07	0.74* (26**±39) aA	0.66* (18**±27) aA	0.23	0.82
F value	2.24	0.18			1.14	5.75		
P	0.12	0.83			0.33	0.007		
<i>Tylenchorhynchus</i>								
Sampling date 1	0.07* (0.4**±1) aA	0 aA	1	0.32	0 aA	0.26* (7**±18) aA	-1.46	0.16
Sampling date 2	0 aA	0 aA	-	-	0 aA	0 aA	-	-
Sampling date 3	0 aA	0.22* (3**±8) aA	-1.48	0.15	0.84* (57**±104) bB	0 aA	2.74	0.01
F value	1.46	2.2			7.52	2.14		
P	0.25	0.13			0.02	0.13		
<i>Criconea</i>								
Sampling date 1	0 aA	0.32* (4**±8) aA	-1.5	0.16	0.33* (5**±9) aA	0.28* (8**±20) aA	0.18	0.86
Sampling date 2	0.25* (5**±12) aA	0.11* (2**±5) aA	0.67	0.51	0.25* (5**±12) aA	0.36* (7**±13) aA	-0.44	0.66
Sampling date 3	0 aA	0 aA	-	-	0.32* (5**±8) aA	0.22* (3**±8) aA	0.45	0.66
F value	2.17	1.06			0.13	0.15		
P	0.13	0.36			0.88	0.87		
<i>Nanidorus</i>								
Sampling date 1	0 aA	0 aA	-	-	0 aA	0 aA	-	-
Sampling date 2	0 aA	0 aA	-	-	0 aA	0 aA	-	-
Sampling date 3	0.11* (2**±6) aA	0 aA	1	0.33	0 aA	0.22* (4**±9) aA	-1.48	0.15
F value	1					2.19		
P	0.38					0.13		

*Log₁₀ (x+1) transformed nematode mean data; **Real means in parentheses followed by the Standard Deviation; Small letters in the same row (for each crop and each sampling date) that differ indicate a significant difference at $P \leq 0.05$ between the two treatments according to Student's t-test, while the same applies for capital letters in the same column indicating significant differences ($P \leq 0.05$) among the three dates for each crop and each treatment according to One-Way ANOVA; - No nematodes recovered.

6.3.2.2.2 Terrestrial non-parasitic nematode diversity and numbers per 200 g rhizosphere soil

Fourteen non-parasitic nematode genera were found in the the rhizosphere soil: *Acrobeles* (bacterivore), *Acrobeloides* (bacterivore), *Aphelenchus* (fungivore), *Aphelenchoides* (fungivore), *Aporcelaimellus* (predator), *Cephalobus* (bacterivore), *Ditylenchus* (fungivore), *Discolaimium* (predator), *Eucephalobus* (bacterivore), *Leptonchus* (fungivore), *Panagrolaimus* (bacterivore), *Teratocephalus* (bacterivore), *Tylencholaimus* (fungivore) and *Tylenchus* (fungivore) (Table 6.5). In general, higher numbers of non-parasitic nematodes were recorded during the 2014/15 compared to the 2013/14 growing season.

The fungivore genera *Aphelenchoides* and *Aphelenchus* were the most abundant genera, followed by (in decreasing order) the bacterivore genera *Acrobeloides*, *Panagrolaimus*, *Cephalobus*, *Eucepalobus* and *Acrobeles* (Table 6.5).

Population densities of the majority of the non-parasitic nematode genera differed significantly ($P \leq 0.05$) in the glyphosate-treated and non-treated plot halves, particularly for maize at the 1st sampling date and for both crops at the 2nd and 3rd sampling dates (Table 6.5). In the glyphosate-treated plot half, population densities of *Acrobeles*, *Acrobeloides* and *Cephalobus* were significantly ($P \leq 0.05$) higher compared to the non-treated maize plot half at the 1st sampling date. At the 2nd sampling date, significantly ($P \leq 0.05$) higher population densities of only *Acrobeles* and *Acrobeloides* were observed in the glyphosate-treated compared to non-treated soybean plot half. For maize this was the case for *Cephalobus*, *Eucephalobus*, *Panagrolaimus* and *Tylencholaimus*. At the 3rd sampling date, *Acrobeles*, *Eucephalobus* and *Tylenchus* population densities were significantly ($P \leq 0.05$) higher in the glyphosate-treated soybean plot half compared to the non-treated plot half. For maize, this was the case for *Acrobeles*, *Acrobeloides*, *Aphelenchus*, *Aphelenchoides*, *Eucephalobus*, *Panagrolaimus* and *Tylencholaimus*.

At the 1st sampling date, significantly ($P \leq 0.05$) higher population densities of *Aphelenchoides* and *Panagrolaimus* were observed in the non-treated soybean plot half and of *Eucephalobus*, *Panagrolaimus* and *Teratocephalus* in the non-treated maize plot half compared to the glyphosate-treated plot halve, (Table 6.5). At the 2nd

sampling date, significantly ($P \leq 0.05$) higher population densities of *Acrobeles*, *Acrobeloides*, *Ditylenchus*, *Eucephalobus*, *Leptonchus* and *Panagrolaimus* were observed in the non-treated plot half compared to the glyphosate-treated plot half. This was also the case for *Acrobeloides*, *Cephalobus*, *Eucephalobus*, *Panagrolaimus*, *Tylenchus* and *Tylencholaimus* in the maize plot half. At the 3rd sampling date, significantly ($P \leq 0.05$) higher population densities of *Acrobeles*, *Aphelenchoides*, *Cephalobus*, *Eucephalobus*, *Panagrolaimus* and *Tylenchus* were observed in the non-treated plot half compared to the glyphosate-treated plot half. This was also the case for *Acrobeles*, *Acrobeloides*, *Aphelenchus*, *Aphelenchoides*, *Cephalobus*, *Eucephalobus*, *Panagrolaimus*, *Teratocephalus* and *Tylencholaimus* in the maize plot half.

At the three sampling dates, the population densities of *Acrobeles*, *Aphelenchus*, *Cephalobus*, *Eucephalobus*, *Leptonchus*, *Tylenchus* and *Tylencholaimus* differed significantly ($P \leq 0.05$) in the glyphosate-treated soybean plot half (Table 6.6). In the non-treated soybean plot half, the population densities of *Acrobeloides*, *Aphelenchus*, *Aphelenchoides*, *Ditylenchus*, *Eucephalobus*, *Leptonchus* and *Tylencholaimus* differed significantly ($P \leq 0.05$) at the three sampling dates. In the maize plot half, the population densities of *Acrobeles*, *Acrobeloides*, *Aphelenchus*, *Aphelenchoides*, *Cephalobus*, *Eucephalobus*, *Panagrolaimus* and *Teratocephalus* differed significantly ($P \leq 0.05$) in the glyphosate-treated plot half at the three sampling dates. In the non-treated maize plot half, the population densities of *Acrobeloides*, *Aphelenchus*, *Cephalobus*, *Eucephalobus*, *Panagrolaimus*, *Teratocephalus*, *Tylenchus* and *Tylencholaimus* differed significantly ($P \leq 0.05$) at the three sampling dates.

Table 6.5. Number of non-parasitic nematodes per 200 g rhizosphere soil of soybean cv. LS 6164 R and maize cv. DKC 80-30 RR plants in glyphosate-treated and non-treated small-field plot halves at three sampling dates during the 2013/14 and 2014/15 growing seasons.

	Nematode genus	Soybean				Maize			
		Glyphosate-treated	Non-treated	t-value	P	Glyphosate-treated	Non-treated	t-value	P
Sampling date 1	<i>Acrobeles</i>	-	-	-	-	3.02* (1 625**±1 247) b	0 a	19.82	0.001
	<i>Acrobelloides</i>	1.43* (81**±90) a	1.55* (68**±54) a	-0.35	0.72	3.23* (2 692**±2 345) b	0 a	22.24	0.001
	<i>Aphelenchus</i>	0.49* (12**±23) a	0.83* a (25**±32) a	-1.02	0.31	2.57* (474**±265) a	2.6* (453**±240) a	-0.2	0.84
	<i>Aphelenchoides</i>	0 a	0.76* (33**±49) b	-2.78	0.01	-	-	-	-
	<i>Aporcelaimellus</i>	-	-	-	-	-	-	-	-
	<i>Cephalobus</i>	1.69* (189±211) a	1.88 (169**±136) a	-0.44	0.66	2.38* (431**±377) b	0 a	13.88	0.001
	<i>Discolaimium</i>	-	-	-	-	-	-	-	-
	<i>Ditylenchus</i>	-	-	-	-	-	-	-	-
	<i>Eucephalobus</i>	-	-	-	-	0 a	2.37 (67±159) b	-35.76	0.001
	<i>Leptonchus</i>	-	-	-	-	-	-	-	-
	<i>Panagrolaimus</i>	0 a	2.13* (338**±272) b	-7.26	0.001	0 a	2.84 (800±478) b	-42.77	0.001
	<i>Teratocephalus</i>	-	-	-	-	0 a	1.33 (22±12) b	-28.43	0.001
	<i>Tylenchus</i>	-	-	-	-	-	-	-	-
	<i>Tylencholaimus</i>	-	-	-	-	1.07 (22±22) a	1.23 (35±28) a	-0.5183	0.61
Sampling date 2	<i>Acrobeles</i>	2.53* (758**±718) b	0 a	9.89	0.001	-	-	-	-
	<i>Acrobelloides</i>	1.76* (108**±103) a	2.23* b (210**±158) b	-2.19	0.04	0 a	2.42* (289**±147) b	-42.92	0.001
	<i>Aphelenchus</i>	1.79* (101**±70) a	1.47* (91**±95) a	0.96	0.35	2.86* (801**±403) a	3* (3 707**±2 485) a	-2	0.06
	<i>Aphelenchoides</i>	-	-	-	-	-	-	-	-
	<i>Aporcelaimellus</i>	-	-	-	-	-	-	-	-
	<i>Cephalobus</i>	2.38* (462**±347) a	2.49* (389**±294) a	-0.42	0.68	3.1* (1 805**±1 325) b	2.73* (589**±398) a	2.5172	0.02
	<i>Discolaimium</i>	-	-	-	-	-	-	-	-
	<i>Ditylenchus</i>	0 a	1.57* (122**±126) b	-5.45	0.001	-	-	-	-
	<i>Eucephalobus</i>	0 a	2.7* (628**±475) b	-31.02	0.001	3.1* (1 825**±1 325) b	0 a	22.67	0.001
	<i>Leptonchus</i>	0 a	0.88* (14**±16) b	-5.06	0.001	-	-	-	-
	<i>Panagrolaimus</i>	0 a	2.4* (314**±238) b	-27.63	0.001	3.24* (2 492**±1 829) b	0 a	23.66	0.001
	<i>Teratocephalus</i>	-	-	-	-	0.35* (6**±11) a	0 a	1.91	0.06
	<i>Tylenchus</i>	-	-	-	-	0 a	2.84* (801**±459) b	-41.72	0.001
	<i>Tylencholaimus</i>	0.87* (22**±27) a	0.99* a (21**±25) a	-0.39	0.7	0.5* (10**±17) b	0 a	2.47	0.02
Sampling date 3	<i>Acrobeles</i>	1.93* (138**±113) b	0 a	10.15	0.001	2.95* (1 039**±579) b	0 a	39.68	0.001
	<i>Acrobelloides</i>	2.2* (275**±226) a	2.09* (314**±550) a	0.37	0.71	2.43* (312**±174) b	1.8* (157**±154) a	2.34	0.03
	<i>Aphelenchus</i>	2.59* (543**±272) a	2.84* (1 298**±801) a	-0.91	0.38	3.81* (6 628**±1 621) b	3* (1 126**±280) a	15.93	0.001
	<i>Aphelenchoides</i>	0 a	2.58* (667**±418) b	-10.62	0.001	3.45* (3 678**±2 014) b	0 a	30.07	0.001
	<i>Aporcelaimellus</i>	0.07* (1**±2) a	0 a	1	0.33	-	-	-	-
	<i>Cephalobus</i>	0 aA	2.52* (942**±1 650) b	-9.73	0.001	0 a	1.85* (178**±174) b	-6.99	0.001
	<i>Discolaimium</i>	0.08* (1**±2) a	0 a	1	0.33	-	-	-	-
	<i>Ditylenchus</i>	-	-	-	-	-	-	-	-
	<i>Eucephalobus</i>	1.9* (138**±113) b	0 a	10.01	0.001	3* b (1 195**±676) b	0 a	38.99	0.001
	<i>Leptonchus</i>	0.88* (16**±17) a	0.73* (16**±21) a	0.51	0.61	-	-	-	-
	<i>Panagrolaimus</i>	0 a	2.62* (944**±1651) b	-9.75	0.001	3.13* (1 558**±869) b	0 a	42.04	0.001
	<i>Teratocephalus</i>	-	-	-	-	0 a	1.2* (25**±17) b	-6.99	0.001
	<i>Tylenchus</i>	2.89* (919**±550) b	0 a	38.57	0.001	-	-	-	-
	<i>Tylencholaimus</i>	-	-	-	-	1.12* (40**±51) b	0 a	4.5	0.001

*Log₁₀ (x+1) transformed data subjected to the Student's *t*-test test; **Real means in parentheses followed by the Standard Deviation; Small letters in the same row (for each crop and each sampling date) that differ indicate a significant difference at $P \leq 0.05$ between the two treatments according to Student's *t*-test, while the same applies for capital letters in the same column indicating significant differences ($P \leq 0.05$) among the three dates for each crop and each treatment according to One-Way ANOVA; - No nematodes recovered.

Table 6.6. One-Way ANOVA of Log₁₀(x+1) transformed non-parasitic nematode numbers per 200 g rhizosphere soil of soybean cv. LS 6164 R and maize cv. DKC 80-30 RR plants grown in glyphosate-treated and non-treated small-field plot halves at three sampling dates during the 2013/14 and 2014/15 growing seasons.

Nematode genus	Soybean				Maize			
	Glyphosate-treated		Non-treated		Glyphosate-treated		Non-treated	
	<i>F</i> -value	<i>P</i>	<i>F</i> -value	<i>P</i>	<i>F</i> -value	<i>P</i>	<i>F</i> -value	<i>P</i>
<i>Acrobeles</i>	52	0.001	-	-	310	0.001	-	-
<i>Acrobeloides</i>	3	0.07	4	0.03	319	0.001	69	0.001
<i>Aphelenchus</i>	40	0.001	15	0.001	72	0.001	72	0.001
<i>Aphelenchoides</i>	-	-	27	0.001	904	0.001	-	-
<i>Aporcelaimellus</i>	1	0.4	-	-	-	-	-	-
<i>Cephalobus</i>	30	0.001	3	0.07	164	0.001	164	0.001
<i>Discolaimium</i>	1	0.4	-	-	-	-	-	-
<i>Ditylenchus</i>	-	-	26	0.001	-	-	-	-
<i>Eucephalobus</i>	103	0.001	1 270	0.001	379	0.001	379	0.001
<i>Leptonchus</i>	19	0.001	8	0.002	-	-	-	-
<i>Panagrolaimus</i>	-	-	1	0.5	418	0.001	418	0.001
<i>Teratocephalus</i>	-	-	-	-	4	0.04	4	0.04
<i>Tylenchus</i>	1 488	0.001	-	-	-	-	1 740	0.001
<i>Tylencholaimus</i>	14	0.001	23	0.001	3	0.1	31	0.001

Non-parasitic nematode data projected according to a faunal analysis showed a substantial difference between glyphosate-treated (plotted below the red line) and the non-treated plot halves (plotted above the red line) for the two crops (Fig. 6.1). Data for the non-treated soil at all sampling dates plotted above the red line in Quadrant A and B, with EI > 50 % for most of the samples due to c-p2 bacterial nematode genera dominating (*viz. Acrobeles, Acrobelloides, and Eucephalobus*). This indicates a bacterial-mediated soil. One sample from the non-treated maize plants plotted in Quadrant B with a high SI (86 %) due to the presence of c-p5 genera, *viz. predators* belonging to *Aporcelaimellus* and *Discolaimium*. In contrast, all samples from the glyphosate-treated plot half, except for one maize plant, plotted in Quadrants C and D and had a low EI (< 35 %). This is substantiated by the presence of c-p2 fungivores belonging to *Aphelenchus*, and *Aphelenchoides*, and the c-p4 fungivore classified under *Tylencholaimus* indicating a fungal-mediated soil.

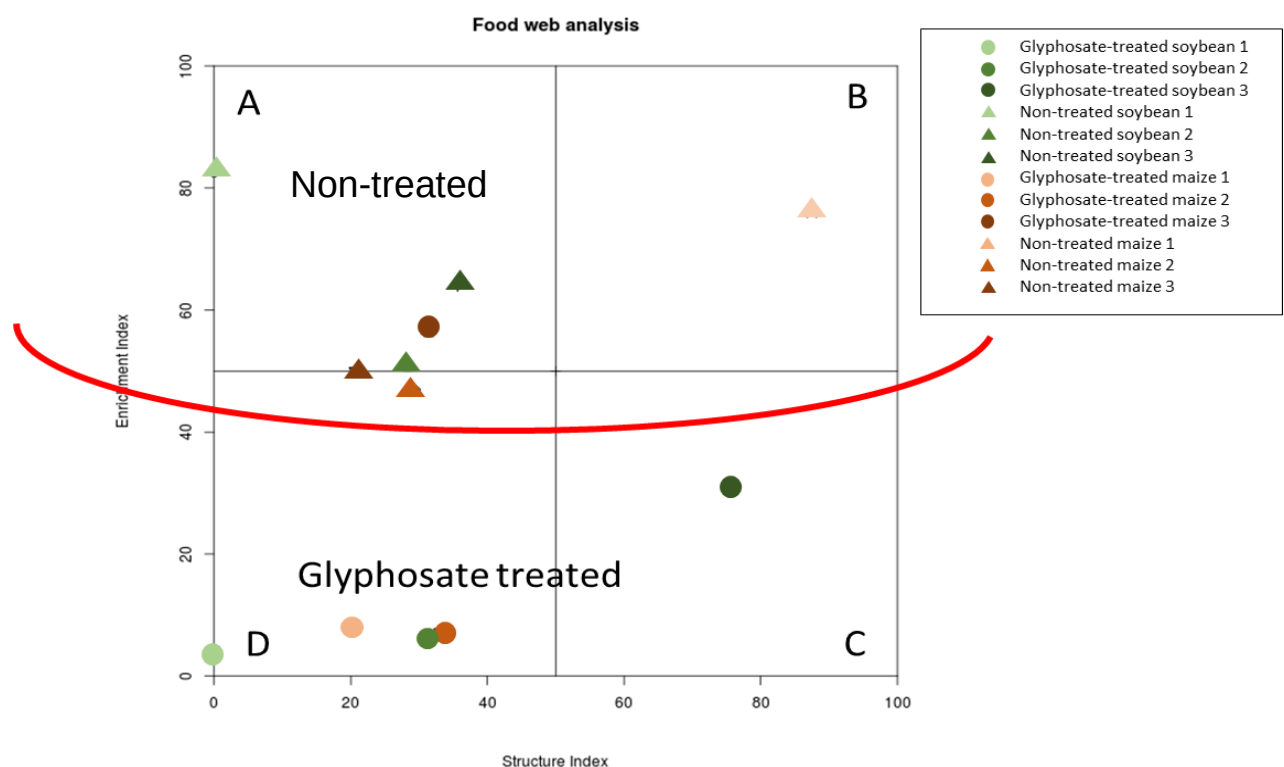


Figure 6.1. A faunal, soil food web profile representing the enrichment and structural indices (EI and SI, respectively) of non-parasitic nematode assemblages identified at three sampling dates in glyphosate-treated and non-treated plot halves planted with soybean (during the 2013/14 growing season) and maize (during the 2014/15 growing season).

6.4 Discussion

The trophic status of *Tylenchus* is inconclusive. For example, feeding habits for *Tylenchus* including both fungivorous and herbivorous have been observed (Wood, 1973; Sohlenius *et al.*, 1977). Yeates *et al.* (1993) considered these nematodes herbivores because they feed on algae, lichens and mosses. However, most *Tylenchus* nematodes may also consume mycorrhizal hyphae which would classify them as fungivores (Brmež *et al.*, 2006; Kibet *et al.*, 2014; Li *et al.*, 2016). To avoid any confusion we consider the genus *Tylenchus* fungivorous in our study. For the purpose of our study, data were analysed and discussed at genus level.

Results showed that glyphosate application as a leaf spray twice per growing season during two consecutive growing seasons had no effect on the population densities of *M. incognita* and *M. javanica*, and *Pratylenchus* spp. For the *Meloidogyne* spp., results from this study are in agreement with that by Cerdeira *et al.* (2007) who reported that glyphosate application did not affect population densities of the four most commonly found nematode pest species in Brazilian soybean fields, viz. *M. arenaria*, *M. incognita*, *M. javanica* and *H. glycines*. Results of this study also correspond with those by Liphadzi *et al.* (2005) who did not find glyphosate application to affect both non-parasitic and plant-parasitic nematode population densities. However, results from this study are in contrast with Vega *et al.* (1993) who reported that high dosage rates of glyphosate (400 and 600 ppm) significantly reduced *M. incognita* root gall and egg mass indices, and population densities in roots of treated soybean (genotype CES 434) plants compared to untreated control plants. *Meloidogyne incognita* egg mass numbers were also significantly reduced (compared to untreated controls) in soybean roots (cv. Williams) (Osman & Viglierchio, 1981).

The substantial increase in the root-knot nematode population densities and to a lesser extent *Pratylenchus*, over time in the roots of both soybean and maize observed in this study accentuates that glyphosate application did not adversely affect the reproduction potential of these nematode pests. In general, plant-parasitic nematodes increase in numbers during a growing season (Pan *et al.*, 2010) because the increase in root mass provides more penetration sites and food for these pests. Concerning the plant-parasitic nematodes identified during this study other than

Meloidogyne and *Pratylenchus*, no conclusive results regarding the effect of glyphosate application could be obtained. Their population densities in the rhizosphere soil and in the roots were either too low or zero.

Regarding the non-parasitic nematodes identified during this study, the significant differences observed over time between the glyphosate-treated and non-treated soybean and maize plot halves were caused by the absence of these nematodes at one or more of the sampling dates. However, this study showed that fungivores of the genera *Aphelenchus* and *Aphelenchoides* were generally more abundant in the rhizosphere soil of the non-treated plot half of both crops, except at the 3rd sampling date during the maize growing season (the last sampling date of this study). Hence, after the 4th and last glyphosate application these two fungivore genera dominated in terms of abundance in the glyphosate-treated maize plot half. This observation is in agreement with Liphadzi *et al.* (2005) who reported an increase in numbers of fungivorous nematodes, in particular of *Aphelenchus*, in glyphosate-treated fields. The increased abundance of fungivores in glyphosate-treated soils may be attributed to increased fungal growth due to root exudation which in turn may increase the population densities of fungivorous nematodes (Kremer & Means, 2009; Pan *et al.*, 2010; Zobiolo *et al.*, 2010).

Food web analysis shows that the majority of the nematodes in the glyphosate-treated plot halves during both the growing seasons plotted in Quadrant D and that glyphosate application affected the non-parasitic nematode assemblage. This result is not in agreement with that by Jansen (2014). This author found no differences in the abundance and diversity of non-parasitic nematodes in glyphosate-treated soybean fields compared to soybean fields that during five years had not been treated with glyphosate. The observed differences in abundance and diversity of non-parasitic nematodes between glyphosate-treated and non-treated plot halves in this study indicate that the rhizosphere soil in the glyphosate-treated plot half was altered. Therefore, it is suggest that glyphosate application may have changed the rhizosphere environment in such a way that the nematode assemblage were different in terms of the most abundant genera.

Terrestrial non-parasitic nematodes are instrumental in ecological processes in the soil and enhance the concentration of plant nutrients in the soil such as organic matter and nitrogen (Wardle *et al.*, 2005). The data generated during this study are novel and add useful information to the limited and not well documented knowledge about the effects of glyphosate application on non-parasitic nematode assemblages and, indirectly, on soil quality. Given the widespread use of glyphosate in South Africa, this practice may adversely affect soil quality in many agro-ecosystems in the country. Yeates *et al.* (1999) stated that the effect(s) of herbicides on nematode population densities may vary with the herbicide applied and the cropping system. Hence, to obtain a more comprehensive insight in the adverse effects of glyphosate application on terrestrial non-parasitic nematode assemblages and soil quality, a longer study period and inclusion of glyphosate-tolerant crops other than soybean and maize (examined during this study are necessary).

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Chapter 7

Conclusions and suggestions for future research

This PhD study focused on plant-parasitic nematodes, in particular *Meloidogyne* spp. (root-knot nematodes), but also included novel information about the non-parasitic assemblages occurring in soils where local soybean crops are cultivated.

The motivation for this study is based on the exceptionally high population densities and widespread occurrence of economically important plant-parasitic nematodes, notably *Meloidogyne* and *Pratylenchus*, in current expanded local soybean production areas (Fourie *et al.*, 2017; Mc Donald *et al.*, 2017). Furthermore, while the use of nematicides remains the first choice to control these pests on soybean crops in other countries (Mueller, 2015), no nematicide is registered for use on soybean in South Africa (Van Zyl, 2016). The withdrawal of Class I nematicides (Warnock *et al.*, 2017) from world markets furthermore limit chances that such products will be registered on soybean in future. Hence, generating information that can assist producers to sustainably produce food was the main objective of this study and broadly included:

- i) to assess the host status of local commercially available soybean cultivars to the predominant nematode pest *Meloidogyne incognita* (Kofoed & White, 1919) Chitwood, 1949 in order to recommend resistant cultivars for use as an alternative environmentally-friendly nematode management strategy,
- ii) to obtain up-to-date and novel information on the identity of nematode assemblages (plant-parasitic and non-parasitic) in local soybean fields, which is currently dominated by glyphosate-tolerant crops (Dlamini *et al.*, 2014) and
- iii) to assess the abundance, diversity and occurrence of both plant-parasitic and non-parasitic nematodes in glyphosate-tolerant and conventional soybean fields as well as adjacent natural veld.

Conducting this study was further substantiated by limited information being available on the host status of local glyphosate-tolerant soybean cultivars to *M. incognita* (Venter, 2014) and none for nematode assemblages in soybean production areas where glyphosate is readily and frequently applied (Fourie *et al.*, 2001; 2017). Although regarded as less toxic to the environment the extensive use of glyphosate has raised concerns about their non-target effects on soil nematodes (Newman *et*

al., 2016). Furthermore, information on non-parasitic nematodes associated with soybean agro-ecosystems and their use as bio-indicators of soil quality prevailing in local soybean agro-ecosystems is also lacking and was another initiative undertaken as part of this study during which novel information was generated.

This study commenced with the screening of 30 commercially available genetically modified, glyphosate-tolerant soybean cultivars to *M. incognita* under glasshouse conditions (see Chapter 2). Also included were four soybean lines with varying levels of *M. incognita* resistance (*viz.* PRF-GCI4, PRF-GCI5, PRF-GCI6 and PRF-GCI7; Fourie *et al.*, 2015). *Meloidogyne incognita*, the predominant root-knot nematode species found in soybean fields during the first official soybean-nematode survey (Fourie *et al.*, 2001), was used as the target nematode species. The study, conducted at two sets of ambient temperature ranges, showed that apart from the resistant standard cultivar LS5995, only cultivar PRF-GCI7 consistently had low values for both resistance parameters (reproduction factor: R_f , and relative percentage susceptibility: %R). Noteworthy is that cultivar DM 6.2i RR had a %R of < 10 % for Experiment 1 (lower temperature regime), but not in Experiment 2 (higher temperature regime). Resistance to *M. incognita* exhibited by cultivars LS5995, PRF-GCI7 and DM 6.2i RR was hence confirmed by this study as previously reported (Fourie *et al.*, 2006; Venter, 2014). The other cultivars screened differed substantially with regard to their susceptibility levels to *M. incognita*, with cultivars DM 5.1i RR and DM 5953 RSF being the most susceptible with exceptionally high R_f values. Results from this study demonstrated the significant reduction in *M. incognita* population densities by using resistant cultivars, which should be the preferred choice in areas where this species poses problems. However, should such cultivars not be adapted to such areas, cultivars with a R_f value ≤ 5 and/or a %R value ≤ 10 % should be used. An important deduction from this study was that the host response of local cultivars to *M. incognita* (and other *Meloidogyne* spp. that are prevalent in the local soybean-based production areas) should be done on a regular basis to provide producers with the most up-to-date and relevant information. This way *Meloidogyne* spp. population densities can be reduced substantially over time and enable producers to produce soybean and other rotation crops sustainably.

⁵Chapters 3 and 4 of this thesis dealt with nematode surveys that were conducted in local soybean production areas to determine the abundance, diversity and occurrence of plant-parasitic nematodes. The first survey was done during one season only (2014/15) and included localities where the National Cultivar Trials of the Agricultural Research Council's Grain Crops Institute, Potchefstroom were done. In these trials, 29 glyphosate-tolerant soybean cultivars evaluated for their agronomical traits were sampled. The second survey was conducted over two growing seasons (2011/12 and 2102/13), with sampling done in both glyphosate-tolerant and conventional soybean fields as well as adjacent natural vegetation. This was a first-time assessment of nematodes present in soils treated with glyphosate, compared to those where glyphosate was not applied for 5 years. Plant-parasitic nematodes, particularly *Meloidogyne* and *Pratylenchus*, were the predominant genera in soybean fields which conforms to reports from the first nematode-soybean survey (Fourie *et al.*, 2001). *Meloidogyne incognita* was the predominant root-knot nematode pest, followed by *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, differing from previous reports that at least four *Meloidogyne* spp. have been associated with local soybean crops (Fourie *et al.*, 2001; Fourie *et al.*, 2017; SAPPNS¹). An interesting anomaly was, however, recorded for the predominant *Pratylenchus* spp. identified in the respective surveys of this study. According to the 2014/15 survey, *Pratylenchus brachyurus* (Godfrey, 1929), Filipjev & Schuurmans Stekhoven, 1941 was predominantly followed by *Pratylenchus zeae* Graham, 1951, which was in agreement with reports of the 1995/96 survey (Fourie *et al.*, 2001). The opposite was, however, true for the 2011/12 and 2012/13 surveys with *P. zeae* dominating, followed by *P. brachyurus*. The latter species has become a major pest of soybean in Brazilian soybean production areas (Ribeiro *et al.*, 2010; Lima *et al.*, 2015). Cognizance of its abundance in local production areas has to be taken since this species (and the other *Pratylenchus* spp. identified) may have the same damage potential under South African environmental conditions. Also important is that both *P. brachyurus* and *P. zeae* are abundant in roots/tubers of crops used in rotation with soybean in South Africa, for instance, maize (*Zea mays* L.) (Mc Donald *et al.*, 2017), and groundnut (*Arachis hypogaeae* L.) (Duarte *et al.*, 2011). This may lead to

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⁶high population densities of these nematode pests that will result in unsustainable crop production.

Concerning plant-parasitic nematode pests identified from natural vegetation adjacent to soybean fields, the presence of particularly *Meloidogyne* and *Pratylenchus* again raised concern. However, a wide diversity of other plant-parasitic nematodes were also found in such natural vegetation sites. The main concern in this regard is that natural vegetation areas are increasingly being cleared to allow crop production. The presence of the said economically important nematode pests in such areas can hence act as the primary source of pathogen inoculum (Van der Putten & Van der Stoel, 1998). Hence, planting soybean or other annual crops on a cleared natural field may have an adverse impact on crop production. Although a range of other plant-parasitic nematode species were identified during these surveys, the most interesting findings were first records for the following seven plant-parasitic nematode species, viz. *Pratylenchus flakkensis* Seinhorst, 1968, *Pratylenchus scribneri* Steiner, 1943 *Pratylenchus vulnus* Allen & Jensen, 1951, *Rotylenchulus brevicaudatus* (Williams, 1960) Sher, 1961, *Telotylenchus avaricus* Kleynhans, 1975, *Tylenchorhynchus brevicaudatus* Williams, 1960 and *Quinisulcius capitatus* (Allen, 1955) Siddiqi, 1971 (SAPPNS¹). The ultimate conclusion from the 2011/2012 and 2012/13 surveys was that no significant differences existed for the abundance and diversity of plant-parasitic nematode assemblages identified from glyphosate-tolerant versus conventional soybean fields. Further and more extensive research is needed to elucidate the effect of glyphosate on nematode assemblages since these surveys just represented a baseline study.

Except for the focus being mainly on plant-parasitic nematodes, the abundance and diversity of non-parasitic nematodes were also addressed in Chapters 5 and 6 of this study. The two-year, consecutive surveys referred to in the paragraph above for plant-parasitic nematodes (Chapter 4), also served as a platform to generate information on the non-parasitic nematode genera present in three ecosystems, viz. glyphosate-tolerant and conventional soybean fields as well as adjacent, natural

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vegetation as constituted in Chapter 5. Twenty-seven non-parasitic nematode genera were collectively identified from soils of glyphosate-tolerant and conventional soybean fields (21 in glyphosate-tolerant and 23 in conventional soybean) and 28 from natural vegetation sites. Bacterivores, represented by *Acrobeles*, *Acrobeloides*, *Eucephalobus* and *Panagrolaimus*, generally dominated in soils of all three ecosystems, followed by fungivores (mainly *Aphelenchus* and *Aphelenchoides*). The abundance and diversity of predator and omnivore nematodes were low in all three ecosystems. Results from this study agreed with those by Xiao *et al.* (2014) that bacterivores are the most abundant nematodes in the soil. Bekker (2016) also reported this trend from South African maize fields and adjacent natural vegetation. However, no correlations were apparent for non-parasitic genera in either of the three ecosystems. According to soil food web analysis, soils from all the sites sampled from all three ecosystems (glyphosate-tolerant and conventional soybean as well as natural vegetation) were disturbed and degraded.

Chapter 6 of this study dealt with a small field experiment that was conducted over two consecutive summer-growing seasons to determine the response of nematode communities (plant-parasitic and non-parasitic nematodes) to glyphosate in a soybean-maize rotation sequence. Six plant-parasitic nematode species/genera and 14 non-parasitic nematode genera were identified. Significant differences ($P \leq 0.005$) were recorded between the glyphosate-treated and non-treated (control) treatments for some non-parasitic nematode genera such as *Acrobeles*, *Acrobeloides*, *Aphelenchoides*, *Aphelenchus*, *Cephalobus*, *Eucephalobus*, *Leptonchus*, *Panagrolaimus*, *Teratocephalus* and *Tylencholaimus* during some of the sampling dates achieved during each season. The high abundance of fungivorous nematodes, particularly *Aphelenchoides* and *Aphelenchus* in glyphosate-treated plots compared to those in non-treated plots was not surprising. Liphadzi *et al.* (2005) also observed high populations of fungal feeding nematodes in glyphosate treated fields and suggested that root exudates from weeds after glyphosate application stimulated soil fungal populations that caused an increase in fungivorous nematodes. Faunal analyses (Ferris *et al.*, 2001; Sieriebriennikov *et al.*, 2014) showed that soils from glyphosate-treated plots were degraded, less enriched and fungal-mediated. Conversely, soils from non-treated plots were disturbed and enriched, and bacterial-mediated. Ultimately, results from this study as well as from Chapters 4 and 5 are

insufficient to draw any final conclusions about the potential impact of glyphosate on both parasitic and non-parasitic nematode assemblages.

Some data generated during this PhD study are novel and add useful information to existing knowledge about plant-parasitic and non-parasitic nematodes associated with local soybean and adjacent natural veld. Given especially the widespread use of glyphosate in South Africa, obtaining more comprehensive insights on the effects of glyphosate application on terrestrial nematode assemblages and soil quality should be considered. This should include longer study periods for field and controlled experiments, and glyphosate-tolerant crops other than soybean and maize examined during this study.

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