

**THE INFLUENCE OF PLANT-PARASITIC NEMATODES ON SOYBEAN  
PRODUCTION IN SOUTH AFRICA WITH SPECIAL REFERENCE TO ROOT-  
KNOT NEMATODES (*MELOIDOGYNE* SPECIES).**

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## CHAPTER 1

### ABSTRACT

Soybean is an important oilseed crop with an increase in production in South Africa since the early 1970's to approximately 138 000 tons produced during 1997. Several plant-parasitic nematode genera and species are associated with this crop world-wide. *Meloidogyne incognita*, *Heterodera glycines*, *Pratylenchus brachyurus* and *Rotylenchulus reniformes* are regarded important nematode species on soybean due to yield losses ascribed to them. Although several plant-parasitic nematode genera and species were previously reported on soybean in South Africa, little is known about the nematode-host interaction in this crop. A survey was, therefore, conducted to identify different plant-parasitic nematodes associated with soybean in the soybean production areas of this country. Host suitability of 38 commercial soybean cultivars for *M. javanica* and *M. incognita* race 1, 2 and 4 were also conducted in greenhouse trials.

Twelve plant-parasitic nematode genera were present, including 15 plant-parasitic species. Two genera and nine species were first reports on soybean in South Africa, which included *Rotylenchulus* spp., *Mesocriconema* spp., *Pratylenchus zae*, *P. crenatus*, *P. neglectus*, *P. thornei*, *Meloidogyne ethiopica*, *Paratrichodorus minor*, *Longidorus pisi*, *Xiphinema vanderlinde* and *X. elongatum*.

*P. zae*, *P. brachyurus* and *Meloidogyne* spp. were predominant endoparasites and *Helicotylenchus* and *Scutellonema* spp. the predominant semi-ectoparasitic nematodes associated with soybean throughout the survey. Mixed populations of *Pratylenchus* spp. and *Meloidogyne* spp. occurred at several localities. *Pratylenchus* spp. included *P. zae* and *P. teres* at Vaalharts, *P. zae* and *P. brachyurus* at Piet Retief, Viljoenskroon, Bethal, Ventersdorp and Oberholzer and *P. brachyurus*, *P. crenatus* and *P. thornei* at Beestekraal. *Meloidogyne* spp. were present in mixed populations at Badplaas (*M. incognita* and *M. javanica*), Greytown (*M. incognita* and *M. ethiopica*) and Vaalharts (*M. incognita* and *M. hapla*). *Heterodera* spp. was not present in soil and root samples collected during this survey. Important is that *Longidorus pisi*, *X. vanderlinde* and *X. elongatum* are not associated with virus transmission in South Africa, while *P. minor* is known to transmit tobacco rattle virus in this country.

The prominence values (PV) of different nematode genera and species differed for different soybean cultivars. *P. zae* and *P. brachyurus* had the lowest PV on A5308, while *Helicotylenchus* spp., *Scutellonema* spp. and *D. africanus* had the lowest PV on Highveld Top.

*Meloidogyne* species had the lowest PV on PAN812 and the highest on Prima. PAN812 was found a poor host for *M. javanica* and *M. incognita* race 1 while Prima was highly susceptible to *M. javanica* and *M. incognita* race 1, 2 and 4 in greenhouse experiments.

Screening of commercial soybean cultivars for host suitability to *M. javanica* and *M. incognita* race 1, 2 and 4 resulted in substantial variation between the 38 cultivars. Although egg mass indices are continuously used as a criterium for expressing nematode resistance in soybean cultivars, this study clearly indicated that RF-values and numbers of eggs/egg mass are more reliable parameters to indicate host suitability of cultivars for a specific root-knot nematode species. Several cultivars were identified as poor hosts for *M. javanica* and *M. incognita* race 1 and 4. A7119 was a poor host for both *M. javanica* and *M. incognita* race 1 and 4, while CRN2233, A7119, Hutton and PAN723 were poor hosts for *M. incognita* race 1 and 4. No cultivars had RF-values lower than 1 for *M. incognita* race 2, but Hutton was the poorest host, with a RF-value of 1.1. Highly susceptible cultivars to *M. javanica* and *M. incognita* race 1, 2 and 4 included Dumela, Wilge, Knap, Hennops, Prima and Tamboti. Valuable information was also obtained regarding the mechanism of resistance in some soybean cultivars, antibiosis as well as antixenosis resistance seem to be present in several cultivars.

## CHAPTER 2

### INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is an important primary source of protein and oil, currently showing increased production world-wide (Shane & Barker, 1986). In South Africa an increase in soybean production is experienced since 1970. Approximately 138 000 tons of soybean will be produced during 1997 (M.A.Smit, 1997- personal communication). According to Corbin (as quoted by Acosta & Negrón, 1982) soybean is the most important cash crop in the United States that produces approximately 75% of the world supply. Soybean is, according to Hill (as quoted by Acosta & Negrón, 1982), widely utilized in human foods, animal feeds and in industrial applications due to its high protein content and oil and meal production from the seeds. Due to an increase in acreage planted to soybean and continuous cropping, an increase in diseases and pests has become evident (Acosta & Negrón, 1982). Production and quality is, therefore, limited world-wide due to parasitizing nematode species, including root-knot (*Meloidogyne* spp.) nematodes (Anon., 1979; Kinloch, 1982; Shane & Barker, 1986).

Endoparasitic nematodes such as *Meloidogyne*, *Heterodera* and *Pratylenchus* spp., feed internally within the plant roots (Sinclair, 1982), with damage caused by these nematodes more easily recognized than damage caused by ectoparasitic nematodes (Good, 1973).

Extensive tissue alteration and destruction in soybean roots, with consequently visual above-ground symptoms may be present. Furthermore, nematode damage to roots reduces the uptake of water and nutrients, often resulting in stunting, reduced yields and in some cases (*Heterodera* and *Meloidogyne* species) reduction of nodulation and N<sub>2</sub> fixation (Good, 1973). Root-knot nematodes are, however, of the most important plant-parasitic nematodes

on a variety of crops world-wide and may infest soybeans wherever it is grown (Schmitt & Noel, 1984). Root-knot nematodes cause enlargements of root tissue, resulting in galling after second stage larvae entered soybean roots and develop into mature, pear-shaped females inside root tissue. Adult females produce egg masses and three to four root-knot nematode generations can be produced per growing season. When soybean roots decay after harvest, larvae and eggs are released into the soil where they over-winter and infest the next crop (Good, 1973).

Although plant-parasitic nematodes are pests of major importance in a variety of agricultural crops, their damage to soybean often remains undetected (Good, 1973). More than 50 plant-parasitic nematode species, representing about 20 genera, have been reported to feed on or to be associated with soybean root systems (Good, 1973).

According to Sinclair (1982) cyst (*Heterodera* spp.), root-knot (*Meloidogyne* spp.) reniform (*Rotylenchulus* spp.) and lesion nematodes (*Pratylenchus* spp.) are the most important nematode genera associated with soybean. Rodríguez-Kábana and Williams (1981) regard root-knot nematode and cyst nematode species to be the economically most important in soybean. Several plant-parasitic nematode species are usually present in soybean fields (Sinclair, 1982), but only one genus (usually *Meloidogyne* spp.) is generally responsible for most damage (Good, 1973).

Plant-parasitic nematodes are a major yield-limiting pest of soybean in the USA, Brazil, Peru, Nigeria, Egypt and Japan (Ibrahim *et al.*, 1972 & 1976; Kinloch, 1980; Lewis *et al.*, 1993; Lordello, 1955) with yield losses ranging from negligible to 100% on a field-by-field basis in

the USA (Schmitt & Noel, 1984). Overall yield losses to soybeans are estimated at about 10% in the USA, to which root-knot nematodes contribute 4%, soybean cyst nematodes 4% and other nematodes 2%. Yield losses due to root-knot nematodes ranging from 30-90% have, however, been reported from Florida (USA) in nematode susceptible cultivars (Sinclair, 1982). These yield losses could increase since soybean are grown more intensively (Good, 1973). According to literature, *M. incognita* is the most important root-knot nematode species on soybean and often cause major yield losses in warmer areas. The extent of damage caused by *M. incognita* depends on environmental conditions (Shane & Barker, 1986). Kinloch (1974) demonstrated that *M. incognita* may cause up to 90% yield loss on susceptible soybean cultivars while Rebois (1973) reported yield reductions of 33% due to this species on both resistant and susceptible cultivars. *M. javanica* and *M. arenaria* are becoming increasingly important in warmer areas of the world, while *M. hapla* occurs in colder areas, also causing yield losses to soybean (Schmitt & Noel, 1984). In warmer areas, more damage also occur on light-textured sandy soils than heavy soils (Sinclair, 1982).

No information on the extent of soybean yield loss due to root-knot nematodes is available under South African conditions at present. Since soybean production in South Africa was in the past limited to areas with heavier soils, less nematode damage occurred. Soybean production is, however, extended to lighter textured soils of South Africa where particularly maize was previously produced. Soybean is also more frequently planted in rotation with maize. *M. javanica* and *M. incognita* are present in maize producing areas and their presence in high numbers often results in significant maize yield losses (Riekert, 1996). When soybean is introduced into these areas, the risk of the crop to be increasingly exposed to root-knot nematode parasitism, with an increase in risk of yield losses is imminent.

Management strategies for plant-parasitic nematodes on soybean are limited (Lewis *et al.*, 1993). Methods for managing *Meloidogyne* species vary with the species or species complex in a field. The first step in devising a nematode management scheme is to correctly identify the species present. Control of soybean-parasitic nematodes must then be aimed at reducing the initial nematode inoculum or preventing nematode infection, because damage to soybean during its early growth stages has the greatest effect on reducing yield potential (Garcia & Rich, 1985). The following control strategies are available for reducing yield loss due to root-knot nematodes: a) use of resistant cultivars, b) crop rotation and c) the application of nematicides (Garcia & Rich, 1985; Sinclair, 1982). While resistant cultivars prevent or inhibit nematode feeding and development, crop rotation and nematicides reduce the initial nematode population density present in a field (Schmitt & Noel, 1984). Each of these control strategies will be discussed separately.

a) Resistant cultivars: Development of soybean cultivars with resistance to certain nematode species is important in crop protection (Schmitt & Noel, 1984). Resistant soybean varieties have proven highly successful in minimizing yield losses if correctly matched to species and races present in a particular field (Good, 1973; Lewis *et al.*, 1993). Resistance is also the most cost-effective control measure in reducing losses due to root-knot nematode infection (Hussey & Boerma, 1981). Levels of resistance in soybean to *Meloidogyne* spp. have been reported from the USA (Boquet *et al.*, 1975; Good, 1973; Hussey & Boerma, 1981; Luzzi *et al.*, 1987) limiting yield losses in *Meloidogyne*-infested fields (Herman *et al.*, 1991). The sensitivity of soybean cultivars to a given nematode species varies considerably (Schmitt & Noel, 1984), with not all resistant cultivars possessing the same level of resistance for a given root-knot nematode species (Sinclair, 1982). According to Rodríguez-Kábana and Williams

(1981) some commercial cultivars have resistance to one or two root-knot nematode species but not combined resistance to all *Meloidogyne* species.

Types of resistance were, however, classified and grouped in three main categories (Painter 1941 & 1951):

i) antibiosis includes all adverse effects exerted by the plant on the biology of the nematode (survival, development and reproduction) (Horber, 1980) when a resistant host-plant variety or species is used for food (Painter, 1951).

ii) antixenosis / non-preference includes the response of plants lacking in characteristics to serve as a host, resulting in negative reactions or total avoidance by the nematode during feeding or reproduction (Horber, 1980). Knowledge of the ways in which nematodes locate plants for shelter, food, or reproduction involves a study of the behaviour of such nematodes in the presence of various stimuli derived from the plant. The lack of such stimuli or presence of counteracting repellent or distracting stimuli constitutes the phase of resistance referred as antixenosis or non-preference (Painter, 1951).

iii) tolerance includes all plant responses resulting in the ability to withstand nematode infestation and to support nematode populations that would severely damage susceptible plants. Tolerance differs from antibiosis and antixenosis resistance in the predominant part played by the plant, while antibiosis and antixenosis require an active response from the nematode, or a lack of response. These three categories do not exclude each other but may interact and complement each other in the sense of intensifying resistance expressions, e.g. when a nonpreferred host also exerts antibiotic effects (Painter, 1951).

The inclusion of these resistance categories as reliable and important parameters are common

in insect resistance studies (Van den Berg, *et al.*, 1994; Wiseman, 1994; Wiseman & Davis, 1990; White, 1990). It is, therefore, essential for nematologists to be informed of these mechanisms of resistance in the management of nematode populations because the continuous change in hybrids by industries requires a continuous search for new sources or higher levels of resistance and the incorporation of these resistance sources into acceptable germplasm (Wiseman & Davis, 1990).

b) Crop rotation: Root-knot nematodes have wide host ranges and reproduce on a wide variety of cultivated crops and weeds. As a result crop rotations do not always control populations of this species successfully (Sinclair, 1982). Maize can be rotated with soybean to control *M. hapla* but other *Meloidogyne* species cannot be controlled in this way (Sinclair, 1982). Crop rotation is also limited by the lack of resistant or non-host alternative high-value crops (Garcia & Rich, 1985).

c) Nematicides: In addition to the use of resistant cultivars and crop rotation, nematicides should be included in an integrated control programme as a complementary control measure for nematode damage to soybean (Rodríguez-Kábana & Williams, 1981; Schmitt & Noel, 1984). The most effective nematicidal treatment is fumigation (Kinloch, 1980) with methyl bromide or EDB (ethylene dibromide) but it is seldom economically justifiable.

Since no nematicides are registered on nematodes associated with soybean in South Africa, the combination of resistant cultivars and crop rotation is the only management strategies available at present for protection of soybean yields against plant-parasitic nematodes.

In contrast to knowledge about the distribution and damage of the more important nematode species associated with soybean in the USA (Good, 1973), very little knowledge concerning nematodes associated with soybeans in South Africa is available. The objectives of this study was therefore to:

1. undertake a country-wide survey, including the most important soybean producing areas to establish what plant-parasitic nematodes are associated with soybean in South Africa.
2. screen commercially available soybean cultivars of South Africa in the greenhouse for resistance to the root-knot nematode species identified to be important according to the survey.
3. determine the mechanism of resistance present in soybean cultivars (antibioses or antixenoses).

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1. Soybean survey

##### 3.1.1 Extraction of nematodes from soybean root and soil samples.

Nematodes were extracted from soil using the decanting and sieving method, described by Cobb (1918). This method was followed by the sugar centrifugal-flotation method (Caveness & Jensen, 1955). Nematodes in the roots were extracted from 5g root samples using the sugar centrifugal-flotation method (Coolen & D'Herde, 1972) and from 50g root samples using an adapted NaOCl method, specifically developed for the extraction of root-knot nematode eggs and larvae from plant roots by Riekert (1995).

##### 3.1.1.1 Decanting and sieving method.

###### Principle:

This method is based upon the density and size of nematodes. Soil samples were stirred and allowed to settle for 30 seconds. This way about 90% of the nematodes stay in suspension. About 20% of the nematodes originally present in the soil sample will be lost during this method (Cobb, 1918).

###### Procedure:

After a soil sample was soaked in water, soil particles with a diameter of more than 1mm were removed by passing the sample through a 710  $\mu\text{m}$ -mesh sieve nested on a 5 l bucket. The residue on the sieve was washed for about 2 minutes and then discarded. The bucket was filled up to 5 liter. The soil sample was thoroughly mixed with water and the mixture allowed to settle for about 30 seconds. The mixture was then decanted through a 25  $\mu\text{m}$ -mesh sieve, leaving behind the sediment that had settled to the bottom of the bucket. The procedure was repeated once. The nematodes and fine soil particles retained on the sieves were washed into centrifuge tubes.

#### a) Water centrifugation

The soil was washed with water into 50ml centrifuge tubes and centrifuged for 5 minutes with a Relative Centrifugal Force (RCF) of 1800g. After centrifugation the supernatant was carefully decanted and discarded. Nematodes were collected in the bottom of the centrifuge tubes. This method was followed by the sugar centrifugal-flotation method.

#### 3.1.1.2. Sugar centrifugal-flotation method

##### **Principle:**

This method is based on the specific gravity of nematodes. Terrestrial nematodes have a specific gravity of about 1.08 and marine nematodes about 1.13. After centrifugation in water, only organic material with a specific gravity lower than 1.0 will remain in suspension and can be discarded. When centrifuged in a solution with a higher specific gravity than the nematodes' density (i.e. sucrose solution with a specific gravity of 1.15) the nematodes will remain in suspension, separated from soil particles with a specific gravity larger than 1.15.

##### **Procedure:**

A sucrose solution with a specific gravity of 1.15 was added to the centrifuge tubes. The sucrose solution was prepared by adding 624g sugar to 1000ml water. This solution and the sediment with nematodes in the centrifuge tubes were thoroughly stirred with a spatula and centrifuged for 1 minute at a RCF of 1800g. When the centrifugation was completed, the supernatant (with nematodes in suspension) was decanted on a 25 $\mu$ m-mesh sieve and gently rinsed with water in order to remove the sucrose as quickly as possible. The nematodes were washed into a sample bottle for examination and counting.

Because the sucrose solution is osmotically active the nematodes can plasmolyse. It was therefore critical that the nematodes were kept in the sucrose solution as shortly as possible, not exceeding a total of four minutes.

### **3.1.1.3. Centrifugal - flotation method for the extraction of nematodes from plant roots.**

#### **Principle:**

One of the most successful methods for extraction of nematodes from plant roots is the centrifugal-flotation method described by Coolen and D'Herde (1972). The modified version of this method by De Waele *et al.* (1987) is described below. This method is based on maceration and centrifugal-flotation.

#### **Procedure:**

- a) A 5g subsample was taken from 18 complete soybean root systems that were combined, cut into 1cm pieces and mixed thoroughly.
- b) The sample was macerated in 250ml tap water at high speed in a domestic blender for 90 seconds to release nematodes from the root tissue.
- c) The suspension of nematodes and root fragments were then decanted on a 710 $\mu$ m-mesh sieve nested on a 25 $\mu$ m-mesh sieve.
- d) The root pieces on the 710 $\mu$ m-mesh sieve were washed thoroughly with running tap water.
- e) The residue on the 25 $\mu$ m-mesh sieve was collected and decanted into a 50ml centrifuge tube.
- f) 2 cm<sup>3</sup> kaolin was added to the tube, stirred well and centrifuged at 1800g for 1 minute.
- g) The supernatant was decanted and sucrose solution (specific gravity = 1.15g/cm<sup>3</sup>) added to the tube. The mixture was stirred well and centrifuged at 1800g for 1 minute.
- h) The supernatant was decanted onto a 25 $\mu$ m-mesh sieve, rinsed well with tap water to remove sucrose. The residue was collected in a sample bottle for examination and counting of the nematodes.

#### **3.1.1.3.1. The importance of kaolin.**

Kaolin is a clay mineral with a specific gravity of 2.6 and consists of particles 2-3 $\mu$ m in size. Although the density of kaolin is greater than that of nematodes, kaolin particles are small and flat, which makes them sink more slowly to the bottom of the centrifuge tube than the

nematodes. This way kaolin spreads out to form a layer over the loose sediment and seals it off when the supernatant is decanted. When the sucrose solution is added to the sediment the mixture must be stirred thoroughly to break the kaolin layer. By doing this the nematodes are brought into suspension in the sugar solution. Another advantage of kaolin is that it also precipitates during the second centrifugation, thus preventing re-mixing of the sedimented debris when the sugar solution is decanted. A suspension of nematodes in clear water is obtained (Coolen & D'Herde, 1972).

#### **3.1.1.4. Adapted NaOCl method for the extraction of root-knot nematode eggs and larvae.**

##### **Procedure:**

- a) A 50g subsample was taken from three complete soybean root systems that were combined, cut into 1cm pieces and mixed thoroughly.
- b) The sample was shaken for 4 minutes in 400ml of a 1% NaOCl solution. (This weak bleach solution breaks down the gelatinous matrix surrounding eggs and releases them from the roots).
- c) This mixture was decanted through a range of nested sieves, from bottom to top: 10-, 25-, 45-, 63-, 75-, 250- and 710 $\mu$ m-mesh. (This ensures less clogging on the bottom 10 $\mu$ m sieve. A vacuum pump was also connected to the 10 $\mu$ m sieve to apply suction and enhance passing of liquid through the sieves.)
- d) Root fragments were washed thoroughly for about 4 minutes on the top sieve (710 $\mu$ m).
- e) Eggs and larvae collected on the 10 $\mu$ m sieve were collected with running tap water into a sample bottle for counting.

#### **3.1.2. Fixation of nematodes.**

Nematodes in water in sample bottles, extracted from soil (200ml) and root (5g) samples, were killed and fixed by adding the same volume of hot 8% formaldehyde (boiling at about 90°C) to a sample bottle. As a result (because of dilution) the final concentration of the

formaldehyde was 4%. It is important to use a hot fixative because killing of nematodes by means of a cold fixative often results in distortion of tissues.

### **3.1.3. Counting of nematodes.**

Root-knot nematode eggs from 50g root samples were counted without prior fixation. Nematode population levels were determined in a counting dish under a stereo microscope and expressed either as the number of nematodes per 200ml soil, 5g roots and 50g roots.

#### **3.1.3.1. Species identification.**

For species identification, plant-parasitic nematodes were transferred to anhydrous glycerin and mounted on slides within paraffin wax rings.

#### **3.1.3.2. Transfer of nematodes to anhydrous glycerin.**

In order to study the finer morphological structures of nematodes under a light microscope (important for identification of nematodes at species level), the nematodes were mounted in glycerin, a medium having a refraction index nearly the same as that of glass. If living or fixed nematodes are suddenly placed in pure glycerin they will plasmolyse. The transfer of nematodes to glycerin therefore must be done gradually. The following method was described by De Grisse (1965).

#### **Procedure:**

- a) Nematodes were transferred from the 4% formaldehyde solution to an eyeglass containing a Glycerin-I solution (99ml 4%formaldehyde + 1ml glycerin).
- b) The eyeglass with the nematodes were placed in a dessicator containing 96% ethanol to saturate the atmosphere (inside the dessicator) in an oven at 30°C for 12 hours. In the saturated atmosphere the formaldehyde solution was replaced by alcohol within 12 hours.
- c) The eyeglass with nematodes were removed from the dessicator after 12 hours, placed in an

oven at 30°C and every two hours two drops of a Glycerin-II solution (95ml 96% ethanol + 5ml glycerin) was added. The ethanol slowly evaporated so that the nematodes remained in pure glycerin in the end. The nematodes were then mounted on slides in anhydrous glycerin.

### **3.1.3.3. Mounting of nematodes on slides.**

#### **3.1.3.3.1. The paraffin-ring method.**

##### **Procedure:**

A copper tube (diameter = 1.5cm) was heated, pressed in paraffin wax and touched on the surface of a glass slide. As a result a paraffin ring was formed on the slide. A small drop ( $\pm$  2mm in diameter) of glycerin was placed in the middle of the wax ring. Nematodes were individually picked up with a needle, transferred to the middle of the ring and sealed off by slipping a cover glass over the paraffin ring. The slide was carefully heated over an open flame to melt the paraffin wax and sealing in the nematodes. Finally the outside edge of the cover glass was sealed off with colourless acrylic nail hardener (Cutex).

#### **3.1.4. Rearing of *Meloidogyne* populations from different localities.**

Soil was collected from each locality and put in two plastic pots (20cm-d) in the greenhouse. Susceptible tomato plants ('Moneymaker' cv) were planted in each pot to attract root-knot nematode larvae and establish root-knot nematode populations for each locality. Infected tomato plants with visible galling were removed approximately 60 days later for identification of *Meloidogyne* species.

### **3.1.4.1. Identification of *Meloidogyne* species from different localities.**

#### **3.1.4.1.1. Staining of root knot nematode infected tomato roots.**

Lactophenol were prepared by mixing 500ml phenol, 500ml lactic acid, 500ml glycerin and 500ml distilled water (Daykin & Hussey, 1985). The stain solution were prepared by adding 5ml of a 1% stock solution of acid fucshin to 100ml of lactophenol. Tomato root systems from each pot, representing a certain locality, were cut into smaller pieces and immersed in the boiling staining solution for 3- 4 minutes and then destained in clear lactophenol for 24 hours. Red stained *Meloidogyne* females were dissected from root fragments and identified. The procedure described above was executed in a fume cabinet.

#### **3.1.4.1.2. Identification of *Meloidogyne* females from infected tomato roots.**

Galls containing mature females were selected on root fragments. Root tissue was gently forced open with a forceps and scalpel to remove adult females. The female was transversely dissected behind the region where the body was swollen and the body tissues pushed out. The female's head was transferred to a drop of glycerin/lactophenol on a glass microscope slide. The area on the cuticle containing the perineal pattern was removed and placed on a perspex cutting block in a drop of glycerin/lactophenol with the dome-like posterior end facing upwards. The perineal pattern were trimmed to a flat square and transferred next to the female's head on the microscope slide, exterior side facing upwards (Hartman & Sasser, 1985). Three to four heads and perineal patterns were transferred, next to each other on a single microscope slide which was sealed with a cover slip and colourless Cutex. The slide was properly labelled and identification was done under a light microscope. Species ratio was determined by identifying 21 female heads and perineal patterns per locality (K.P.N. Kleynhans, 1997- personal communication).

### **3.2. Screening of 38 soybean cultivars in the greenhouse for resistance to *Meloidogyne javanica* and *M. incognita* race 1, 2 and 4.**

#### **3.2.1. Planting procedure**

Plastic pots ( 4dm<sup>3</sup> , 20cm-d) were filled with methyl bromide fumigated and pasteurized soil and watered. Soil nutrients were added (Table 2) according to soil nutrient analysis (Table 1) (Venter, 1994). Soybean seeds were inoculated with *Bradyrhizobium japonicum* bacteria. Five soybean seeds per pot were planted and thinned to one per pot 14 days after seedling emergence.

#### **3.2.2. *Meloidogyne* species and races inoculum**

*Meloidogyne* species and races were maintained in the greenhouse at the ARC - Grain Crops Institute on tomatoes ('MoneyMaker' cv). *Meloidogyne javanica* and *M. incognita* race 1, 2 and 4 were inoculated in the respective trials.

#### **3.2.3. Extraction of *Meloidogyne* species eggs**

Infected tomato roots were washed free of soil , cut into 1 cm pieces and eggs were extracted by means of the NaOCl method (Riekert, 1995) as described in 2.2.4.

#### **3.2.4. Preparation of inoculum for screening trials**

In the screening with *M. javanica* and *M. incognita* race 1 and 2 , 10 000 eggs were inoculated. Five thousand eggs per plant (available inoculum) were inoculated in the screening trial with *M. incognita* race 4.

The egg-water suspension was put on a magnetic stirrer and stirred for 5 minutes to ensure an even dispersion of the eggs during inoculation. The suspension was divided into six aliquots for the six replicates of each trial with a Socorex nematode stepper 411 model. Each of these

suspensions was topped up to 190ml (5ml x 38 cultivars in each replicate) with tap water. The suspensions were stirred for 5 minutes and 38 aliquotes (for the 38 cultivars in each replicate) of 5ml each were poured into sample bottles. The suspensions were continuously stirred throughout the process.

### **3.2.5. Inoculation of soybean seedlings with *Meloidogyne* spp. eggs.**

Plants were watered prior to inoculation. Fourteen days after planting soil surrounding the soybean roots were gently removed to expose the root systems. Inoculation was done by pipeting 5ml aliquots of egg-water suspension containing  $10\ 000 \pm 500$  eggs for *M. javanica*, *M. incognita* race 1 and 2 and  $5\ 000 \pm 500$  eggs for *M. incognita* race 4 on the exposed parts of the roots. Each sample bottle was rinsed with 5ml tap water (to ensure all the eggs were inoculated) and poured onto the roots. The roots were covered with soil. Replicates were rotated every two weeks to ensure all replications were exposed to equal conditions in the greenhouse.

### **3.2.6. Counting of egg masses.**

Soybean roots were removed 56 days after inoculation (Hussey & Boerma, 1981), washed free from excessive soil and debris and weighed. Staining of egg masses to facilitate counting was done by submerging individual root systems in a 0.1 % phloxine B solution (0.1g phloxine B/100ml water) for 20 minutes. Each root system was placed in  $\pm 200$ ml water in a white plastic container (30cm x 20cm x 5cm) and cut into 2cm segments. Egg masses were counted under a commercial magnifying glass.

### **3.2.7. Egg mass rating index.**

Egg masses were rated according to the following index: 0 = 0; 1 = 1; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; 5  $\geq$  100 (Hussey. & Boerma, 1981).

### 3.2.8. Extraction and counting of eggs.

Eggs were extracted from root systems using the adapted NaOCl method (Riekert, 1995 - described in 3.1.1.4.) and counted under a stereo dissection microscope.

**Table 1. Nutrient analyses of soil from Viljoenskroon.**

NUTRIENT	AMBIC mg.kg-1
P	10.34
Ca	134
Mg	35
Na	5
K	59
Zn	2.31
Fe	33
Cu	1
Mn	1

**Table 2. Soil nutrients added per pot (4dm<sup>3</sup>).**

NUTRIENT	QUANTITY APPLIED(g)
Super phosphate (10.5%)	7.90
Potassium chloride	0.88
Dolomitic lime	4.99
Limestone ammonium nitrate (28% nitrogen)	0.11

## CHAPTER 4

### A SURVEY OF NEMATODES ON SOYBEAN IN SOUTH AFRICA.

The characterization of nematode communities is important for research, advisory purposes and disease diagnosis (Barker, 1985). Most nematode assays are based on numbers of nematodes per sample. Measurement of distribution uniformity or rate of occurrence of different nematode groups can, however, also be included as a valuable parameter. Inclusion of both population density and frequency of occurrence in relation to each other are expressed as the prominence value of a particular nematode group with regard to a specific area. Prominence value is thus an ecologically important index for nematode groups included in surveys (De Waele & Jordaan, 1988a + b; Jordaan *et al.*, 1989; Jordaan *et al.*, 1992; Norton, 1984; Van Eeden *et al.*, 1993; Venter *et al.*, 1992). High prominence values indicate predominant nematode groups while low prominence values usually indicate nematode groups of minor importance to a specific crop. The objective of this study was, therefore, to undertake a survey to identify the most important plant-parasitic nematode genera and species associated with soybean in this country and determine their prominence values.

#### 4.1. MATERIALS AND METHODS

##### 4.1.1. Soybean survey

Soil and root samples for nematode extractions were collected at flowering during March and April 1996 at 17 localities representative of the soybean producing areas in South Africa (Fig. 1). These localities were selected from a list of the National Soybean Cultivar Trials. Soil properties, total seasonal rainfall and irrigation figures are provided in Tabel 3. Crop history at the different localities is listed in Table 4.

##### 4.1.2. Sampling of nematodes

###### 4.1.2.1. Trial layout

Cultivars included in the National Soybean Cultivar Trials were planted in a randomized complete block design (Table 5; Fig. 2), consisting of three replicates of 30 cultivars. Each plot consisted of four 5m rows and all seeds were inoculated with *Bradyrhizobium japonicum* bacteria before

planting.

#### **4.1.2.2 Nematode samples**

Six soybean plants were selected randomly and their root systems with adhering soil removed with a spade during flowering from the two border rows of each replicate and combined (totalling 12 soybean root systems per cultivar per locality).

#### **4.1.2.3. Handling of samples**

Samples were labelled, kept in closed plastic bags in coolbags and transported by car to the ARC-Grain Crops Institute (GCI). The samples were stored at 8°C - 10°C for 1 week before nematode extraction (Barker & Nusbaum, 1971).

#### **4.1.2.4. Extraction of nematodes**

Different extraction methods were used for nematodes from 200ml soil samples, 5g root samples and 50g root samples as described in Chapter 3: Materials and Methods.

#### **4.1.2.5. Data analyses**

##### **4.1.2.5.1. Prominence values.**

Prominence values ( $PV = \text{population density} \times \sqrt{\text{frequency of occurrence} / 10}$ ) were calculated for plant-parasitic nematode population densities in soil and roots (De Waele & Jordaan, 1988 a + b).

#### 4.1.2.5.2. Frequency of occurrence of nematode species

- a) Frequency of occurrence of each nematode species at 510 plots (17 localities x 30 cultivars) is expressed as a percentage and was calculated using the formula:

$$\frac{(\text{number of plots on which the species occurred})}{510 \text{ plots}} \times 100$$

- b) Frequency of occurrence of each nematode species at each locality is expressed as a percentage and was calculated using the formula:

$$\frac{(\text{number of cultivars on which the species occurred at each locality})}{(30 \text{ cultivars})} \times 100$$

- c) Frequency of occurrence of each nematode species on each cultivar is expressed as a percentage and was calculated using the formula:

$$\frac{(\text{number of times the species occurred on each cultivar})}{(17 \text{ localities})} \times 100$$

#### 4.1.2.5.3. Population density of each species

- a) Population density of each nematode species at 510 plots (17 localities x 30 cultivars) was calculated as follows:

$$\frac{(\text{total number of a species present})}{(\text{number of plots on which the species occurred})}$$

b) Population density of each nematode species at each locality was calculated as follows:

$$\frac{\text{(total number of that species present)}}{\text{(number of cultivars on which the species occurred)}}$$

c) Population density of each nematode species on each soybean cultivar was calculated as follows:

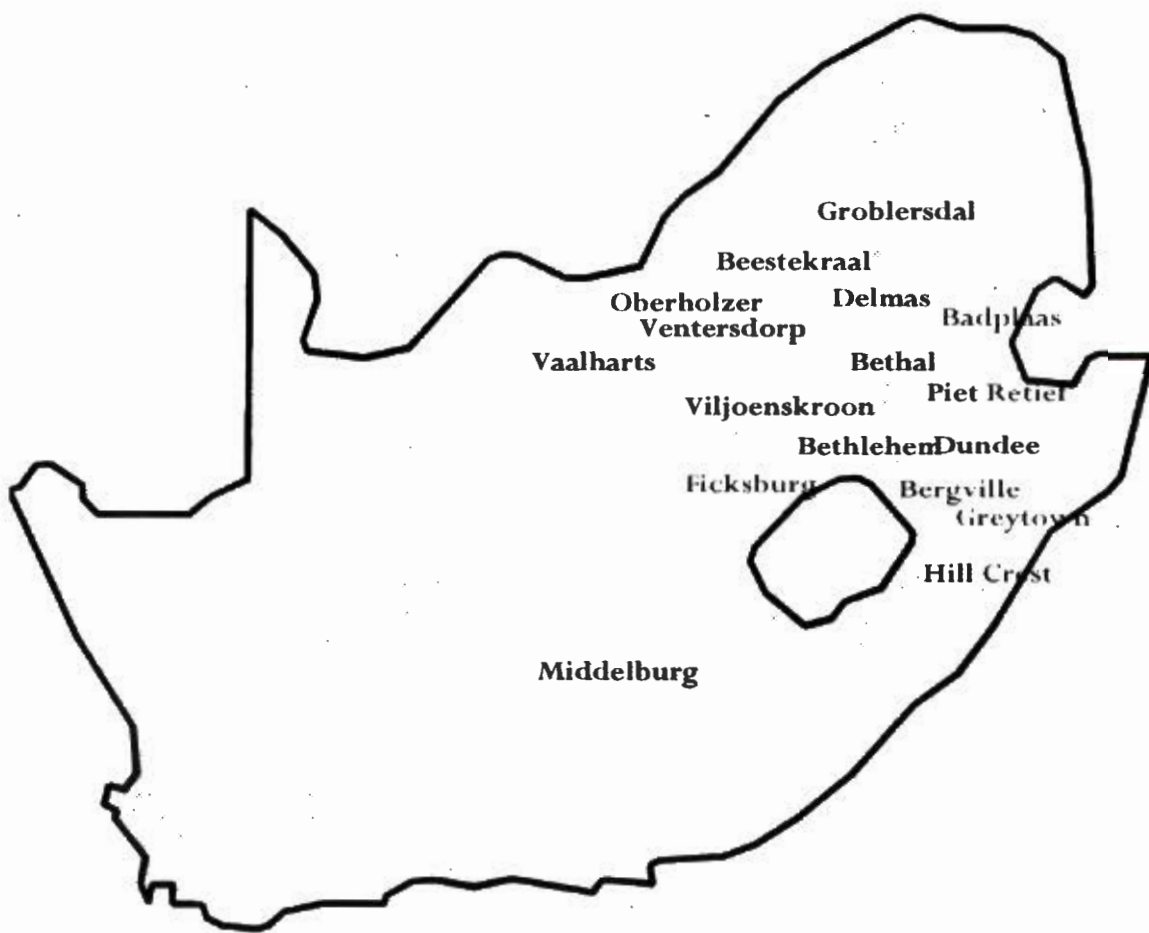
$$\frac{\text{(total number of a species present on each cultivar)}}{\text{(number of localities on which the species occurred on each cultivar)}}$$

**4.1.2.6. Correlation of clay%, sand%, silt%, organic material%, pH and rainfall (independent variables) versus nematode numbers (dependent variable).**

- a) Simple regressions were done for correlations between clay %, sand %, silt %, organic material %, pH and rainfall figures, individually, and nematode numbers.
- b) Stepwise regressions were done for inclusion of independent and dependent variables into a model for interpretation of relationships between combinations of these independent variables and nematode numbers.

The independent variables clay %, sand %, organic material % and rainfall were forced into a model to predict their combined effect on nematode numbers . The independent variable with the lowest R<sup>2</sup>-value were consequently removed from the model to determine which variable had the strongest correlation with regard to nematode numbers.

**Figure 1. Localities where soybean fields were sampled for nematodes during the 1996 soybean survey in the major soybean production areas of South Africa.**



**Table 3. Soil type, pH, rainfall and irrigation figures for the 17 localities where samples were taken during the 1995/96 season.**

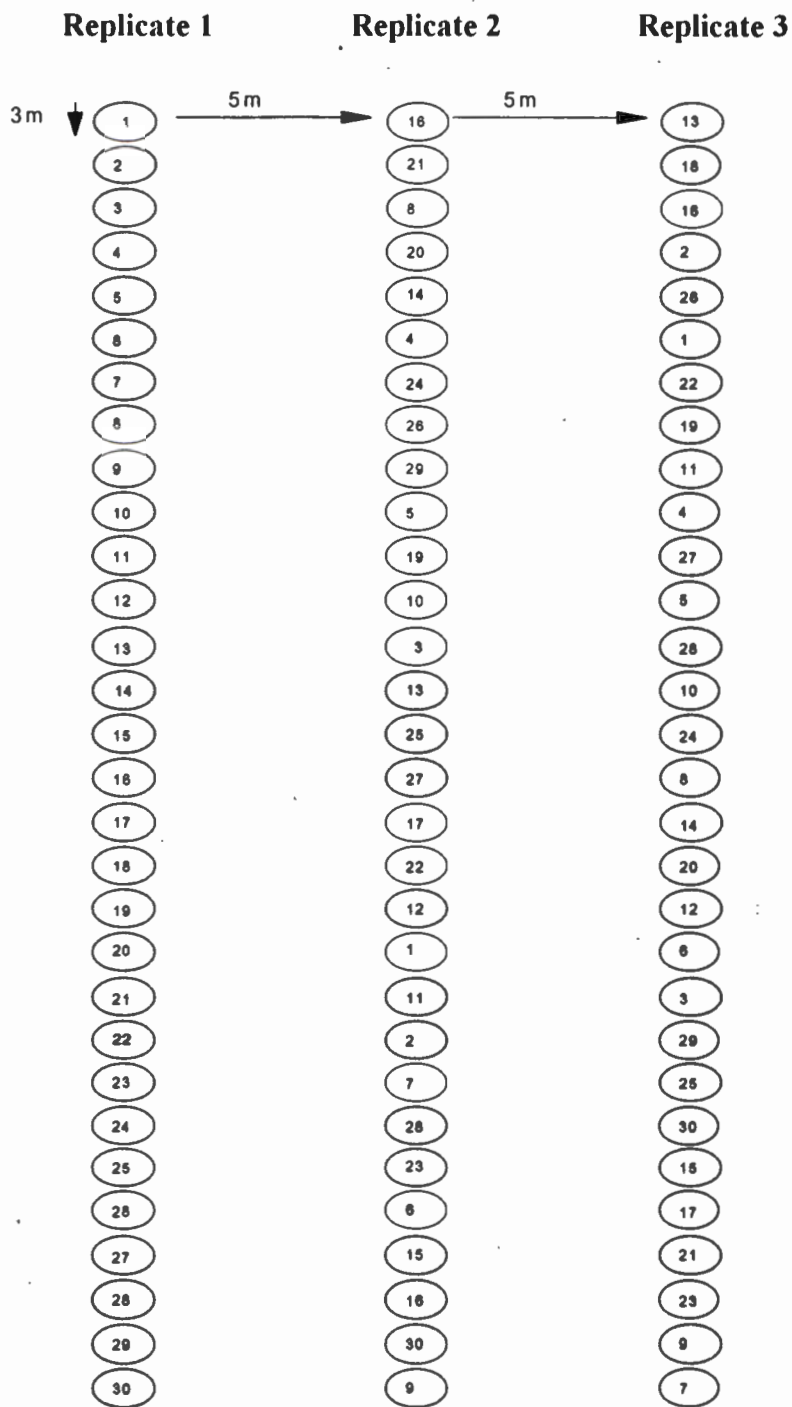
Locality	Soil type				pH (H <sub>2</sub> O)	Rainfall (mm) [Irrigation figures in parentheses]
	% Clay	%Sand	%Silt	%Organic material		
1. Badplaas	10.7	79.8	8.4	0.82	5.5	979
2. Greytown	24.5	23.2	50.2	3.2	4.2	875
3. Dundee	28.4	46.9	23.2	1.3	4.3	1053
4. Vaalharts	6.6	88.9	3.1	0.6	6.5	841(350)
5. Hill Crest	4.7	80.6	8.7	3.63	4.3	802
6. Middelburg (Cape)	17.4	52.9	27.3	0.98	8.1	876(410)
7. Groblersdal	24.6	65.0	10.1	0.82	5.4	751(118)
8. Bergville	30.9	45.9	21.2	1.4	4.5	1193
9. Ficksburg	16.6	62.0	18.7	0.5	4.3	584
10. Piet Retief	27.7	50	22.1	0.65	5.5	875
11. Delmas	18.8	71.3	9.7	0.67	6.0	949
12. Viljoenskroon	7.6	88.2	4.1	0.2	4.3	723
13. Bethlehem	12.6	76.1	11.3	0.4	5.1	568
14. Bethal	17.3	74.7	8.8	0.7	4.8	750
15. Ventersdorp	9.7	79.1	10.8	0.3	5.5	655
16. Beestekraal	18.8	57.1	24.6	1.16	7.2	507
17. Oberholzer	12.5	71.5	16.2	0.7	5.1	630

**Table 4. Crop history at 17 localities before soybean was planted during the 1995/96 season.**

Locality	1993/94	1994/95
1. Badplaas	Maize	Soybean
2. Greytown	Maize	Soybean
3. Dundee	Sunflower	Drybean
4. Vaalharts	Fallow	Cotton
5. Hill Crest	Maize	Maize
6. Middelburg (Cape)	Maize	Wheat
7. Groblersdal	Maize	Soybean
8. Bergville	Maize	Maize
9. Ficksburg	Maize	Maize
10. Piet Retief	Soybean	Maize
11. Delmas	Maize	Maize
12. Viljoenskroon	Maize	Maize
13. Bethlehem	Lupine	Maize
14. Bethal	Maize	Maize
15. Ventersdorp	Maize	Maize
16. Beestekraal	Maize	Maize
17. Oberholzer	Unknown	Soybean

**Table 5. Thirty commercial soybean cultivars included in the National Soybean Cultivar Trials at 17 localities in South Africa.**

NUMBER	CULTIVAR NAME
1	Crawford
2	Bakgat
3	Prima
4	Tamboti
5	CRN2233
6	A5308
7	A5409
8	PAN430
9	Sonop
10	Wenner
11	PAN494
12	Hutcheson
13	Highveld Top
14	Knap
15	Amstel
16	PAN717
17	Wilge
18	Dumela
19	A7119
20	PAN790
21	PAN812
22	Forrest
23	SNK60
24	PAN723
25	Kiaat
26	Mukwa
27	Ibis
28	Nyala
29	Gazelle
30	SCS1



**Figure 2. Trial layout of thirty commercial soybean cultivars included in the National Soybean Cultivar Trials during 1995/1996.**

## 4.2. RESULTS

4.2.1. Soil samples (200ml) - 17 Localities x 30 cultivars (510 plots): Prominence values (PV), frequency of occurrence (%) and mean population density (mean numbers of nematodes / 200 ml soil) of free-living, predatory and plant-parasitic nematodes in soil samples (200ml) pooled over 30 cultivars and 17 localities (510 plots) in soybean production areas of South Africa are presented in Table 6.

**Table 6. Prominence value (PV), frequency of occurrence and mean population density (mean numbers of nematodes/ 200ml soil) of free-living, predatory and plant-parasitic nematodes pooled over 30 cultivars and 17 localities (510 plots) in soybean production areas of South Africa.**

	Prominence values(PV)	Frequency of occurrence (%)	Mean population density
<i>P. zae</i>	317	80.2	354
<i>P. brachyurus</i>	39	31.2	69
<i>P. crenatus</i>	84	5.9	346
<i>P. teres</i>	5	1.4	45
<i>P. neglectus</i>	46	5.5	196
<i>P. thornei</i>	41	5.7	173
<i>Meloidogyne</i> spp.	74	50.8	103
<i>D. africanus</i>	1	1.2	11
<i>Helicotylenchus</i> spp.	222	82.9	243
<i>Scutellonema</i> spp.	205	71.2	243
<i>Rotylenchus</i> spp.	73	65.3	90
<i>Rotylenchulus</i> spp.	44	13.5	119
<i>Paratrichodorus minor</i>	28	36.5	46
<i>Mesocriconema</i> spp.	15	21.0	34
<i>Tylenchorhynchus</i> spp.	11	7.10	40
<i>Longidorus pisi</i>	2	16.3	5
<i>Xiphinema vanderlinde</i>	0.95	5.3	4
<i>X. elongatum</i>	0.3	1.6	2.3
Rhabditida	694	98.2	700
Mononchida	9	13.7	25

Nematodes recovered from the rhizosphere of soybean plants consisted of free-living Rhabditida, predatory Mononchida and 12 plant-parasitic genera.

#### 4.2.1.1. Endoparasitic nematodes

Endoparasitic lesion nematodes species identified included *P. zaeae*, *P. brachyurus*, *P. crenatus*, *P. teres*, *P. neglectus* and *P. thornei*.

Predominant endoparasitic nematodes included *P. zaeae*, *P. crenatus* and the genus *Meloidogyne* with PV of 317, 84 and 74 respectively. *P. brachyurus* had a lower PV (39) and lower mean population density of 69 than *P. crenatus* (84 and 346), *P. neglectus* (46 and 196) and *P. thornei* (41 and 173) but occurred on 31.2% of the plots, while *P. crenatus*, *P. neglectus* and *P. thornei* occurred only on 5.9%, 5.5% and 5.7% of the plots, respectively. *P. teres* was the root-lesion nematode species with the lowest PV (5), occurring only in 1.4% of the plots, with a low mean population density of 45. *D. africanus* had a low mean population density of 11 and occurred only in 1.2% of the plots, with a PV of 1. (Table 6).

#### 4.2.1.2. Semi-ectoparasitic nematodes

The genera *Helicotylenchus*, *Scutellonema*, *Rotylenchus* and *Rotylenchulus* were present.

The predominant semi-ectoparasitic nematodes were *Helicotylenchus* spp. and *Scutellonema* spp. with PV of 222 and 205, occurring at 82.9% and 71.2% of the plots, respectively, with a mean population density of 243. The genera *Rotylenchus* and *Rotylenchulus* had PV of 73 and 44, respectively, occurred in 65.3% and 13.5% of the plots, with mean population densities of 90 and 119, respectively (Table 6).

#### 4.2.1.3. Ectoparasitic nematodes

*P. minor*, *Mesocriconema* spp., *Tylenchorhynchus* spp., *X. vanderlinde*, *X. elongatum* and *L. pisi* were identified with low PV, frequency of occurrence (except *P. minor* occurring at 36.5% of the plots) as well as low mean population densities (Table 6).

4.2.2. Soil samples (200ml) - Localities (pooled over 30 cultivars): Prominence values (PV), frequency of occurrence (%) and mean population density (mean nematode numbers / 200ml soil) of free-living, predatory and plant-parasitic nematodes in soil samples (200ml) from 17 localities (pooled over 30 cultivars) in soybean production areas of South Africa are presented in Tables 7, 8 and 9.

**Table 7. Prominence values (PV) of free-living, predatory and plant-parasitic nematodes in soil samples (200ml) from 17 localities (pooled over 30 cultivars) in soybean production areas of South Africa.**

	Prominence value (PV)																
	Localities *																
	Badpl	Greyt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	Ober
<i>P. zeae</i>	535	482	864	309	102	0	362	242	166	161	33	25	63	29	951	0	554
<i>P. brachyurus</i>	0	0	0	0	0	0	0	0	0	58	0	139	0	70	93	3	18
<i>P. crenatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	346	0
<i>P. teres</i>	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. neglectus</i>	0	0	0	0	0	190	0	0	0	0	0	0	0	0	0	0	0
<i>P. thornei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	170	0
<i>Meloidogyne</i> spp. larvae	325	63	95	154	45	180	28	8	21	2	23	21	31	0	6	24	8
<i>D. africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
<i>Helicotylenchus</i> spp.	215	55	42	11	288	138	76	67	41	1065	44	16	48	990	43	46	310
<i>Scutellonema</i> spp.	647	40	118	0	36	0	264	390	157	139	25	0	20	890	137	77	73
<i>Rotylenchus</i> spp.	28	56	0	14	217	50	0	0	0	143	73	59	44	300	14	15	29
<i>Rotylenchulus</i> spp.	0	0	0	0	61	0	0	0	0	160	0	0	0	84	0	0	0
<i>Paratrichodorus minor</i>	2	2	1	0	1	1	0	1	0	6	22	49	326	33	12	40	32
<i>Mesocriconema</i> spp.	3	57	3	3	2	0	0	0.2	0	2	64	9	0	0	0	0	0
<i>Tylenchorhynchus</i> spp.	0	0	0	0	19	0	0	0	0	0	40	0	0	0	0	0	0
<i>Longidorus pisi</i>	0	2	2	0	0.2	0.4	0	0	2	1	0	9	0.4	1	2	1	0
<i>X. vanderlindeii</i>	0	0	0	0.6	0	0	0	0	0	0	0	4	0	0	0	0	0
<i>X. elongatum</i>	1.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhabditida	166	195	127	4370	1327	1067	622	691	771	118	458	576	355	153	292	231	239
Mononchida	18	0	0	6	10	6	5	17	10	0	0	8	3	0	30	0	0

\* Badpl = Badplaas, Greyt = Greytown, Dundee = Dundee, Vaalh = Vaalharts, Hcrest = Hill Crest, Midb = Middelburg, Grobl = Groblersdal, Berg = Bergville, Ficks = Ficksburg, Pretief = Piet Retief, Delm = Delmas, Viljk = Viljoenskroon, Bethl = Bethlehem, Bethal = Bethal, Vdorp = Ventersdorp, Bkr = Beestekraal and Ober = Oberholzer

**Table 8. Frequency of occurrence (%) of free-living, predatory and plant-parasitic nematodes from soil samples (200ml) from 17 localities (pooled over 30 cultivars) in soybean production areas in South Africa.**

	Frequency of occurrence (%)																
	Localities *																
	Badpl	Greyt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	Ober
<i>P. zaeae</i>	100	93	100	100	100	0	93	97	97	100	90	43	90	60	100	0	100
<i>P. brachyurus</i>	0	0	0	0	0	0	0	0	0	93	0	97	0	87	97	93	63
<i>P. crenatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. teres</i>	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. neglectus</i>	0	0	0	0	0	93	0	0	0	0	0	0	0	0	0	0	0
<i>P. thornei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97	0
<i>Meloidogyne</i> spp.	93	87	53	73	70	83	37	10	47	20	60	67	47	0	30	53	33
<i>D. africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
<i>Helicotylenchus</i> spp.	100	83	80	30	100	83	73	87	74	100	83	47	83	100	100	87	100
<i>Scutellonema</i> spp.	100	80	97	0	70	0	97	90	90	97	43	0	67	100	100	83	87
<i>Rotylenchus</i> spp.	100	83	0	30	97	77	0	0	0	90	87	87	90	100	100	87	83
<i>Rotylenchulus</i> spp.	0	0	0	0	63	0	0	0	0	87	0	0	0	80	0	0	0
<i>P. minor</i>	13	7	0	0	30	13	0	23	0	30	37	83	90	77	53	73	90
<i>Mesocriconema</i> spp.	17	83	17	10	73	0	0	7	0	47	77	27	0	0	0	0	0
<i>Tylenchorhynchus</i> spp.	0	0	0	0	37	0	0	0	0	0	83	0	0	0	0	0	0
<i>Longidorus pisi</i>	0	37	47	0	3	13	0	0	3	30	0	63	13	17	43	7	0
<i>X. vanderlinde</i>	0	0	0	23	0	0	0	0	0	0	0	68	0	0	0	0	0
<i>X. elongatum</i>	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhabditida	100	93	100	100	100	97	100	97	100	97	97	97	93	100	100	100	100
Mononchida	83	0	0	10	17	10	7	30	13	0	0	23	10	0	30	0	0

\* Badpl = Badplaas, Greyt = Greytown, Dundee = Dundee, Vaalh = Vaalharts, Hcrest = Hill Crest, Midb = Middelburg, Grobl = Groblersdal, Berg = Bergville, Ficks = Ficksburg, Pretief = Piet Retief, Delm = Delmas, Viljk = Viljoenskroon, Bethl = Bethlehem, Bethal = Bethal, Vdorp = Ventersdorp, Bkr = Beestekraal and Ober = Oberholzer

**Table 9.** Mean population density (nematodes/200ml soil) of free-living, predatory and plant-parasitic nematodes from soil samples (200ml) from 17 localities (pooled over 30 cultivars) in soybean production areas in South Africa.

	Mean population density																
	Localities *																
	Badpl	Greyt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	Ober
<i>P. zae</i>	535	499	864	309	102	0	375	246	168	161	35	37	66	37	951	0	554
<i>P. brachyurus</i>	0	0	0	0	0	0	0	0	0	60	0	141	0	75	94	3	23
<i>P. crenatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	346	0
<i>P. teres</i>	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. neglectus</i>	0	0	0	0	0	196	0	0	0	0	0	0	0	0	0	0	0
<i>P. thornei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	173	0
<i>Meloidogyne</i> spp.	336	68	130	180	54	197	46	26	31	5	30	26	45	0	11	32	13
<i>D. africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0
<i>Helicotylenchus</i> spp.	215	60	47	20	288	151	89	72	48	1065	48	23	53	990	43	49	310
<i>Scutellonema</i> spp.	647	45	120	0	43	0	269	411	165	141	38	0	24	890	137	80	78
<i>Rotylenchus</i> spp.	28	61	0	26	220	57	0	0	0	151	78	64	47	300	14	16	31
<i>Rotylenchulus</i> spp.	0	0	0	0	77	0	0	0	0	172	0	0	0	94	0	0	0
<i>P. minor</i>	5	7	0	0	2	2	0	3	0	11	36	54	133	38	16	46	34
<i>Mesocriconema</i> spp.	7	63	8	8	3	0	0	1	0	2	73	17	0	0	0	0	0
<i>Tylenchorhynchus</i> spp.	0	0	0	0	32	0	0	0	0	0	43	0	0	0	0	0	0
<i>Longidorus pisi</i>	0	3	2	0	1	1	0	0	10	2	0	11	0	2	3	3	0
<i>X. vanderlinde</i>	0	0	0	1	0	0	0	0	0	0	0	5	0	0	0	0	0
<i>X. elongatum</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhabditida	166	202	127	4370	1327	1086	622	702	771	120	466	585	367	153	292	231	239
Mononchida	19	0	0	19	24	18	19	31	26	0	0	16	10	0	55	0	0

\* Badpl = Badplaas, Greyt = Greytown, Dundee = Dundee, Vaalh = Vaalharts, Hcrest = Hill Crest, Midb = Middelburg, Grobl = Groblersdal, Berg = Bergville, Ficks = Ficksburg, Pretief = Piet Retief, Delm = Delmas, Viljk = Viljoenskroon, Bethl = Bethlehem, Bethal = Bethal, Vdorp = Ventersdorp, Bkr = Beestekraal and Ober = Oberholzer

#### 4.2.2.1. Endoparasitic nematodes

*P. zae* had PV ranging between 25 at Viljoenskroon to 951 at Ventersdorp (Table 7) and occurred at 16 localities (Table 8), with a mean population density ranging between 35 at Delmas to 951 at Ventersdorp (Table 9). *P. brachyurus* with a PV ranging between 3 at Beestekraal to 139 at Viljoenskroon (Table 7), were present at 6 localities (Table 8) with a mean population density ranging between 3 at Beestekraal to 141 at Viljoenskroon (Table 9). *P. crenatus* occurred at Beestekraal only (Table 8), with a PV of 346 (Table 7) and a mean population density of 346 (Table 9). *P. teres* was present at Vaalharts (Table 8) only with a PV of 22 (Table 7) and a mean population density of 45 (Table 9). *P. neglectus* was present at Middelburg (Cape) (Table 8) only with a PV of 190 (Table 7) and a mean population density of 196 (Table 9). *P. thornei* occurred at Beestekraal (Table 8) only with a PV of 170 (Table 7) and a mean population density of 173 (Table 9). *Meloidogyne* spp. was present at 16 localities (Table 8) with PV ranging between 2 at Piet Retief to 325 at Badplaas (Table 7) and a mean population density ranging between 5 at Piet Retief to 336 at Badplaas (Table 9). *Ditylenchus africanus* was present at Beestekraal only (Table 8) with a low PV of 5 (Table 7) and a mean population density of 11 (Table 9).

#### 4.2.2.2. Semi-ectoparasitic nematodes

*Helicotylenchus* spp. was present at all 17 localities (Table 8) with PV ranging between 11 at Vaalharts to 1065 at Piet Retief (Table 7) and a mean population density ranging between 20 at Vaalharts to 1065 at Piet Retief (Table 9). *Scutellonema* spp. had PV ranging between 20 at Bethlehem to 890 at Bethal (Table 7), were present on 14 localities (Table 8) with a mean population density ranging between 24 at Bethlehem to 890 at Bethal (Table 9). *Rotylenchus* spp. was present at 13 localities (Table 8) with PV ranging between 14 at Vaalharts and Ventersdorp to 300 at Bethal (Table 7) and a mean population density ranging between 14 at Ventersdorp to 300 at Bethal (Table 9). *Rotylenchulus* spp. was present at 3 localities (Table 8) with PV ranging between 61 at Hill Crest to 160 at Piet Retief (Table 7) and a mean population density ranging between 77 at Hill Crest to 172 at Piet Retief (Table 9).

#### 4.2.2.3. Ectoparasitic nematodes

*Mesocriconema* spp. occurred at 9 localities (Table 8) with PV ranging between 0.2 at Bergville to 64 at Delmas (Table 7) and a mean population density ranging between 1 at Bergville to 73 at Delmas (Table 9). *P. minor* had PV ranging between 1 at Dundee, Hill Crest and Middelburg - Cape to 326 at Bethlehem (Table 7), occurred at 14 localities (Table 8) with a mean population density ranging between 2 at Hill Crest & Middelburg - Cape to 133 at Bethlehem (Table 9). *L. pisi*, *X. vanderlindei*, *X. elongatum* and *Tylenchorhynchus* spp. had low PV (Table 7) and mean population densities (Table 9) throughout the survey. *L. pisi* occurred at 11 localities, *X. vanderlindei* and *Tylenchorhynchus* spp. at 2 localities (Hill Crest & Delmas) while *X. elongatum* was present only at Badplaas (Table 8).

#### 4.2.2.4. Rhabditida and Monochidae

Prominence values for Rhabditida ranged between 118 at Piet Retief to 4370 at Vaalharts (Table 7). Rhabditida were present at all localities (Table 8) with a mean population density ranging from 120 at Piet Retief to 4370 at Vaalharts (Table 9). The order Monochida had low PV throughout the survey, ranging between 3 at Bethlehem and 30 at Ventersdorp (Table 7). They were present at 10 localities (Table 8) with a mean population density ranging between 10 at Bethlehem to 55 at Ventersdorp (Table 9).

4.2.3. Soil samples (200ml) : Cultivars (pooled over 17 localities) : Prominence values (PV), frequency of occurrence (%) and mean population density of free-living, predatory and plant-parasitic nematodes in soil samples (200ml) from 30 cultivars (pooled over 17 localities) in soybean production areas of South Africa presented in Tables 10, 11 and 12.

**Table 10. Prominence values of free-living, predatory and plant-parasitic nematodes from soil samples (200ml) of 30 cultivars pooled over 17 localities in soybean production areas of South Africa.**

	Prominence values (PV)																			
	Nematodes *																			
	<i>Rhb</i>	<i>Mon</i>	<i>Pz</i>	<i>Pb</i>	<i>Pc</i>	<i>Pt</i>	<i>Pn</i>	<i>Pth</i>	<i>Hel</i>	<i>Scu</i>	<i>Rot</i>	<i>Mel</i>	<i>Mcr</i>	<i>Par</i>	<i>Lp</i>	<i>Xvd</i>	<i>Xel</i>	<i>Tyl</i>	<i>Roc</i>	<i>Dit</i>
Crawford	442	10	583	60	28	0.5	82	126	125	135	70	51	2	44	0	0.3	0	1	82	2
Bakgat	762	10	276	33	31	0	88	91	322	207	123	50	4	24	1	0	0.7	0.7	5	94
Prima	464	4	260	60	135	0	57	60	142	154	77	130	26	28	3	0	0	5	27	2
Tamboi	911	14	349	30	16	70	0	19	32	176	196	83	46	31	32	4	0	0	20	40
CRN2233	490	16	300	40	0.3	0	44	0	230	256	42	26	20	20	0.9	0	0	3	0	0
A5308	544	13	311	76	85	3	25	22	148	199	68	171	5	16	2	0	0	13	72	3
A5409	610	8	363	26	15	3	107	28	216	320	76	64	17	22	1	0	0.7	8	18	0
PAN430	648	60	385	35	32	26	19	29	150	174	60	57	10	28	7	0	0.2	19	54	0
Sonop	826	12	300	33	56	24	44	73	225	125	71	214	7	14	1	0	0	5	40	0
Wenner	714	5	340	40	16	0	0	31	184	133	40	15	6	6	0.5	0.3	0.3	6	23	5
PAN494	532	9	193	31	81	0	82	64	310	191	107	46	2	29	3	0	0	8	47	3
Hutcheson	508	0	399	42	83	0	32	38	187	183	72	57	15	27	1	0	0	4	60	0
H. Top	499	6	279	19	88	0	76	41	166	136	60	228	11	16	2	0	0.5	4	27	1
Knap	674	9	243	20	82	0	38	25	242	146	90	49	11	24	2	0	0	0	24	0
Amstel	869	2	277	54	13	0	12	19	173	157	73	16	13	23	0.7	0.3	0	5	30	0
PAN717	907	0.3	276	42	123	0	38	47	413	172	116	35	21	29	2	0.3	0	19	47	0
Wilge	800	0.5	392	38	233	0	70	35	244	161	51	317	12	60	0.7	0.3	0.9	25	49	0
Dumela	716	1	300	32	112	0	44	38	208	326	59	326	5	24	0	0	0	13	33	0
A7119	663	8	231	21	121	0	57	41	250	234	75	21	4	20	4	0	0	0.18	19	0
PAN790	726	7	304	66	53	13	101	16	171	219	46	36	24	20	2	0	0	0	16	0
PAN812	966	2	251	52	122	0	25	38	170	189	37	40	48	47	1	0	0	3	23	0
Forrest	695	3	337	26	36	0	4	37	362	344	71	32	37	43	1	0.3	0.7	0	110	0
SNK60	574	6	402	53	203	6	19	79	177	231	110	53	26	37	2	0	0	6	80	0
PAN723	685	13	235	36	150	0	25	42	268	228	60	19	26	26	2	0	0	18	9	0
Kiaat	1082	6	382	33	58	0	32	50	169	300	63	39	10	23	0	0	0.3	0	41	0
Mukwa	893	8	288	21	61	0	152	16	248	385	91	15	6	11	0.6	0	0	3	227	0
Ibis	594	0.5	407	46	207	0	12	24	271	120	70	39	26	42	0.6	0	0	18	48	0
Nyala	768	0.5	361	40	97	0	25	48	265	176	101	35	12	28	1	0	0	17	32	0
Gazelle	677	3	205	38	0.5	0	0	0.5	148	142	60	14	13	27	3	0	0	21	9	0
SCS1	592	13	268	29	21	0	6	18	338	207	69	33	3	32	0	0.5	0	32	3	0

\* *Rhb* = Rhabditida, *Mon* = Monochidae, *Pz* = *P. zaeae*, *Pb* = *P. brachyurus*, *Pc* = *P. crenatus*, *Pt* = *P. teres*, *Pn* = *P. neglectus*, *Pth* = *P. thornei*, *Hel* = *Helicotylenchus* spp., *Scu* = *Scutellonema* spp., *Rot* = *Rotylenchus* spp., *Mel* = *Meloidogyne* spp., *Mrc* = *Mesocriconema* spp., *Par* = *P. minor*, *Lp* = *Longidorus pisi*, *Xvd* = *X. vanderlinde*, *Xel* = *X. elongatum*, *Tyl* = *Tylenchorhynchus* spp., *Roc* = *Rotylenchulus* spp. and *Dit* = *Ditylenchus* spp.

Table 11. Frequency of occurrence (%) of free-living, predatory and plant-parasitic nematodes from soil samples (200ml) of 30 cultivars pooled over 17 localities in soybean production areas of South Africa.

	Frequency of occurrence (%)																			
	Nematodes*																			
	<i>Rhb</i>	<i>Mon</i>	<i>Pz</i>	<i>Pb</i>	<i>Pc</i>	<i>Pt</i>	<i>Pn</i>	<i>Pth</i>	<i>Hel</i>	<i>Scu</i>	<i>Rot</i>	<i>Mel</i>	<i>Mcr</i>	<i>Par</i>	<i>Lp</i>	<i>Xvd</i>	<i>Xel</i>	<i>Tyl</i>	<i>Roc</i>	<i>Dit</i>
Crawford	100	24	88	24	6	6	6	6	94	71	71	71	24	35	0	6	0	6	12	6
Bakgat	100	18	82	29	6	0	6	6	94	77	53	59	24	30	12	0	6	12	12	0
Prima	100	24	77	18	6	0	6	6	88	71	59	53	35	41	18	0	0	12	12	6
Tamboti	100	6	82	30	6	0	6	6	88	77	71	71	18	41	24	0	0	12	18	0
CRN2233	100	35	88	24	6	0	6	0	77	71	77	53	34	29	35	0	0	6	0	0
A5308	100	6	82	29	6	6	6	6	100	77	71	71	24	35	12	0	0	6	18	6
A5409	100	18	88	24	6	6	6	6	100	77	71	53	30	17	18	0	6	12	18	0
PAN430	100	30	88	30	6	6	6	6	94	77	71	71	24	41	24	0	6	6	12	0
Sonop	94	18	88	29	6	6	6	6	88	65	71	35	24	35	24	0	0	12	18	0
Wenner	88	12	71	24	6	0	0	6	65	59	53	35	6	6	12	6	6	6	18	6
PAN494	94	12	82	35	6	0	6	6	77	59	65	41	18	41	12	0	0	12	18	6
Hutcheson	100	0	82	35	6	0	6	6	94	71	71	59	24	47	29	0	0	12	18	0
H.Top	100	6	82	35	6	0	6	6	88	71	71	47	29	47	29	0	6	6	18	6
Knap	100	18	77	29	6	0	6	6	82	77	71	47	24	35	29	0	0	0	18	0
Amstel	94	18	65	29	6	0	6	6	71	59	64	53	18	29	18	6	0	6	18	0
PAN717	100	6	77	35	6	0	6	6	82	82	59	53	35	29	6	6	0	6	18	0
Wilge	100	6	82	35	6	0	6	6	88	71	65	53	18	47	18	6	6	6	18	0
Dumela	94	12	77	29	6	0	6	6	88	71	65	29	24	35	0	0	0	6	12	0
A7119	94	24	71	35	6	0	6	6	65	77	65	65	12	24	18	0	0	12	6	0
PAN790	100	12	71	35	6	6	6	6	82	59	53	59	24	41	12	0	0	0	12	0
PAN812	100	18	82	35	6	0	6	6	82	82	71	53	18	29	24	0	0	6	18	0
Forrest	100	12	82	35	6	0	6	6	77	65	65	47	18	29	18	6	6	0	6	0
SNK60	100	6	82	35	6	6	6	6	82	67	71	41	29	41	12	0	0	6	12	0
PAN723	100	24	82	35	6	0	6	6	77	65	71	47	24	41	18	0	0	12	18	0
Kiaat	100	6	77	35	6	0	6	6	77	71	65	41	24	47	0	0	6	0	18	0
Mukwa	94	12	77	35	6	0	6	6	88	77	59	41	6	29	18	0	0	6	6	0
Ibis	100	12	71	35	6	0	6	6	71	59	65	29	24	53	29	0	0	6	18	0
Nyala	94	6	77	35	6	0	6	6	77	77	65	65	24	53	12	0	0	6	6	0
Gazelle	100	12	88	29	6	0	0	6	82	71	59	41	12	42	12	0	0	12	12	0
SCS1	100	6	88	29	6	0	6	6	71	82	59	41	12	41	0	6	0	6	6	0

\* *Rhb* = Rhabditida, *Mon* = Monochidae, *Pz* = *P. zaeae*, *Pb* = *P. brachyurus*, *Pc* = *P. crenatus*, *Pt* = *P. teres*, *Pn* = *P. neglectus*, *Pth* = *P. thornei*, *Hel* = *Helicotylenchus* spp., *Scu* = *Scutellonema* spp., *Rot* = *Rotylenchus* spp., *Mel* = *Meloidogyne* spp., *Mcr* = *Mesocriconema* spp., *Par* = *P. minor*, *Lp* = *Longidorus pisi*, *Xvd* = *X. vanderlinde*, *Xel* = *X. elongatum*, *Tyl* = *Tylenchorhynchus* spp., *Roc* = *Rotylenchulus* spp. and *Dit* = *Ditylenchus* spp.

Table 12. Mean population density (mean numbers of nematodes 200ml / soil) of free-living, predatory and plant-parasitic nematodes from soil samples (200ml) of 30 cultivars pooled over 17 localities in production areas of South Africa.

	Mean population density																			
	Nematodes *																			
	<i>Rhb</i>	<i>Mon</i>	<i>Pz</i>	<i>Pb</i>	<i>Pc</i>	<i>Pt</i>	<i>Pn</i>	<i>Pth</i>	<i>Hel</i>	<i>Scu</i>	<i>Rot</i>	<i>Mel</i>	<i>Mcr</i>	<i>Par</i>	<i>Lp</i>	<i>Xvd</i>	<i>Xel</i>	<i>Tyl</i>	<i>Roc</i>	<i>Dit</i>
Crawford	442	21	621	124	115	2	338	520	129	160	83	61	4	74	0	1	0	4	241	7
Bakgat	762	24	304	60	126	0	364	377	332	237	168	66	9	44	4	0	3	16	273	0
Prima	465	8	298	143	555	0	234	247	151	183	100	179	45	43	6	0	0	16	79	9
Tamboi	911	59	385	55	700	0	79	130	187	224	99	55	75	50	7	0	0	59	95	0
CRN2233	490	26	319	81	1	0	182	0	263	305	48	36	42	36	2	0	0	12	0	0
A5308	544	52	342	141	350	13	104	92	148	227	81	204	11	28	6	0	0	52	171	12
A5409	610	19	386	53	63	12	442	117	216	366	90	87	31	52	3	0	3	22	43	0
PAN430	648	111	410	65	132	106	78	120	154	199	71	68	20	44	14	0	1	78	156	0
Sonop	852	28	319	60	230	101	182	299	239	155	95	361	15	24	3	0	0	15	95	0
Wenner	760	16	405	82	66	0	0	131	229	174	55	25	26	23	2	1	1	25	55	20
PAN494	549	27	213	52	335	0	338	263	355	249	133	71	5	45	10	0	0	25	111	13
Hutcheson	508	0	440	70	341	0	130	156	193	218	86	74	32	39	2	0	0	13	143	0
H.Top	499	26	308	32	363	0	312	170	177	162	72	332	19	24	4	0	2	16	65	6
Knap	674	21	278	38	340	0	155	104	268	167	107	71	23	40	3	0	0	0	57	0
Amstel	896	6	344	100	55	0	51	79	207	205	90	22	32	42	2	1	0	19	72	0
PAN717	907	1	315	70	502	0	156	195	456	190	152	48	43	53	10	1	0	78	112	0
Wilge	800	2	432	63	960	0	285	143	260	191	63	435	28	86	2	1	4	104	116	0
Dumela	738	4	343	59	462	0	182	156	221	388	74	602	11	41	0	0	0	52	96	0
A7119	684	17	276	36	500	0	234	169	311	268	93	27	13	42	10	0	0	52	78	0
PAN790	726	19	362	112	220	52	416	65	188	286	63	46	49	31	6	0	0	0	46	0
PAN812	966	4	276	88	501	0	103	156	187	209	44	55	113	86	2	0	0	13	54	0
Forrest	695	8	372	44	150	0	15	152	415	428	88	47	87	80	3	1	3	0	455	0
SNK60	574	26	443	89	840	26	78	325	196	265	130	82	48	58	7	0	0	26	234	0
PAN723	685	27	259	61	621	0	103	195	307	284	71	27	54	41	5	0	0	52	21	0
Kiaat	1082	25	437	55	241	0	130	208	193	357	79	61	21	34	0	0	1	0	99	0
Mukwa	921	25	329	35	255	0	628	66	264	441	118	23	26	21	1	0	0	13	936	0
Ibis	594	2	485	78	853	0	51	100	323	157	86	72	53	59	1	0	0	78	114	0
Nyala	792	2	413	68	400	0	103	196	303	202	126	44	24	39	4	0	0	72	130	0
Gazelle	677	10	219	69	2	0	0	2	163	169	78	22	40	43	10	0	0	60	26	0
SCS1	593	52	284	54	86	0	26	76	402	228	90	51	8	50	0	2	0	130	13	0

\* *Rhb* = Rhabditida, *Mon* = Monochidae, *Pz* = *P. zaeae*, *Pb* = *P. brachyurus*, *Pc* = *P. crenatus*, *Pt* = *P. teres*, *Pn* = *P. neglectus*, *Pth* = *P. thornei*, *Hel* = *Helicotylenchus* spp., *Scu* = *Scutellonema* spp., *Rot* = *Rotylenchus* spp., *Mel* = *Meloidogyne* spp., *Mcr* = *Mesocriconeema* spp., *Par* = *P. minor*, *Lp* = *Longidorus pisi*, *Xvd* = *X. vanderlinde*, *Xel* = *X. elongatum*, *Tyl* = *Tylenchorhynchus* spp., *Roc* = *Rotylenchulus* spp. and *Dit* = *Ditylenchus* spp.

#### 4.2.3.1. Endoparasitic nematodes

*Pratylenchus* spp. differed in prominence, with *P. zae* being least prominent in the rhizosphere of PAN494, *P. brachyurus* in the rhizosphere of Highveld Top, *P. crenatus* in the rhizosphere of CRN2233, *P. teres* in the rhizosphere of Crawford, *P. neglectus* in the rhizosphere of Forrest and *P. thornei* in the rhizosphere of Gazelle (Tables 10 and 12). *P. teres*, *P. neglectus* and *P. thornei* did not occur on certain cultivars while other *Pratylenchus* spp. were present on all 30 cultivars (Table 11).

The genus *Meloidoyne* were least prominent in the rhizosphere of Gazelle (Table 10 and 12) and occurred on all 30 cultivars (Table 11). *D. africanus* had low PV and low mean population densities and were present only in the rhizosphere of Crawford, Bakgat, Prima, Tamboti A5308, Wenner, PAN494 and Highveld Top (Tables 10, 11 and 12).

#### 4.2.3.2. Semi-ectoparasitic nematodes

The genera *Helicotylenchus*, *Scutellonema*, *Rotylenchus* and *Rotylenchulus* were least prominent in the rhizosphere of Tamboti, Sonop, PAN812 and SCS1, respectively (Table 10 and 12) and were present in the rhizosphere of all 30 cultivars except for *Rotylenchulus* not being present on CRN2233 (Table 11).

#### 4.2.3.3. Ectoparasitic nematodes

*P. minor* and *Mesocriconema* spp. were least prominent in the rhizosphere of Wenner and Crawford, respectively (Table 10 and 12), and occurred on all 30 cultivars (Table 11). *L. pisi*, *X. vanderlinde*, *X. elongatum* and *Tylenchorhynchus* spp. had low prominence (Table 10 and 12) and did not occur on all 30 cultivars (Table 11).

#### 4.2.3.4. Rhabditida and Monochida

Orders Rhabditida and Monochida were most prominent in the rhizosphere of Kiaat and PAN430,

respectively, and least prominent on Crawford and PAN717, respectively (Table 10 and 12).

4.2.4. Root samples (5g) - 17 localities x 30 cultivars (510 plots): Prominence value (PV,) frequency of occurrence (%) and mean population density (mean numbers of nematodes / 5g roots) of plant-parasitic nematodes from root samples (5g) pooled over 30 cultivars and 17 localities (510 plots) in soybean production areas in South Africa presented in Table 13.

**Table 13. Prominence values (PV), frequency of occurrence (%) and mean population density (mean numbers of nematodes / 5g roots) of plant-parasitic nematodes from root samples (5g) pooled over 30 cultivars and 17 localities (510 plots) in soybean production areas of South Africa.**

	Prominence value(PV)	Frequency of occurrence (%)	Mean population density (5g)
<i>P.zaeae</i>	112	87	120
<i>P.brachyurus</i>	64	33	111
<i>P.crenatus</i>	2	6	9
<i>P.teres</i>	4	6	18
<i>P.neglectus</i>	7	6	28
<i>P.thornei</i>	5	6	22
<i>Meloidogyne</i> spp. larvae	17	49	25
<i>D. africanus</i>	0.8	4	4
<i>Scutellonema</i> spp.	14	61	18
<i>Helicotylenchus</i> spp.	2	51	3
<i>Rotylenchus</i> spp.	2	30	4
<i>Rotylenchulus</i> spp.	0.6	7	2

#### 4.2.4.1. Endoparasitic nematodes

Endoparasitic nematodes had low PV and mean population densities in root samples (Table 13). Six *Pratylenchus* spp., namely *P. zaeae*, *P. brachyurus*, *P. crenatus*, *P. teres*, *P. neglectus*, and *P. thornei* (Table 13) were identified from soybean root samples, as well as *Meloidogyne* spp. and *D. africanus*.

*P. zaeae* and *P. brachyurus* were the predominant endoparasites with PV of 112 and 64, occurred at 87% and 33% of the plots with mean population densities ranging from 120 and 111, respectively. *P. crenatus*, *P. teres*, *P. neglectus* and *P. thornei* had low PV, occurred less frequently with low mean population densities. *Meloidogyne* spp. had a PV of 17, occurred at 49% of the plots with a mean population density of 25. *D. africanus* was present at 4% of the plots, had PV of 0.8 with a mean population density of 4 (Table 13).

#### 4.2.4.2. Semi-ectoparasitic nematodes

The genera *Helicotylenchus*, *Rotylenchus*, *Scutellonema* and *Rotylenchulus* were present. *Scutellonema* spp. were the predominant ectoparasites with a PV of 14, occurring at 61% of the plots with a mean population density of 18 (Table 13).

The genera *Helicotylenchus*, *Rotylenchus* and *Rotylenchulus* had low PV and low mean population densities. The genera *Helicotylenchus* and *Rotylenchus* occurred at 51% and 30% of the plots while *Rotylenchulus* spp. occurred at only 7% of the plots (Table 13).

4.2.5. Root samples (5g) - Localities (pooled over 30 cultivars): Prominence value (PV) frequency of occurrence (%) and mean population density (mean numbers nematodes / 5g roots) of plant-parasitic nematodes in root samples (5g) of 30 cultivars at 17 localities (pooled over 30 cultivars) in soybean production areas in South Africa presented in Tables 14, 15 and 16 respectively.

**Table 14. Prominence value (PV) of plant-parasitic nematodes from root samples (5g) of at 17 localities (pooled over 30 cultivars) in soybean production areas in South Africa.**

	Prominence values (PV)																
	Localities *																Ober
	Badpl	Greyt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	
<i>P. zaeae</i>	431	249	272	6	8	0	14	77	83	157	33	98	65	32	229	0	29
<i>P. brachyurus</i>	0	0	0	0	0	0	0	0	0	64	0	454	0	72	31	2	7
<i>P. crenatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
<i>P. teres</i>	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. neglectus</i>	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0
<i>P. thornei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0
<i>Meloidogyne</i> spp.	122	7	17	29	12	15	1	1	4	1	1	1	1	0	0	1	1
<i>D. africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
<i>Scutellonema</i> spp.	137	2	2	0	2	0	3	1	1	12	2	4	12	3	0	3	8
<i>Helicotylenchus</i> spp.	10	1	1	1	2	1	1	1	1	3	1	2	3	3	1	1	0.8
<i>Rotylenchus</i> spp.	4	1	0	1	1	0.5	0	0	0.4	2	1	5	4	4	0	2	2
<i>Rotylenchulus</i> spp.	0	0	0	0	1	0	0	0	0	2	0	0	0	2	1	0	0

\* Badpl = Badplaas, Greyt = Greytown, Dundee = Dundee, Vaalh = Vaalharts, Hcrest = Hill Crest, Midb = Middelburg, Grobl = Groblersdal, Berg = Bergville, Ficks = Ficksburg, Pretief = Piet Retief, Delm = Delmas, Viljk = Viljoenskroon, Bethl = Bethlehem, Bethal = Bethal, Vdorp = Ventersdorp, Bkr = Beestekraal and Ober = Oberholzer

**Table 15. Frequency of occurrence (%) of plant-parasitic nematodes from root samples (5g) at 17 localities (pooled over 30 cultivars) in soybean production areas in South Africa.**

	Frequency of occurrence (%)																
	Localities*																
	Badpl	Grevt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	Ober
<i>P. zaeae</i>	100	100	100	83	100	0	100	100	100	100	100	100	100	100	100	0	100
<i>P. brachyurus</i>	0	0	0	0	0	0	0	0	0	100	0	100	0	100	97	70	100
<i>P. crenatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
<i>P. teres</i>	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. neglectus</i>	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
<i>P. thornei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
<i>Meloidogyne</i> spp.	93	97	97	97	100	90	40	27	50	20	17	23	17	0	0	43	20
<i>D. africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	0
<i>Scutellonema</i> spp.	100	53	53	0	87	0	93	40	23	100	77	83	90	83	0	97	60
<i>Helicotylenchus</i> spp.	100	53	30	17	73	40	23	60	23	90	37	47	80	90	43	37	23
<i>Rotylenchus</i> spp.	100	37	0	17	13	7	0	0	10	43	20	93	53	73	0	27	20
<i>Rotylenchulus</i> spp.	0	0	0	0	23	0	0	0	0	57	0	0	0	40	3	0	0

**Table 16. Mean population density of plant-parasitic nematodes from root samples (5g) at 17 localities (pooled over 30 cultivars) in soybean production areas in South Africa.**

	Mean population density (nematodes/5g roots)																
	Localities*																
	Badpl	Grevt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	Ober
<i>P. zaeae</i>	431	249	272	6	8	0	14	77	83	157	33	98	65	32	229	0	29
<i>P. brachyurus</i>	0	0	0	0	0	0	0	0	0	64	0	454	0	72	32	2	7
<i>P. crenatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
<i>P. teres</i>	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. neglectus</i>	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0
<i>P. thornei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0
<i>Meloidogyne</i> spp.	126	7	18	29	12	15	2	2	5	2	3	2	2	0	0	2	3
<i>D. africanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
<i>Scutellonema</i> spp.	137	3	2	0	2	0	3	1	1	12	2	4	12	4	0	3	11
<i>Helicotylenchus</i> spp.	10	2	1	1	2	2	2	2	1	3	2	3	3	3	2	2	2
<i>Rotylenchus</i> spp.	4	2	0	3	3	2	0	0	1	3	2	5	6	4	0	3	4
<i>Rotylenchulus</i> spp.	0	0	0	0	2	0	0	0	0	2	0	0	0	2	5	0	0

\* Badpl = Badplaas, Grevt = Greytown, Dundee = Dundee, Vaalh = Vaalharts, Hcrest = Hill Crest, Midb = Middelburg, Grobl = Groblersdal, Berg = Bergville, Ficks = Ficksburg, Pretief = Piet Retief, Delm = Delmas, Viljk = Viljoenskroon, Bethl = Bethlehem, Bethal = Bethal, Vdorp = Ventersdorp, Bkr = Beestekraal and Ober = Oberholzer

#### 4.2.5.1. Endoparasitic nematodes

*P. zae* had a PV ranging between 6 at Vaalharts to 431 at Badplaas (Table 14), occurring at 15 localities (Table 15) with a mean population density ranging between 6 at Vaalharts to 431 at Badplaas (Table 16). *P. brachyurus* occurred at 6 localities (Table 15) with PV and mean population densities ranging between 2 at Beestekraal to 454 at Viljoenskroon (Tables 14 and 16). *P. crenatus*, *P. teres*, *P. neglectus* and *P. thornei* had low PV (Table 14), each occurring at one locality only (Table 15), at low mean population densities (Table 16).

*Pratylenchus* spp. occurring in mixed populations included *P. zae* and *P. teres* (Vaalharts), *P. zae* and *P. brachyurus* (Piet Retief, Viljoenskroon, Bethal, Ventersdorp and Oberholzer) and *P. brachyurus*, *P. crenatus* and *P. thornei* (Beestekraal) (Table 14). According to PV (Table 14) and mean population densities (Table 16) *P. zae* dominated *P. brachyurus* at Piet Retief, Ventersdorp and Oberholzer. *P. teres*, on the other hand, dominated *P. zae* at Vaalharts, while *P. brachyurus* dominated *P. zae* at Viljoenskroon and Bethal (Tables 14 and 16). *P. thornei* was the predominant lesion nematode species at Beestekraal, followed by *P. crenatus* and *P. brachyurus* (Tables 14 and 16).

The genus *Meloidogyne* occurred at 15 localities (Table 15) with PV ranging between 1 at Groblersdal, Bergville, Piet Retief, Delmas, Viljoenskroon, Bethlehem, Beestekraal and Oberholzer to 122 at Badplaas (Table 14) and also had low mean population densities (Table 16).

#### 4.2.5.2. Semi-ectoparasitic nematodes

The genus *Scutellonema* had a PV ranging between 1 at Bergville and Ficksburg to 137 at Badplaas, (Table 14) occurred at 14 localities (Table 15) with a mean population density ranging between 1 at Bergville and Ficksburg to 137 at Badplaas (Table 16).

The genera *Helicotylenchus*, *Rotylenchus* and *Rotylenchulus* had low PV (Table 14), occurring at 17, 13 and four localities, respectively (Table 15) with low mean population densities (Table 16).

4.2.6. Root samples (5g) - Cultivars (pooled over 17 localities): Prominence values (PV) of plant-parasitic nematodes from root samples (5g) of 30 cultivars (pooled over 17 localities) from soybean production areas of South Africa presented in Fig 3 - 14 and frequency of occurrence (%) and mean population density (mean numbers of nematodes/5g roots) in Tables 17 and 18.

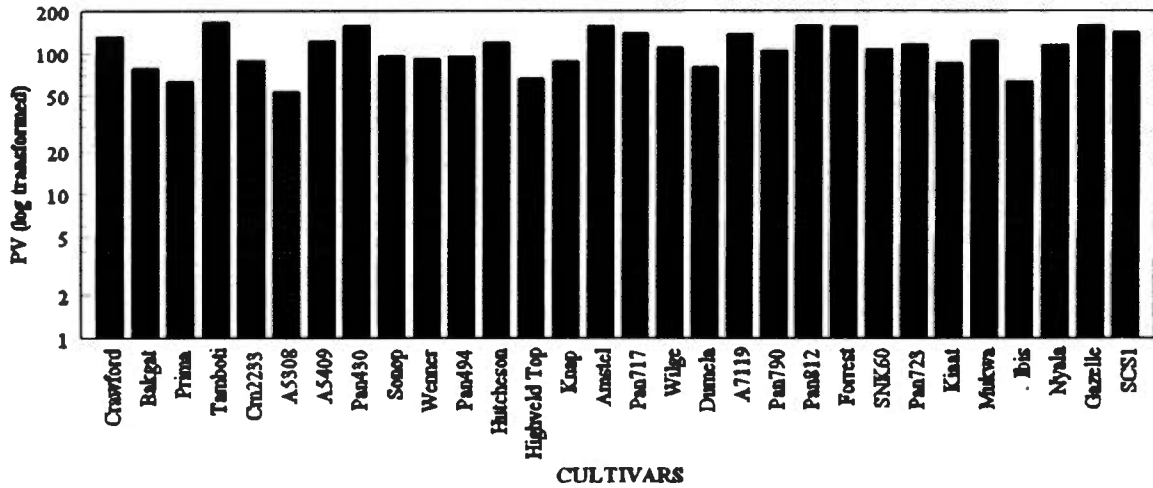


Figure 3. PV of *P. zaei* in roots of 30 commercial soybean cultivars (pooled over 17 localities).

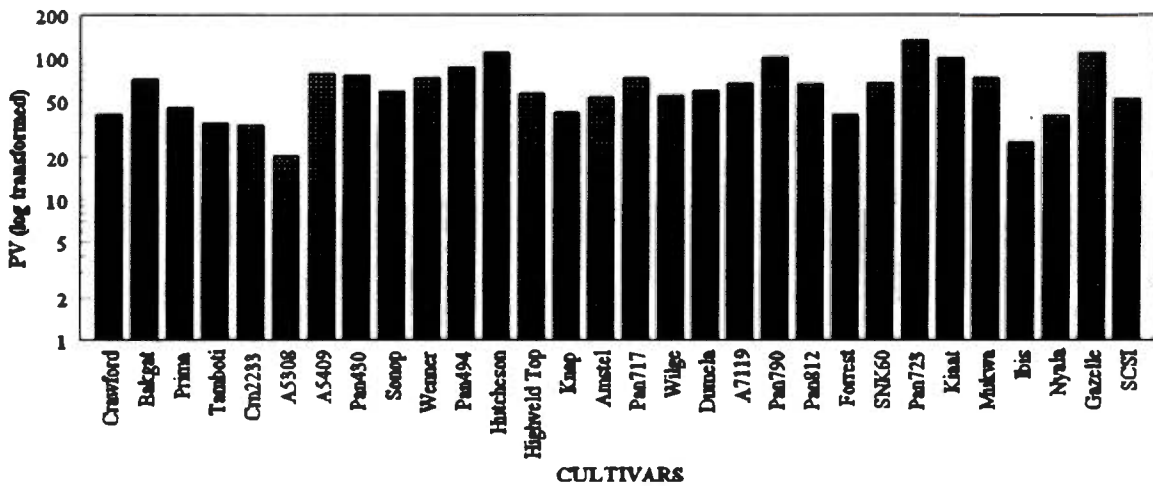


Figure 4. PV of *P. brachyurus* in roots of 30 commercial soybean cultivars (pooled over 17 localities).

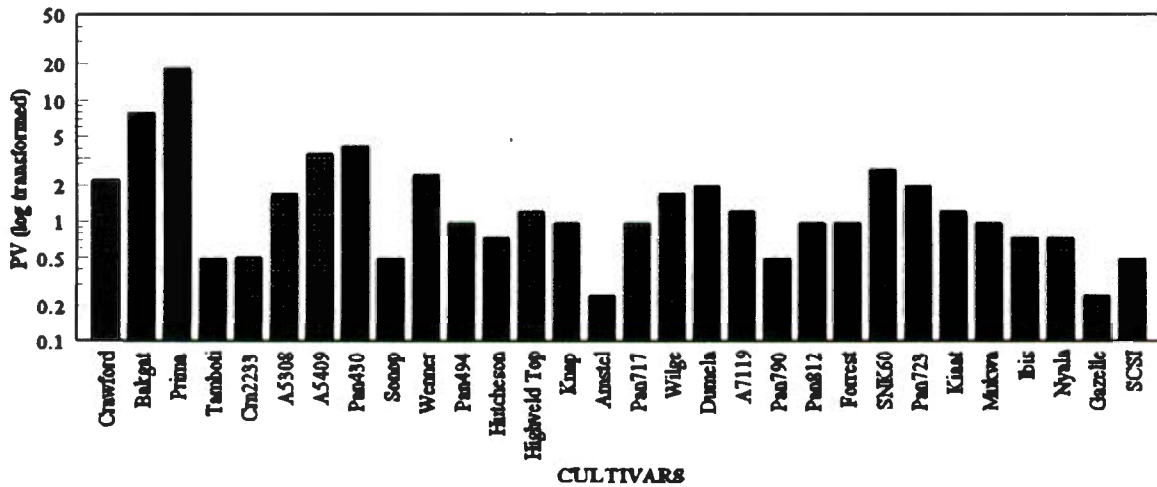


Figure 5. PV of *P. crenatus* in roots of 30 commercial soybean cultivars (pooled over 17 localities).

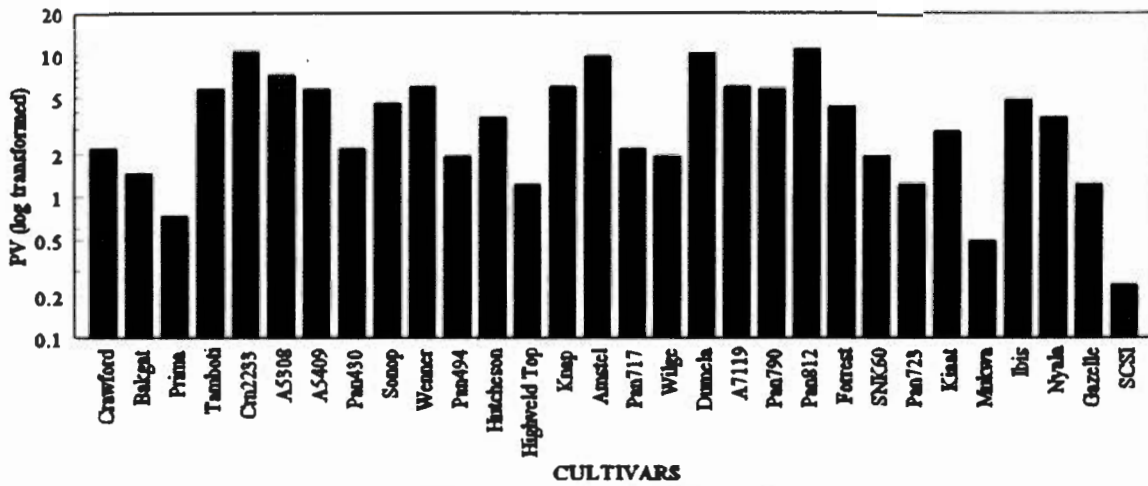


Figure 6. PV of *P. teres* in roots of 30 commercial soybean cultivars (pooled over 17 localities).

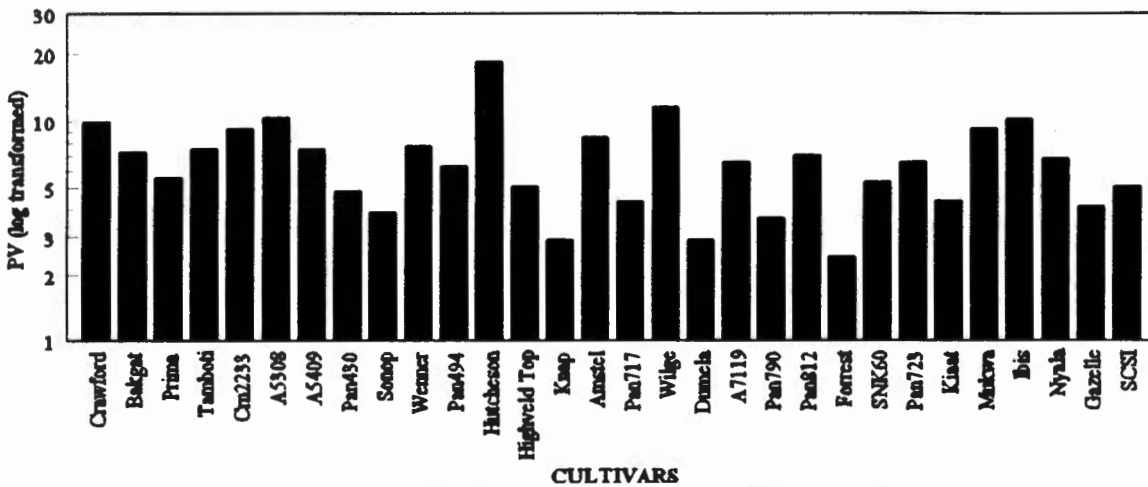


Figure 7. PV of *P. neglectus* in roots of 30 commercial soybean cultivars (pooled over 17 localities).

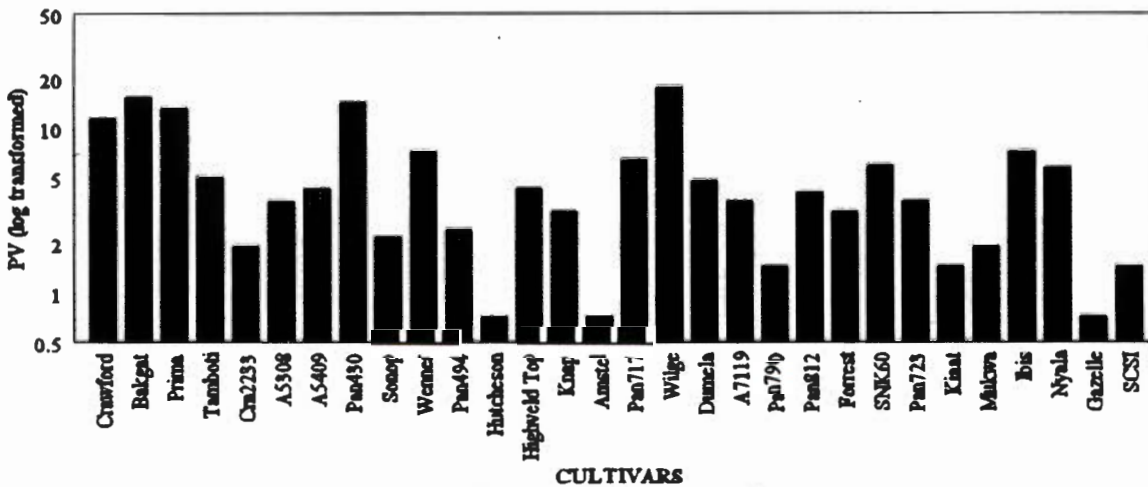


Figure 8. PV of *P. thornet* in roots of 30 commercial soybean cultivars (pooled over 17 localities).

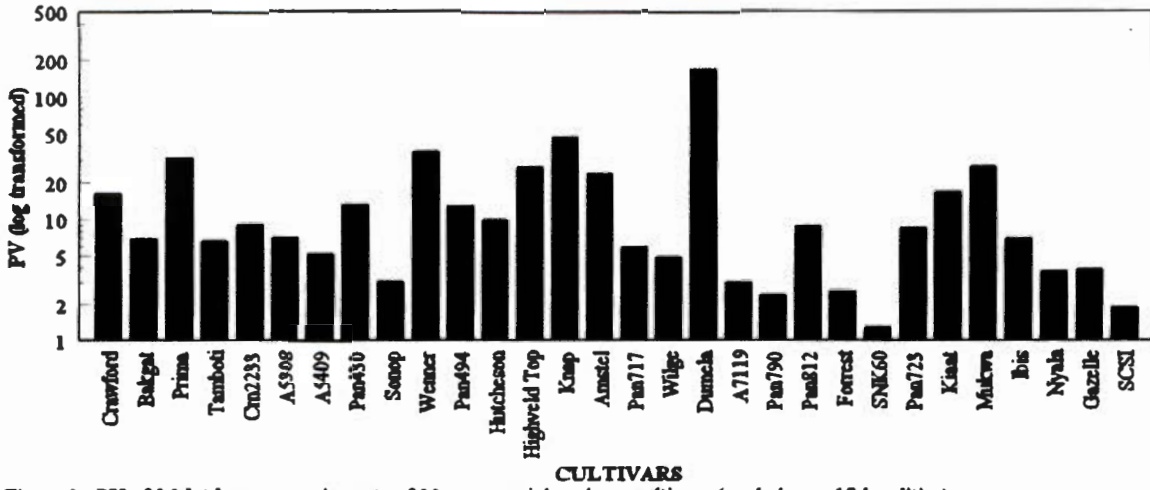


Figure 9. PV of *Meloidogyne* spp. in roots of 30 commercial soybean cultivars (pooled over 17 localities).

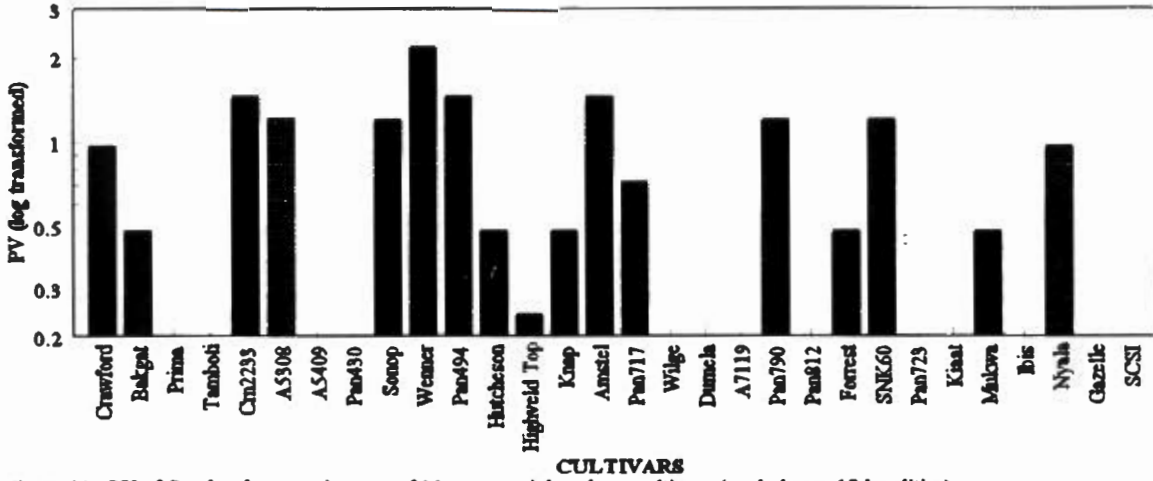


Figure 10. PV of *Ditylenchus* spp. in roots of 30 commercial soybean cultivars (pooled over 17 localities).

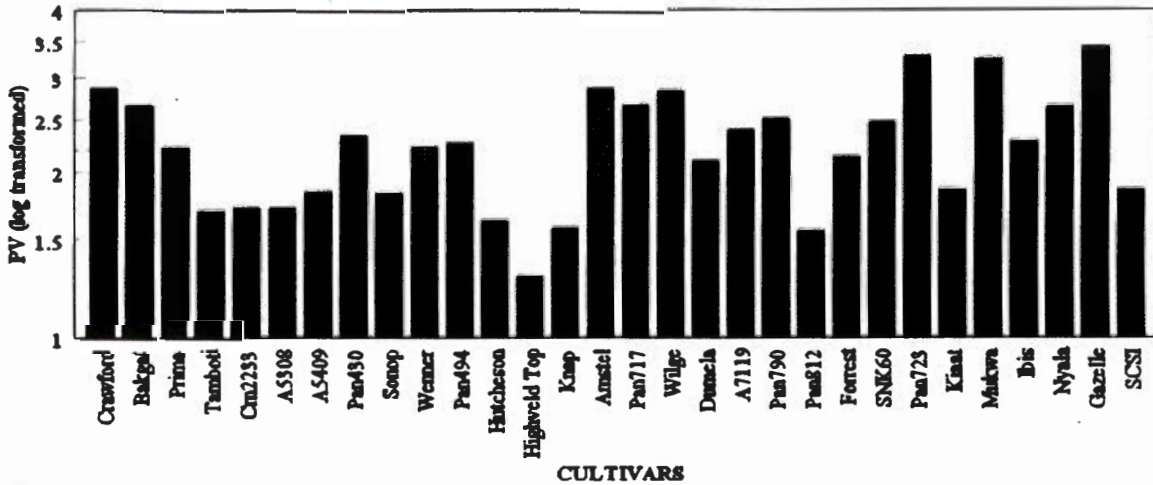


Figure 11. PV of *Helicotylenchus* spp. in roots of 30 commercial soybean cultivars (pooled over 17 localities).

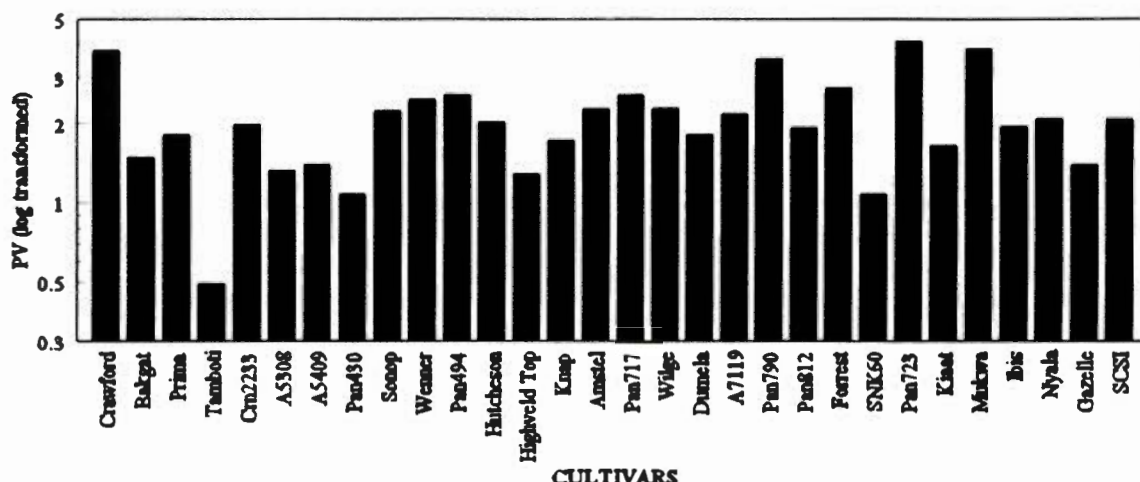


Figure 12. PV of *Rotylenchus* spp. in roots of 30 commercial soybean cultivars (pooled over 17 localities).

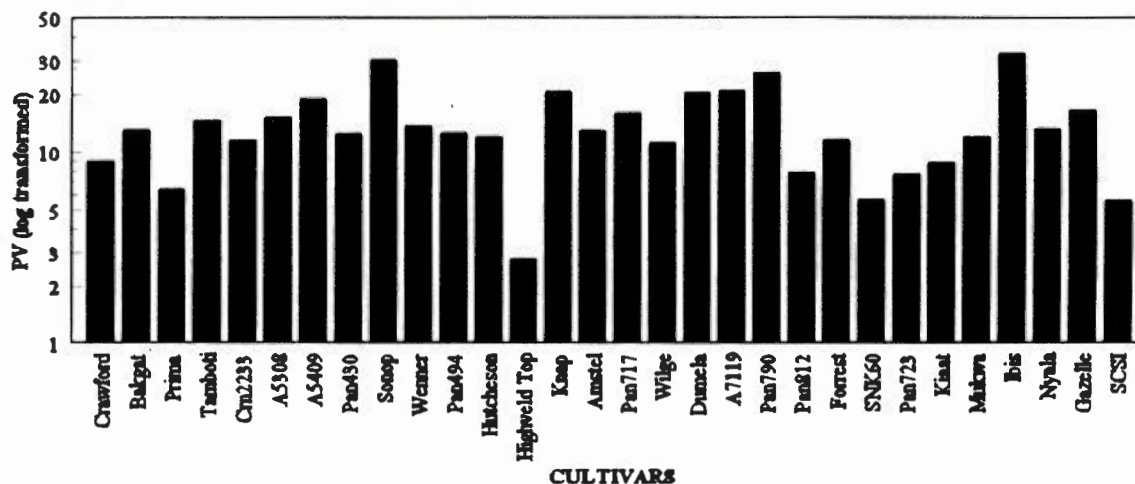


Figure 13. PV of *Scutellonema* spp. in roots of 30 commercial soybean cultivars (pooled over 17 localities).

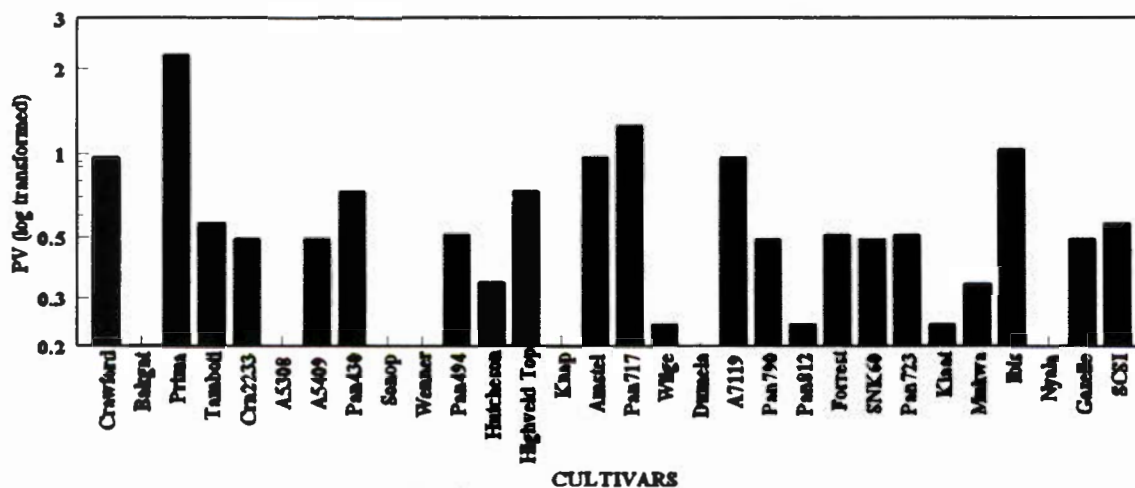


Figure 14. PV of *Replanchulus* spp. in roots of 30 commercial soybean cultivars (pooled over 17 localities).

**Table 17. Frequency of occurrence (%) of plant-parasitic nematodes in root samples (Sg) of 30 cultivars (pooled over 17 localities) from soybean production areas of South Africa.**

	Frequency of occurrence (%)											
	Genera and species *											
	<i>Pz</i>	<i>Pb</i>	<i>Pcr</i>	<i>Pt</i>	<i>Pn</i>	<i>Pth</i>	<i>Mel</i>	<i>Dit</i>	<i>Hel</i>	<i>Rot</i>	<i>Scu</i>	<i>Roc</i>
Crawford	88	35	6	6	6	6	47	6	71	29	71	6
Bakgat	82	24	6	6	6	6	47	6	47	35	59	0
Prima	88	35	6	6	6	6	53	0	59	24	71	12
Tamboti	88	35	6	6	6	6	47	0	59	6	59	18
CRN2233	88	29	6	6	6	6	41	6	47	35	59	6
A5308	88	35	6	6	6	6	53	6	47	24	41	0
A5409	88	35	6	6	6	6	59	0	59	18	65	6
PAN430	82	35	6	6	6	6	47	0	65	29	41	6
Sonop	88	29	6	6	6	6	47	6	41	18	59	0
Wenner	88	35	6	6	6	6	47	6	47	35	59	0
PAN494	88	35	6	6	6	6	41	6	65	41	65	12
Hutcheson	88	35	6	6	6	6	41	6	47	41	77	12
Highveld Top	82	35	6	6	6	6	53	6	53	35	53	6
Knap	88	29	6	6	6	6	41	6	23	12	41	0
Amstel	88	35	6	6	6	6	47	6	35	35	65	6
PAN717	88	35	6	6	6	6	59	6	53	35	71	18
Wilge	88	35	6	6	6	6	65	0	41	41	47	6
Dumela	88	35	6	6	6	6	47	0	71	18	65	6
A7119	88	29	6	6	6	6	35	0	47	35	59	6
PAN790	88	29	6	6	6	6	47	6	71	24	59	6
PAN812	88	35	6	6	6	6	53	0	41	41	71	12
Forrest	88	35	6	6	6	6	53	6	47	47	53	6
SNK60	88	35	6	6	6	6	35	6	47	29	53	12
PAN723	88	35	6	6	6	6	53	0	59	24	71	6
Kiaat	88	35	6	6	6	6	53	0	53	41	65	12
Mukwa	88	29	6	6	6	6	35	6	47	35	71	12
Ibis	88	35	6	6	6	6	59	0	41	29	71	0
Nyala	88	29	6	6	6	6	65	6	47	35	71	6
Gazelle	82	29	6	6	6	6	47	0	47	18	65	18
SCS1	82	35	6	6	6	6	47	6	53	35	65	6

\**Pz* = *P. zaeae*, *Pb* = *P. brachyurus*, *Pcr* = *P. crenatus*, *Pt* = *P. teres*, *Pn* = *P. neglectus*, *Pth* = *P. thornei*, *Mel* = *Meloidogyne* spp. *Dit* = *Ditylenchus* spp., *Hel* = *Helicotylenchus* spp., *Rot* = *Rotylenchus* spp., *Scu* = *Scutellonema* spp. and *Roc* = *Rotylenchulus* spp.

**Table 18. Mean population density of plant-parasitic nematodes from root samples (5g) of 30 cultivars (pooled over 17 localities) from soybean production areas of South Africa.**

	Mean population density											
	Genera and species *											
	<i>Pz</i>	<i>Pb</i>	<i>Pcr</i>	<i>Pt</i>	<i>Pn</i>	<i>Pth</i>	<i>Mel</i>	<i>Dit</i>	<i>Hel</i>	<i>Rot</i>	<i>Scu</i>	<i>Roc</i>
Crawford	141	68	9	9	41	48	24	4	3	7	11	4
Bakgat	87	147	32	6	30	64	10	2	4	3	17	0
Prima	67	76	75	3	23	55	44	0	3	4	8	7
Tamboti	177	58	2	24	31	21	10	0	2	2	19	1
CRN2233	95	62	2	44	38	8	14	6	3	3	15	2
A5308	57	34	7	30	43	15	10	5	3	3	24	0
A5409	130	131	15	24	31	18	7	0	2	3	23	2
PAN430	175	127	17	9	20	60	19	0	3	2	19	3
Sontop	100	107	2	19	16	9	5	5	3	5	39	0
Wenner	98	122	10	25	32	30	53	9	3	4	18	0
PAN494	101	145	4	8	26	10	20	6	3	4	16	2
Hutcheson	128	185	3	15	76	3	15	2	2	3	14	1
Highveld Top	73	95	5	5	21	18	36	1	2	2	4	3
Knap	93	76	4	25	12	13	73	2	3	5	32	0
Amstel	167	89	1	41	35	3	34	6	5	4	16	4
Pan717	149	121	4	9	18	27	8	3	4	4	19	3
Wilge	118	91	7	8	48	74	6	0	4	4	16	1
Dumela	85	99	8	43	12	20	247	0	3	4	25	4
A7119	145	122	5	25	27	15	5	0	4	4	27	2
PAN790	112	186	2	24	15	6	4	5	3	7	34	1
PAN812	168	110	4	46	29	17	12	0	2	3	9	2
Forrest	165	67	4	18	10	13	4	2	3	4	16	2
SNK60	114	113	11	8	22	25	2	5	4	2	8	2
PAN723	123	224	8	5	27	15	12	0	4	9	9	1
Kiaat	91	168	5	12	18	6	23	0	3	3	11	1
Mukwa	131	133	4	2	38	8	46	2	5	7	14	3
Ibis	67	43	3	20	42	30	9	0	4	4	39	0
Nyala	122	73	3	15	28	24	5	4	4	4	16	2
Gazelle	174	199	1	5	17	3	6	0	5	3	20	1
SCS1	156	86	2	1	21	6	3	6	3	4	7	7

\* *Pz* = *P.zeae*, *Pb* = *P.brachyurus*, *Pcr* = *P.crenatus*, *Pt* = *P.teres*, *Pn* = *P.neglectus*, *Pth* = *P.thornei*, *Mel* = *Meloidogyne* spp., *Dit* = *Ditylenchus* spp., *Hel* = *Helicotylenchus* spp., *Rot* = *Rotylenchus* spp., *Scu* = *Scutellonema* spp. and *Roc* = *Rotylenchulus* spp.

#### 4.2.6.1. Endoparasitic nematodes

PV for *P. zaeae* ranged between 53 for A5308 to 166 for Tamboti (Fig. 3) and occurred in the roots of all 30 cultivars (Table 17) with a mean population density ranging between 57 for A5308 to 177 for Tamboti (Table 18).

PV for *P. brachyurus* ranged between 20 for A5308 to 133 for PAN723 (Fig. 4) and occurred on all 30 cultivars (Table 17) with a mean population density ranging between 34 for A5308 to 224 for PAN723 (Table 18).

PV for *P. crenatus* ranged between 0.25 for Amstel to 18 for Prima (Fig. 5) and occurred on all 30 cultivars (Table 17) with a mean population density ranging between 1 for Amstel to 75 for Prima (Table 18).

PV for *P. teres* ranged between 0.25 for SCS1 to 11.1 for PAN812 (Fig. 6) and occurred on all 30 cultivars (Table 17) with a mean population density ranging between 1 for SCS1 to 46 for PAN812 (Table 18).

PV for *P. neglectus* ranged between 2.4 for Forrest to 18 for Hutcheson (Fig. 7) and occurred on all 30 cultivars (Table 17) with a mean population density ranging between 10 for Forrest to 76 for Hutcheson (Table 18).

PV for *P. thornei* ranged between 0.73 for Hutcheson, Amstel and Gazelle to 18 for Wilge (Fig. 8) and occurred on all 30 cultivars (Table 17) with a mean population density ranging between 3 for Hutcheson, Amstel and Gazelle to 74 for Wilge (Table 18).

PV for genus *Meloidogyne* ranged between 1.3 for SNK60 to 169 for Dumela (Fig. 9) and occurred in the roots of all 30 cultivars (Table 17) with a mean population density ranging between 2 for SNK60 to 247 for Dumela (Table 18).

PV for *D. africanus* ranged between 0.25 for Highveld Top to 2.2 for Wenner (Fig. 10) and did not occur on roots of Prima, Tamboti, A5409, PAN430, Wilge, Dumela, A7119, PAN812, PAN723, Kiaat, Ibis and Gazelle (Table 17) and had a low mean population density ranging between 1 for Highveld Top to 9 for Wenner (Table 18).

#### 4.2.6.2. Semi-ectoparasitic nematodes

*Scutellonema* spp. had PV ranging between 2.7 for Highveld Top to 33 for Ibis (Fig. 13) and occurred on all 30 cultivars (Table 17) with a mean population density ranging between 4 for Highveld Top to 39 for Sonop and Ibis (Table 18).

*Helicotylenchus* spp. had PV ranging between 1.3 for Highveld Top to 3.4 for Gazelle (Fig. 11) and occurred on all 30 cultivars (Table 17) with a mean population density ranging between 2 for Tamboti, A5409, Hutcheson, Highveld Top and PAN812 to 5 for Gazelle (Table 18).

*Rotylenchus* spp. had PV ranging between 0.5 for Tamboti to 4.1 for PAN723 (Fig. 12) and occurred on all 30 cultivars (Table 17) with mean population densities ranging between 2 for Tamboti, PAN430, Highveld Top and SNK60 to 9 for PAN723 (Table 18).

*Rotylenchulus* spp. had PV ranging between 0.25 for Wilge, PAN790 and PAN723 (Fig. 14) and did not occur on Bakgat, A5308, Sonop, Wenner, Knap and Ibis ( Table 17 & Fig. 13) and had a low mean population density (Table 18).

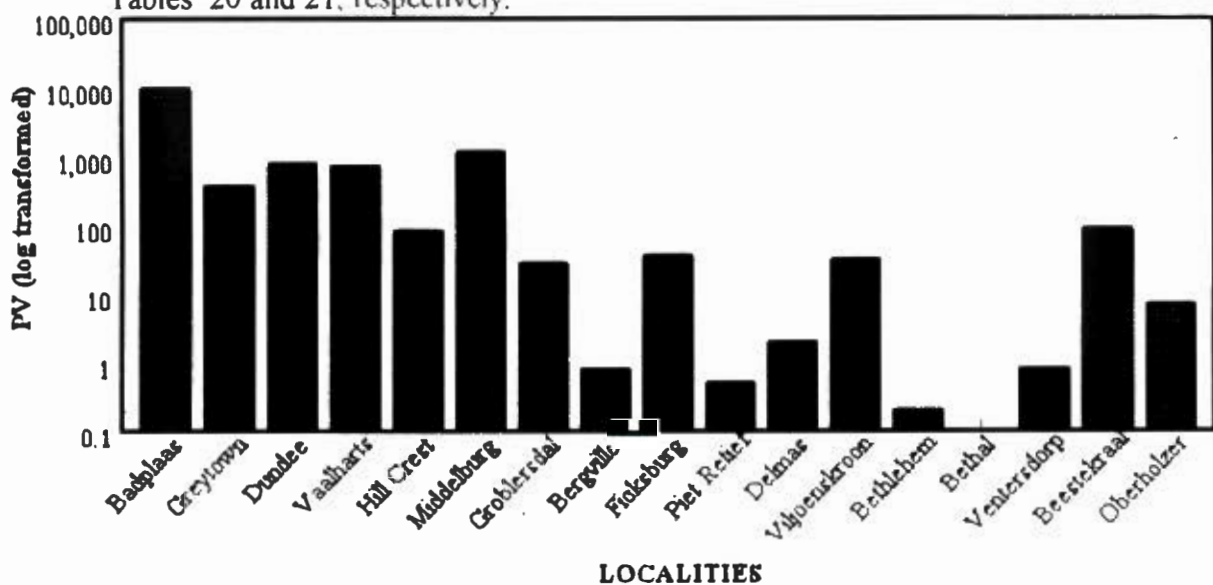
4.2.7. Root samples - (50g - modified NaOCl method for extraction of *Meloidogyne* spp. eggs): Localities x cultivars (510 plots): Prominence values (PV), frequency of occurrence (%) and mean population density (mean numbers of root-knot nematode eggs/50g roots) from root samples of 30 cultivars pooled over 17 localities (510 plots) from soybean production areas of South Africa presented in Table 19.

**Table 19.** Prominence values (PV), frequency of occurrence (%) and mean population density (mean numbers of root-knot nematode eggs/50g roots) from root samples of 30 cultivars pooled over 17 localities (510 plots) from soybean production areas of South Africa presented in Table 14.

	Prominence value (PV)	Frequency of occurrence (%)	Mean population density
<i>Meloidogyne</i> spp.	1036	52	1437

The genus *Meloidogyne* had a PV of 1036, occurred at 52% of the plots with a mean population density of 1437 (Table 19).

4.2.8. Root samples (50g) - Localities (pooled over 30 cultivars): Prominence values (PV), frequency of occurrence (%) and mean population density (mean numbers of root-knot nematode eggs/50g roots) at 17 localities (pooled over 30 cultivars) of root-knot nematodes from soybean production areas of South Africa presented in Figure 15 and Tables 20 and 21, respectively.



**Figure 15.** PV of *Meloidogyne* spp. at 17 localities (pooled over 30 cultivars) from the soybean production areas of S.A.

**Table 20. Frequency of occurrence (%) of root-knot nematodes from root samples (50g) of 30 cultivars (pooled over 17 localities) in soybean production areas in South Africa.**

	Frequency of occurrence (%)																
	Localities*																
	Badpl	Greyt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	Ober
<i>Meloidogyne</i> spp.	97	97	60	93	83	93	63	27	63	3	37	40	3	0	13	47	63

**Table 21. Mean population density of root-knot nematodes from root samples (5g) of 30 cultivars (pooled over 17 localities) in soybean production areas in South Africa.**

	Mean population density																
	Localities*																
	Badpl	Greyt	Dund	Vaalh	Hcrest	Midb	Grobl	Berg	Ficks	Pretief	Delm	Viljk	Bethl	Bethal	Vdorp	Bkr	Ober
<i>Meloidogyne</i> spp.	9969	385	1035	761	92	1216	36	2	47	3	4	51	1	0	2	133	8

\* Badpl = Badplaas, Greyt = Greytown, Dundee = Dundee, Vaalh = Vaalharts, Hcrest = Hill Crest, Midb = Middleburg, Grobl = Groblersdal, Berg = Bergville, Ficks = Ficksburg, Pretief = Piet Retief, Delm = Delmas, Viljk = Viljoenskroon, Bethl = Bethlehem, Bethal = Bethal, Vdorp = Ventersdorp, Bkr = Beestekraal and Ober = Oberholzer

PV for *Meloidogyne* species ranged between 0.18 at Bethlehem to 9801 at Badplaas (Fig. 15). Genus *Meloidogyne* occurred at 16 localities (Table 20) with a mean population density ranging between 1 at Bethal to 9969 at Badplaas (Table 21).

The following *Meloidogyne* species were identified:

- i) Badplaas - *M. incognita* (85%) and *M. javanica* (15%) - mixed population
- ii) Greytown - *M. incognita* (52%) and *M. ethiopica* (48%) - mixed population
- iii) Dundee - *M. javanica* (100%)
- iv) Vaalharts - *M. incognita* (57%) and *M. hapla* (43%) - mixed population
- v) Hill Crest - *M. incognita* (100%)
- vi) Middelburg (Cape) - *M. javanica* (100%)
- vii) Beestekraal - *M. incognita* (100%)

4.2.9. Root samples (50g) - Cultivars (pooled over 17 localities): Prominence values (PV), frequency of occurrence (%) and mean population density (root-knot nematode eggs/50g roots) from root samples of 30 cultivars pooled over 17 localities) presented in Figure 16 and Table 22 .

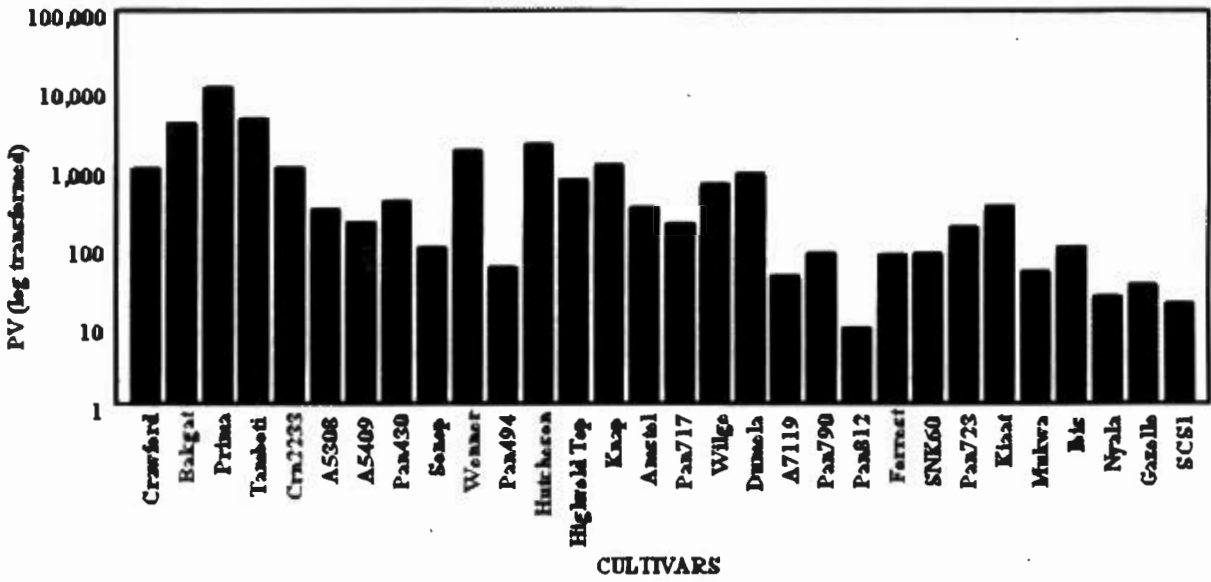


Figure 16. PV of *Meloidogyne* spp. on 30 commercial soybean cultivars (pooled over 17 localities).

**Table 22. Frequency of occurrence (%) and mean population density of root-knot nematodes (mean number of root-knot nematode eggs / 50g roots) from root samples (50g) of 30 cultivars (pooled over 17 localities) from soybean production areas of South Africa.**

Frequency of occurrence (%) and mean population density (mean numbers of root-knot nematode eggs / 50g roots)		
	Frequency of occurrence (%)	Mean population density
Crawford	53	1380
Bakgat	71	4499
Prima	65	13226
Tamboti	29	7831
CRN2233	47	1474
A5308	47	437
A5409	47	295
PAN430	41	596
Sonop	65	120
Wenner	41	2651
PAN494	53	75
Hutcheson	53	2799
Highveld Top	59	945
Knap	59	1449
Amstel	59	412
Pan717	59	263
Wilge	53	874
Dumela	59	1123
A7119	53	58
PAN790	59	107
PAN812	41	14
Forrest	47	116
SNK60	47	117
PAN723	47	261
Kiaat	53	454
Mukwa	53	66
Ibis	53	135
Nyala	58	30
Gazelle	29	60
SCSI	59	25

PV of *Meloidogyne* species ranged between 9 for PAN812 to 10639 for Prima (Fig. 16). This genus occurred on all 30 cultivars (Table 22) with a mean population density ranging between 14 for PAN812 to 13226 for Prima (Table . 22).

4.2.10. Pooled plant-parasitic nematode numbers (200ml soil + 5g roots + 50g roots): Prominence values (PV), frequency of occurrence (%) and mean population density (mean numbers of nematodes/200ml soil + 5g roots + 50g roots) pooled over 30 cultivars and 17 localities (510 plots) from soybean production areas of South Africa presented in Table 23.

**Table 23.** Prominence values (PV), frequency of occurrence (%) and mean population density (mean numbers of nematodes/50g roots) of plant-parasitic nematodes from soil (200ml) and root samples(5g + 50g) pooled over 30 cultivars and 17 localities (510 plots) from soybean production areas of South Africa

	Prominence value (PV)	Frequency of occurrence (%)	Mean population density
<i>Meloidogyne</i> spp.	955	91	999
<i>P. zae</i>	414	88	440
<i>P.brachyurus</i>	101	35	172
<i>P.crenatus</i>	86	6	354
<i>P.neglectus</i>	51	6	212
<i>P.thornei</i>	46	6	189
<i>P. teres</i>	7	6	28
<i>D. africanus</i>	1	4	7
<i>Helicotylenchus</i> spp.	216	88	230
<i>Scutellonema</i> spp.	201	84	220
<i>Rotylenchus</i> spp.	72	68	88
<i>Rotylenchulus</i> spp.	42	15	109
<i>P.minor</i>	28	37	46
<i>Mesocriconema</i> spp.	15	21	34
<i>Tylenchorhynchus</i> spp.	11	7	40
<i>L.pisi</i>	2	16	4
<i>X.vanderlinde</i>	0.9	5	4
<i>X.elongatum</i>	0.3	2	2

#### 4.2.10.1. Endoparasitic nematodes

*Meloidogyne* spp, *P. zae* and *P. brachyurus* were the predominant endoparasites associated on soybean when nematode numbers from soil and root samples were combined (Table 23).

#### 4.2.10.2. Semi-ectoparasitic nematodes

The genera *Helicotylenchus* and *Scutellonema* were the predominant endoparasites when nematode numbers from soil and root samples were combined (Table 23).

#### 4.2.10.3. Ectoparasitic nematodes

Ectoparasitic nematodes had low prominence values throughout the survey.

4.2.11. Correlations coefficients between nematodes (dependant variable) and % clay, % sand, % silt, % organic material, pH and rainfall (independant variables).

Table 24. Correlation coefficients (R) for nematodes with respect to the individual independant variables clay, sand, silt, organic material, pH and rainfall (all localities included).  $P>0.05$

Total numbers of nematodes/locality	% clay	% sand	% silt	% organic material	pH	Rainfall
Total plant-parasitic nematodes	-0.236	0.248	-0.200	-0.139	0.049	0.095
<i>Pratylenchus</i> spp.	-0.239	0.212	-0.120	-0.353	-0.059	-0.217
Hoplolaiminae	0.134	0.038	-0.112	-0.011	-0.144	-0.028
<i>Meloidogyne</i> spp.	-0.194	0.178	-0.147	0.001	0.118	0.223
<i>Mesocriconema</i> spp.	0.184	-0.316	0.310	0.289	-0.091	0.120
<i>P. minor</i>	-0.214	0.293	-0.210	-0.381	-0.023	-0.594
Rhabditida	-0.446	0.426	-0.411	-0.010	0.282	0.375
Monochida	-0.216	0.224	-0.269	0.068	-0.153	0.299

Table 25.  $R^2$  values with inclusion of different numbers of independant variables (all localities included).  $P>0.05$

	5 variables	4 variables	3 variables	2 variables	1 variable
<i>Pratylenchus</i> spp.	0.28	0.27	0.24	0.16	0.12
Hoplolaiminae	0.18	0.14	0.09	0.09	0.02
<i>Meloidogyne</i> spp.	0.13	0.13	0.125	0.12	0.05
<i>Mesocriconema</i> spp.	0.14	0.14	0.13	0.12	0.09
<i>P. minor</i>	0.44	0.44	0.43	0.38	0.35

Table 26.  $R^2$  values with inclusion of different numbers of independant variables (maize monoculture localities included only).  $P>0.05$

	5 variables	4 variables	3 variables	2 variables	1 variable
<i>Pratylenchus</i> spp.	0.61	0.49	0.35	0.30	0.18
Hoplolaiminae	0.43	0.33	0.27	0.14	0.09
<i>Meloidogyne</i> spp.	0.76	0.73	0.61	0.56	0.35
<i>Mesocriconema</i> spp.	0.53	0.53	0.51	0.30	0.13
<i>P. minor</i>	0.51	0.50	0.47	0.28	0.24

**4.2.11.1. Correlation coefficients (R) for nematodes with respect to the individual independent variables clay %, sand %, silt %, organic material %, pH and rainfall (all localities included).**

Low correlation coefficients were obtained between free-living, predatory and endo- as well as ectoparasitic nematodes (Table 24) and clay %, sand %, silt %, organic material %, pH and rainfall individually. These correlations are, however, not significant and not further discussed.

**4.2.11.2. Inclusion of nematodes (dependent variables) and clay %, sand %, silt %, organic material %, pH and rainfall (independent variables) for prediction of associations.**

**4.2.11.2.1. All localities**

Insignificant low  $R^2$ -values were obtained (Table 25).

**4.2.11.2.2. Maize monoculture localities only.**

Although  $R^2$ -values were higher when localities other than maize monoculture were not included, no significant correlations were obtained (Table 26).

#### 4.2.12. Discussion

The low numbers of plant-parasitic nematodes recovered in root samples were probably due to excessive rainfall before and during sampling.

*P. zae*, *P. brachyurus* and *Meloidogyne* spp. were the predominant endoparasitic species on soybean in South Africa during this survey. This corresponds with literature that *Meloidogyne* spp. are usually responsible for most damage on soybean world-wide, while lesion nematodes are also one of the most important nematode genera associated with soybean (Sinclair, 1982).

Although the lesion nematode species *P. zae* were predominant it does, however, not increase as rapidly as *P. brachyurus* on soybean (Endo, 1967). *P. brachyurus* is an important parasite of soybean world-wide (Koenning & Schmitt, 1986; Olowe & Corbett, 1976; Schmitt & Barker, 1988) and is known to suppress soybean plant growth (Koenning *et al.*, 1985; Schmitt, 1976; Schmitt & Barker, 1981) and yield (Acosta, 1982; Koenning *et al.*, 1985; Lindsey & Cairns, 1971). This is the first report of *P. zae*, *P. crenatus*, *P. teres*, *P. neglectus* and *P. thornei* being associated with soybean in South Africa. *P. crenatus* is associated with soybean in the USA (Ferris *et al.*, 1971) and *P. neglectus* with soybean in Japan and the USA (Ferris *et al.*, 1971; Gotoh & Ohshima, 1963; Rebois & Cairns, 1968;). The damage potential of these nematode species on soybean is, however, unknown.

*Meloidogyne* spp. are one of the most important nematode groups associated with soybean world-wide (Good, 1973). *M. hapla*, *M. incognita* and *M. javanica*, previously reported by Keetch and Buckley (1984) on soybean in South Africa, were also identified in this study. *M. ethiopica*, a relatively unknown species, was identified at Greytown during this survey, which is a first report for South Africa. Although *M. arenaria* was identified on soybean in South Africa by Keetch and Buckley (1984), this species was not found where root-knot nematode numbers were adequate for species identification during this survey. *M. incognita* was the predominant root-knot nematode species identified during the survey, followed by *M. javanica*. *M. incognita* is also the most important root-knot nematode species associated with soybean world-wide (Birchfield & Harville, 1984; Kinloch, 1982; Swanson & Van Gundy, 1984).

Species of *Ditylenchus*, previously noted on soybean in South Africa (Keetch & Buckley, 1984), are not regarded as important parasites of this crop (Good, 1973 ; Schmitt & Noel, 1984). This is, however, the first report of *D. africanus* on soybean in South Africa.

*Heterodera* spp. is one of the most important nematode species associated with soybean worldwide (Aeny & Riggs, 1993), causing yield losses to soybean in the USA (Young & Heatherly, 1988). This species was, however, not found during this survey.

*Helicotylenchus* and *Scutellonema* spp. were the predominant semi-ectoparasitic nematodes in soil and root samples and were previously reported on soybean by Keetch and Buckley (1984). These genera were also reported on soybean in the USA (Lewis *et al.*, 1993) and from Senegal (Germani *et al.*, 1984) but are generally considered to cause little or no damage to this crop (Schmitt & Noel, 1984). Although they had low PV, *Rotylenchus* and *Rotylenchulus* spp. were also identified during this survey, being a first report of *Rotylenchulus* spp. associated with soybean in South Africa. *Rotylenchulus reniformes* is, however, reported to be as an important parasite of soybean in the Philippines as *Meloidogyne* spp (Good, 1973). *R. reniformes* is also regarded as an important pest of soybean in the USA and Ghana (Good, 1973).

This is also a first report of the following ectoparasitic nematodes associated with soybean in South Africa: *P. minor*, *Mesocriconema* spp., *L. pisi*, *X. vanderlinde*, and *X. elongatum*. *Tylenchorhynchus* spp., also associated with soybean in South Africa was listed by Keetch and Buckley (1984). The genus *Paratrichodorus*, however, occurred in low numbers in 63% of soybean fields sampled in South Carolina (USA) (Lewis *et al.*, 1993) and 9% of fields sampled in the Coastal Plain of North Carolina (Schmitt & Barker, 1988). This species is also a vector of the American and Japanese isolates of the tobacco rattle virus (Kleynhans *et al.*, 1996). Although *Mesocriconema* spp. was reported from 45% of soybean fields sampled in South Carolina this genus is not associated with yield loss (Lewis *et al.*, 1993). *Xiphinema* spp. were also present on soybean in the USA ( Jenkins *et al.*, 1956; Lewis *et al.*, 1993; Golden & Rebois, 1978; Rebois & Golden, 1978), with *X. americanum* causing significant yield losses (Schmitt, 1977). None of the South African *Longidorus* spp. has been associated with virus transmission, while four of the local *Xiphinema* spp. are vectors for different viruses. *X. vanderlinde* and *X. elongatum*,

identified in this survey, are, however, not regarded as virus transmitters in South Africa (Kleynhans *et al.*, 1996). *Tylenchorhynchus* spp. were also reported from the USA (Jenkins *et al.*, 1956; Lewis *et al.*, 1993; Golden & Rebois, 1978; Rebois & Golden, 1978). *T. claytoni* suppressed yields in microplots by 21% (Ross *et al.*, 1967).

Free-living and predatory nematodes from the orders Rhabditida and Monochida were also present in this survey and is common in South African soils. Free-living nematodes are usually abundant in the presence of decomposing organic material in soil, feeding on bacteria (Heyns, 1971). No significant correlation was, however, obtained between Rhabditida and organic material % in this study. The presence of Rhabditida with regard to plant-parasitic nematode genera and species were also not correlated significantly.

The prominence values of different plant-parasitic nematodes differed on different soybean cultivars in soil and root samples. Nematode numbers could, however, not be subjected to analyses of variance due to high variation over the different localities. Sensitivity of soybean cultivars to a given species varies considerably, however (Schmitt & Noel, 1984). Different plant-parasitic nematode species and genera did not generally share least prominence on the same cultivars, except for *P. zae* and *P. brachyurus*, which were both least prominent on A5308 and *Helicotylenchus*, *Scutellonema* and *Ditylenchus africanus* on Highveld Top. Where the above mentioned species and genera occur in mixed populations, A5308 and Highveld Top will be recommended to avoid a build-up of these nematode populations. Cultivars Amstel, SCS1, Forrest and Tamboti had the lowest prominence for *P. crenatus*, *P. teres*, *P. neglectus*, and *Rotylenchus* spp., respectively, and these cultivars can be recommended when high infestation levels of these species and genus occur. *P. thornei* had the lowest PV on Hutcheson, Amstel and Gazelle and *Rotylenchulus* spp. on Wilge, PAN790 and PAN723 and will thus be the most suitable cultivars to recommend where high infestation levels of these nematodes are encountered. Several resistant and tolerant cultivars are available for *P. brachyurus* in the U.S.A. (Lindsey & Cairns, 1971; Schmitt, 1976; Schmitt & Noel, 1984; Sinclair, 1982) and for *R. reniformes* in the Philippines (Lim & Castillo, 1979) and the USA (Birchfield & Brister, 1969).

*Meloidogyne* spp. (including *M. incognita*, *M. javanica*, *M. hapla* and *M. ethiopica*) were least

prominent on PAN812 while they had the highest prominence on Prima. Results from greenhouse screenings (discussed in Chapter 5) also indicate that PAN812 is a poor host for *M. incognita* race 1 and *M. javanica*, while Prima is highly susceptible to *M. javanica* and *M. incognita* race 1, 2 and 4. Soybean resistance to root-knot nematodes is dependant on the presence of a particular root-knot nematode species as well as physiological races of these nematode species (Good, 1973; Schmitt & Noel, 1984; Sinclair, 1982).

#### 4.2.13. Conclusions

The presence of a variety of nematode species on soybean over a wide and diverse area is an indication that plant-parasitic nematodes have the potential to adversely influence soybean production in South Africa. *P. zae*, *P. brachyurus*, *M. incognita* and *M. javanica*, the predominant species associated with soybean in this study, were also described as plant-parasitic nematodes species of major importance to maize in South Africa (De Waele & Jordaan, 1988a; Riekert, 1996). The present tendency of soybean increasingly being planted in rotation with maize in traditional maize production areas of this country implies that soybean will be increasingly exposed to these economically important plant-parasitic nematodes.

Control strategies, including crop rotation, resistant cultivars and application of nematicides have thus to be attended to in order to minimize yield losses to soybean.

## CHAPTER 5

### HOST SUITABILITY OF SOYBEAN CULTIVARS TO DIFFERENT *MELOIDOGYNE* SPECIES AND RACES UNDER GREENHOUSE CONDITIONS.

Several soybean cultivars resistant to *Meloidogyne* species are available in the USA (Birchfield & Harville, 1984; Boquet *et al.*, 1975; Golden & Birchfield, 1978; Herman *et al.*, 1991; Luzzi *et al.*, 1987; Schmitt & Noel, 1984). No information is, however, available on the host suitability to root-knot nematode species of South African commercial soybean cultivars. The objective of this study was, therefore, to screen South African soybean cultivars for host suitability to different *Meloidogyne* species and races.

#### 5.1. Material and methods

##### 5.1.1. Layout of screening trials

Thirty-eight commercial soybean cultivars, selected from the National Cultivar Trials (Table 27) were screened for resistance to *M. javanica* and *M. incognita* race 1, 2 and 4 in the greenhouse. Trials were conducted for each nematode species using the same experimental layout at a 19 - 26°C night/day temperature regime and a 14 L:10 D photoperiod. The trial layout was a randomized complete block design, each entry replicated six times (Fig. 16).

##### 5.1.1.1. Soil type

Sandy loam soil (3.9% clay, 1.9% silt, 93.6% sand, 0.2 % organic material, pH (KCl) 5.17) was collected from maize plots at the Viljoenskroon trial site.

##### 5.1.1.2. Methyl bromide soil fumigation

One cubic meter of damp Viljoenskroon soil was spread out in a 50cm layer on a concrete floor. Two cans of methyl bromide (581g a.i. each) were put in an open tin on a wooden block with a nail protruding from the bottom of the tin and placed in an upright position on

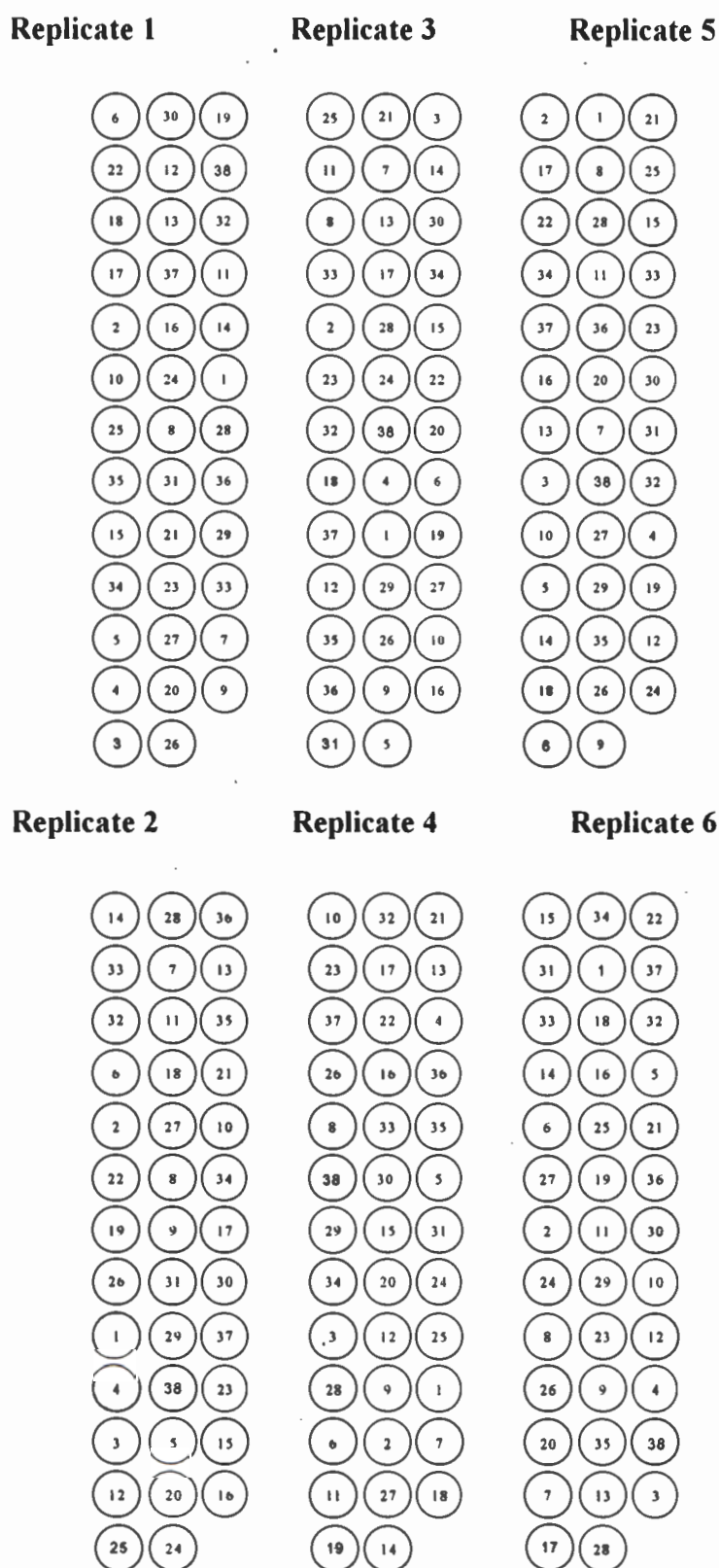
top of the soil layer 1 m apart. The soil with methyl bromide cans were then covered with plastic fumigation cover sheets. Methyl bromide was released by pressing the can from the outside of the cover sheet into the soil to release the gas. The covers were removed after 72 hours.

#### **5.1.1.3. Pasteurization of methyl bromide fumigated soil**

Methyl bromide fumigated soil was transferred to the Oil and Protein seeds Centre where it was pasteurized for 8 hours at 82°C in a commercial pasteurizer.

**Table 27. Thirty-eight commercial soybean cultivars screened against *M. javanica*, *M. incognita* race 1, 2 and 4 in the greenhouse.**

NUMBER	CULTIVAR NAME
1.	PAN581
2.	Columbus
3.	Impala
4.	Highveld Top
5.	Pan 717
6.	A5409
7.	Zebra
8.	Gazelle
9.	Talana
10.	A5308
11.	Bakgat
12.	CRN2233
13.	A7119
14.	SCS1
15.	Bamboes
16.	Hutton
17.	PAN723
18.	PAN494
19.	Dumela
20.	Wilge
21.	Knap
22.	Nyala
23.	Forrest
24.	Hutcheson
25.	SNK60
26.	Hennops
27.	Ibis
28.	PAN790
29.	Prima
30.	PAN812
31.	Crawford
32.	Tambooti
33.	PAN430
34.	Sonop
35.	Wenner
36.	Amstel
37.	Kiaat
38.	Mukwa



**Fig. 17.** Trial layout for the screening of thirty-eight commercial soybean cultivars against *M. javanica*, *M. incognita* race 1, 2 and 4, respectively, in the greenhouse.

5.2. Data collected:

- a) egg mass counts
- b) egg counts

The number of eggs/egg mass and eggs/g of root (eggs/root system/ root mass of plant) was calculated.

Egg laying females (ELF)/root system was indexed and soybean cultivars were grouped into categories on the basis of ELF index, according to the system used by Murray *et al.* (1986) (Table 28).

Table 28. ELF index, egg mass/plant and resistance classification of soybean cultivars (Murray *et al.*, 1986).

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

\* R = resistant: MR = moderately resistant: S = susceptible and VS = very susceptible

Oosterbrink's Reproduction Factor was calculated as follows:  $RF = \text{final egg number} / \text{initial egg number (PF/PI)}$  (Windham & Williams, 1988). RF-values were calculated to determine the reproductive potential of the 38 cultivars for different *Meloidogyne* species and races. Host status of the soybean cultivars were categorized into resistant/poor host, good hosts and excellent hosts, according to the method used by Windham & Williams (1988) (Table 29).

Table 29. RF-values and classification of host status of soybean cultivars (Windham & Williams, 1988).

Category	RF-value	Host status
1.	< 1	resistant/poor host
2.	1 to 5	good hosts
3.	> 5	excellent hosts

### 5.3. Results: Reproductive potential of *M. javanica* on different soybean cultivars.

Data of egg mass index (ELF), number of egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factors are provided in Table 30.

**Table 30.** Egg mass index egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factor of *M. javanica* on 38 commercial South African soybean cultivars.

Cultivars	Egg mass index <sup>1</sup> (ELF)	Egg masses/plant *	Eggs/plant *	Eggs/egg mass	Eggs/g root	RF-value
1 PAN581	3.3	17 ab	9905 abc	592	301	0.9
2 Columbus	2.9	10 a	10751 abc	1091	299	1.1
3 Impala	3.2	15 ab	10436 abc	669	325	1.0
4 H.Top	5	137 ef	62612 cdefg	446	1618	6.2
5 PAN717	3.4	19 ab	10925 abc	511	331	1.0
6 A5409	2.52	7 a	7970 ab	1157	275	0.8
7 Zebra	3.15	14 ab	7612 ab	557	234	0.7
8 Gazelle	2.79	9 a	3076 a	646	88	0.3
9 Talana	2.27	5 a	4664 ab	1207	119	0.4
10 A5308	3.4	19 ab	13394 abc	802	524	1.3
11 Bakgat	3.55	22 ab	24090 abcde	119	988	2.4
12 CRN2233	3.3	17 ab	10193 abc	619	326	1.0
13 A7119	3	11 a	8193 ab	723	302	0.8
14 SCSI	2.79	9 a	6275 ab	866	192	0.6
15 Bamboes	4.04	34 abc	15763 abcd	412	501	1.5
16 Hutton	4.03	33 abc	18738 abcde	697	498	1.8
17 PAN723	4	31 abc	10420 abc	344	298	1.0
18 PAN 494	3.4	19 ab	7941 ab	794	242	0.7
19 Dumela	5	265 hi	122586 i	463	4118	12.2
20 Wilge	5	191 fg	71923 efghij	346	1799	7.1
21 Knap	5	320 i	121562 hi	336	3586	12.1
22 Nyala	2.27	5 a	7004 ab	1270	200	0.7
23 Forrest	3.2	15 ab	8529 abc	505	387	0.8
24 Hutcheson	5	229 gh	113352 ghi	491	3645	11.3
25 SNK60	4.39	61 abcd	8552 abc	295	294	0.8
26 Hennops	5	139 ef	68339 defgh	514	2132	6.8
27 Ibis	3.55	22 ab	16344 abcd	799	418	1.6
28 PAN790	3.45	20 ab	19580 abcde	963	544	1.9
29 Prima	5	455 j	230916 j	452	7309	23.0
30 PAN812	2.9	10 a	7308 ab	904	276	0.7
31 Crawford	4.13	41 abc	14662 abcd	403	433	1.4
32 Tamboti	5	226 gh	105665 fghi	473	2180	10.5
33 PAN430	4.36	59 abcd	24911 abcde	572	536	2.4
34 Sonop	5	105 de	49043 abcde	524	1044	4.9
35 Wenner	4.5	69 bcd	39708 abcde	572	1564	3.9
36 Amstel	5	109 de	58116 bcdef	533	1669	5.8
37 Kiaat	4.17	44 abc	35442 abcde	1062	1468	3.5
38 Mukwa	4.73	87 cde	47291 abcde	540	1047	4.7

<sup>1</sup> Egg mass index: 0 = 0; 1 = 1; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; 5 ≥ 100.

\* Values in columns followed by different letters differ significantly (P > 0.05).

### **5.3.1. ELF-indices**

#### **Category 1: Resistant**

*M. javanica* produced egg masses on all cultivars (Table 28), no one being resistant to this species (Table 30).

#### **Category 2: Moderately resistant**

The following cultivars can be classified as moderately resistant to *M. javanica* (Table 28) on the basis of ELF-indices between 1.0 and 3.2: Columbus, Impala, A5409, Zebra, Gazelle, Talana, A7119, SCS1, Nyala, Forrest and PAN812 (Table 30).

#### **Category 3: Susceptible**

The following cultivars can be classified as susceptible to *M. javanica* on the basis of ELF-indices between 3.3 and 3.7 (Table 28): PAN581, PAN717, A5308, Bakgat, CRN2233, PAN494, Ibis and PAN790 (Table 30).

#### **Category 4: Very susceptible**

The following cultivars can be classified as very susceptible to *M. javanica* (Table 28) on the basis of ELF-indices ranging between 3.8 and 5: Highveld Top, Bamboes, Hutton, PAN723, Dumela, Wilge, Knap, Hutcheson, SNK60, Hennops, Prima, Crawford, Tamboti, PAN430, Sonop, Wenner, Amstel, Kiaat and Mukwa (Table 30).

### **5.3.2. RF-values**

#### **Category 1: Poor hosts**

The following cultivars had RF-values smaller than 1 and were accordingly classified as poor

hosts (Table 29) to *M. javanica*: PAN581, A5409, Zebra, Gazelle, Talana, A7119, SCS1, PAN494, Nyala, Forrest, SNK60 and PAN812 (Table 30).

These cultivars also supported lower numbers of eggs/g root, ranging between 88 for Gazelle to 387 for Forrest (Table 30).

### **Category 2 : Good hosts**

Cultivars with RF-values between 1 and 5, and classified as good hosts (Table 29) for this species are: Columbus, Impala, PAN717, A5308, Bakgat, CRN2233, Bamboes, Hutton, PAN723, Ibis, PAN790, Crawford, PAN430, Sonop, Wenner, Kiaat and Mukwa (Table 30).

These cultivars supported numbers of eggs/g root ranging between 298 for PAN723 to 1564 for Wenner (Table 30).

### **Category 3 : Excellent hosts**

The following cultivars had RF-values greater than 5 and are classified as excellent hosts (Table 29) for *M. javanica*: Highveld Top, Dumela, Wilge, Knap, Hutcheson, Hennops, Prima, Tamboti and Amstel (Table 30).

These cultivars also supported high numbers of eggs/g root ranging between 1618 for Highveld Top to 7309 for Prima (Table 30).

#### **5.3.3. Eggs/egg mass**

A low number of eggs/egg mass is an indication of antibiosis resistance exhibited by a specific cultivar. Number of eggs/egg mass ranged between 119 for Bakgat to 1270 for Nyala (Table 30).

#### 5.4: Results: Reproductive potential of *M.i*<sup>a</sup> race 1 on different soybean cultivars.

Data of egg mass index (ELF), number of egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factor is provided in Table 31.

Table 31. Egg mass index, egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factor of *M. incognita* race 1 on 38 commercial South African soybean cultivars.

Cultivars	Egg mass index <sup>1</sup> (ELF)	Egg masses/plant <sup>a</sup>	Eggs/plant <sup>a</sup>	Eggs/egg mass	Eggs/g root	RF-value
1 PAN581	4.64	80 abcde	19204 ab	265	749	1.9
2 Columbus	4.22	48 abcd	19514 ab	422	795	1.9
3 Impala	4.7	85 bcde	25737 ab	353	1150	2.5
4 Highveld Top	4.85	96 cde	23592 ab	259	717	2.3
5 PAN717	4.26	51 abcd	12189 a	303	512	1.2
6 A5409	4.04	34 abc	13625 ab	373	951	1.3
7 Zebra	3.6	23 abc	13451 ab	647	1586	1.3
8 Gazelle	3.8	27 abc	16169 ab	562	825	1.6
9 Talana	3.7	25 abc	8913 a	361	489	0.8
10 A5308	4.36	59 abcd	14832 ab	258	1269	1.4
11 Bakgat	4.89	99 cde	18091 ab	189	718	1.8
12 CRN2233	4.15	42 abc	19195 a	236	822	0.9
13 A7119	2.39	6 ab	8373 a	1365	400	0.8
14 SCS1	4.21	47 abcd	23214 ab	547	769	2.3
15 Bamboes	3.15	14 ab	7850 a	865	288	0.7
16 Hutton	2.39	6 ab	2473 a	695	108	0.2
17 PAN723	2.14	4 a	7566 a	2869	295	0.7
18 PAN494	4.18	45 abcd	20954 ab	524	796	2.0
19 Dumela	5	484 i	148847 f	297	6974	14.8
20 Wilge	5	459 hij	107587 e	228	3698	10.7
21 Knap	5	386 gh	111573 e	296	3616	11.1
22 Nyala	3.05	12 ab	14332 ab	1256	379	1.4
23 Forrest	2.79	9 ab	7793 a	1084	393	0.7
24 Hutcheson	5	180 f	29201 ab	170	1040	2.9
25 SNK60	2.39	6 ab	4459 a	1202	266	0.4
26 Hennops	5	337 g	97319 ef	605	6936	9.7
27 Ibis	4.5	69 abcd	10406 a	159	346	1.0
28 PAN790	5	123 def	15021 ab	155	755	1.5
29 Prima	5	377 g	92048 ef	319	5538	9.2
30 PAN812	2.65	8 ab	4626 a	653	124	0.4
31 Crawford	4.22	48 abcd	41913 bc	771	1119	4.1
32 Tamboti	5	336 g	67909 cd	201	1426	6.7
33 PAN430	4.28	52 abcd	18454 ab	460	493	1.8
34 Sonop	5	149 ef	41883 bc	285	960	4.1
35 Wenner	4.02	32 abc	12076 a	483	442	1.2
36 Amstel	4.13	41 abc	17552 ab	493	485	1.7
37 Kiaat	4.69	84 bcde	25025 ab	308	900	2.5
38 Mukwa	4.34	57 abcd	18904 ab	359	514	1.8

<sup>1</sup> Egg mass index: 0 = 0; 1 = 1; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; 5 ≥ 100.

<sup>a</sup> Values in columns followed by different letters differ significantly (P = 0.05).

<sup>a</sup> *M. incognita*

### 5.4.1 ELF-indices

#### Category 1: Resistant

*M. incognita* race 1 produced egg masses on all cultivars (Table 28), no one being resistant to this species (Table 31).

#### Category 2: Moderately resistance

The following cultivars are classified as moderately resistant to *M. incognita* race 1 on the basis of the ELF-indices between 1.0 and 3.2 (Table 28): A7119, Bamboes, Hutton, PAN723, Nyala, Forrest, SNK60 and PAN812 (Table 31).

#### Category 3: Susceptible

The following cultivars are classified as susceptible to *M. incognita* race 1 on the basis of ELF-indices ranging between 3.3 and 3.7 (Table 28): Zebra and Talana (Table 31).

#### Category 4: Very susceptible

The following cultivars are classified as very susceptible to *M. incognita* race 1 on the basis of ELF-indices ranging between 3.8 and 5 (Table 28): PAN581, Columbus, Impala, Highveld Top, PAN717, A5409, Gazelle, A5308, Bakgat, CRN2233, SCS1, PAN494, Dumela, Wilge, Knap, Hutcheson, Hennops, Ibis, PAN790, Prima, Crawford, Tamboti, PAN430, Sonop, Wenner, Amstel, Kiaat and Mukwa (Table 31).

### 5.4.2. RF-values

#### Category 1 :Poor hosts

The following cultivars had RF-values smaller than 1 for *M. incognita* race 1 (Table 29) and

were accordingly classified as poor hosts (Table 31): Talana, CRN2233, A7119, Bamboes, Hutton, PAN723, Forrest, SNK60 and PAN812.

These cultivars supported numbers of eggs/g root ranging between 108 for Hutton to 822 for CRN2233 (Table 31).

### **Category 2 : Good hosts**

Cultivars with RF-values between 1 and 5, classified as good hosts (Table 29) for this species were: PAN581, Columbus, Impala, Highveld Top, PAN717, A5409, Zebra, Gazelle, A5308, Bakgat, SCS1, PAN494, Nyala, Hutcheson, Ibis, PAN790, Crawford, PAN430, Sonop, Wenner, Amstel, Kiaat and Mukwa (Table 31).

These cultivars also supported eggs/g root ranging between 346 for Crawford to 1586 for Zebra (Table 31).

### **Category 3 : Excellent hosts**

The following cultivars had RF-values greater than 5 and are classified as excellent hosts (Table 29) for *M.incognita* race1: Dumela, Wilge, Knap, Hennops, Prima and Tamboti (Table 31).

All these cultivars resulted in high numbers of eggs/g root system ranging between 1426 for Tamboti to 6974 for Dumela (Table 31).

#### **5.4.3. Eggs/egg mass**

A low number of eggs/egg mass is an indication of antibiosis resistance exhibited by a specific cultivar. Number of eggs/egg mass ranged between 155 for PAN790 to 2869 for PAN723 (Table 31).

### 5.5. Results: Reproductive potential of *M.i*<sup>a</sup> race 2 on different soybean cultivars.

Data of egg mass index (ELF), number of egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factor is provided in Table 32.

Table 32. Egg mass index, egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factor of *M. incognita* race 2 on 38 commercial South African soybean cultivars

Cultivars	Egg mass index <sup>1</sup> (ELF)	Egg masses/plant <sup>a</sup>	Eggs/plant <sup>a</sup>	Eggs/egg mass	Eggs/g root	RF-value
1 PAN581	5	165 abcde	98052 def	667	9463	9.8
2 Columbus	5	129 abcde	93623 cdef	744	8226	9.3
3 Impala	5	151 abcde	110720 efg	729	10889	11.1
4 Highveld Top	5	191 cde	94290 cdef	519	10489	9.4
5 PAN717	5	180 bcde	94302 cdef	513	8592	9.4
6 A5409	4.58	76 abcde	55605 abcde	734	4645	5.5
7 Zebra	4.35	58 abc	43384 abcde	673	4120	4.3
8 Gazelle	5	104 abcde	43210 abcde	501	3932	4.3
9 Talana	5	118 abcde	61090 abcdef	520	5211	6.1
10 A5308	4.86	97 abcde	92435 cdef	983	12910	9.2
11 Bakgat	5	115 abcde	62729 abcdef	586	8915	6.2
12 CRN2233	4.5	69 abcd	45658 abcde	753	3534	4.5
13 A7119	3.7	25 a	12563 a	781	1134	1.2
14 SCS1	4.76	89 abcde	66587 abcdef	943	6547	6.6
15 Bamboes	4.18	45 ab	27429 abcd	661	3370	2.7
16 Hutton	3.85	28 a	11850 a	506	867	1.1
17 PAN723	4.05	35 a	18208 ab	593	1682	1.8
18 PAN 494	4.73	87 abcde	70989 abcdef	778	6134	7.1
19 Dumela	5	671 gh	277944 i	419	47773	27.7
20 Wilge	5	465 f	177643 gh	389	19402	17.7
21 Knap	5	594 fgh	276994 i	542	27156	27.6
22 Nyala	4.58	75 abcde	71282 abcdef	966	5707	7.1
23 Forrest	5	131 abcde	22852 abc	189	3666	2.2
24 Hutcheson	5	214 de	130445 fgh	595	12766	13.0
25 SNK60	4.36	59 abc	22722 abc	392	2537	2.2
26 Hennops	5	714 hi	202708 h	283	32833	20.2
27 Ibis	4.67	83 abcde	46128 abcde	536	4031	4.6
28 PAN790	5	120 abcde	88060 bcdef	795	8813	8.8
29 Prima	5	543 fg	288201 i	529	32795	28.8
30 PAN812	4.18	45 ab	37278 abcd	878	3333	3.7
31 Crawford	5	217 e	115253 efg	525	2769	11.5
32 Tamboti	5	654 gh	279895 i	482	8479	27.9
33 PAN430	4.43	64 abc	53945 abcde	841	1055	5.3
34 Sonop	5	830 i	319829 i	435	6413	31.9
35 Wenner	4.66	82 abcde	65853 abcdef	682	1768	6.5
36 Amstel	4.29	53 abc	31321 abcd	595	1147	3.1
37 Kiaat	4.36	59 abc	32942 abcd	548	2938	3.2
38 Mukwa	5	110 abcde	73941 abcdef	717	1870	7.3

<sup>1</sup> Egg mass index: 0 = 0; 1 = 1; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; 5 = 100.

<sup>a</sup> Values in columns followed by different letters differ significantly (P < 0.05).

<sup>a</sup> *M. incognita*

### 5.5.1. ELF-indices

#### Categories 1 & 2: Resistant and moderately resistant

No cultivars are classified as moderately resistant or resistant (Table 28) to *M. incognita* race 2 on the basis of ELF-indices (Table 32).

#### Category 3 : Susceptible

One cultivar only A7119 (Table 28) is classified as susceptible to *M. incognita* race 2 with an ELF-index between 3.3 and 3.7 (Table 32).

#### Category 4 : Very susceptible

The majority of cultivars are classified as very susceptible to *M. incognita* race 2 on the basis of ELF-indices between 3.8 and 5 (Table 28). They are: PAN581, Columbus, Impala, Highveld Top, PAN717, A5409, Zebra, Gazelle, Talana, A5308, Bakgat, CRN2233, SCS1, Bamboes, Hutton, PAN723, PAN494, Dumela, Wilge, Knap, Nyala, Forrest, Hutcheson, SNK60, Hennops, Ibis, PAN790, Prima, PAN812, Crawford, Tamboti, PAN430, Sonop, Wenner, Amstel, Kiaat and Mukwa (Table 32).

### 5.5.2. RF-values

#### Category 1 : Poor hosts

No cultivars had RF-values smaller than 1 for *M. incognita* race 2 and could thus be classified as poor hosts (Table 29) (Table 32).

#### Category 2 : Good hosts

Cultivars with RF-values between 1 and 5 classified as good hosts (Table 29) for this species

were: Zebra, Gazelle, CRN2233, A7119, Bamboes, Hutton, PAN723, Forrest, SNK60, Ibis, PAN812, Amstel and Kiaat (Table 32).

These cultivars supported numbers of eggs/g root ranging between 1147 for Amstel to 4120 for Zebra (Table 32).

### **Category 3 : Excellent hosts**

The following cultivars had RF-values greater than 5 and are classified as excellent hosts (Table 29) for *M. incognita* race 2: PAN581, Columbus, Impala, Highveld Top, PAN717, A5409, Talana, A5308, Bakgat, SCS1, PAN494, Dumela, Wilge, Knap, Nyala, Hutcheson, Hennops, PAN790, Prima, Crawford, Tamboti, PAN430, Sonop, Wenner and Mukwa (Table 32).

These cultivars had numbers of eggs/g root ranging between 1055 for PAN430 to 47773 for Dumela (Table 32).

#### **5.5.3. Eggs/egg mass**

Low numbers of eggs/egg mass is an indication of antibiosis resistance exhibited by a specific cultivar. Number of eggs/egg mass ranged between 189 for Forrest to 983 for A5308 (Table 32).

## 5.6. Results: Reproductive potential of *M.i*<sup>a</sup> race 4 on different soybean cultivars.

Data of egg mass index (ELF), number of egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factor is provided in Table 33.

Table 33. Egg mass index egg masses/plant, eggs/plant, eggs/egg mass, eggs/g root and reproduction factor of *M. incognita* race 4 on 38 commercial South African soybean cultivars

Cultivars	Egg mass index <sup>1</sup> (ELF)	Egg masses/plant <sup>a</sup>	Eggs/plant <sup>a</sup>	Eggs/egg mass	Eggs/g root	RF-value
1 PAN581	4.11	39 fghijk	34110 fgh	209	1348	6.8
2 Columbus	4.13	41 ghijk	27103 bcdefg	551	1198	5.4
3 Impala	4.15	42 hijk	35680 fgh	725	1466	7.1
4 Highveld Top	3.5	21 abcdefgh	26557 bcdefg	168	815	5.3
5 PAN717	3.91	30 cdefghij	27506 cdefg	405	849	5.5
6 A5409	3.1	13 abcd	10773 abcdef	637	598	2.1
7 Zebra	3.35	18 abcdefg	10724 abcdef	2168	302	2.1
8 Gazelle	4.04	34 defghijk	17920 abcdefg	593	496	3.5
9 Talana	4.04	34 defghijk	13683 abcdef	2346	336	2.7
10 A5308	4.04	34 defghijk	13588 abcdef	748	701	2.7
11 Bakgat	4.2	46 ijkl	28900 defg	347	1017	5.7
12 CRN2233	3.3	16 abcdef	3037 abc	167	91	0.6
13 A7119	1.99	2 a	4622 abcd	2354	160	0.9
14 SCSI	4.03	33 cdefghij	13163 abcdef	133	477	2.6
15 Bamboes	3.7	25 abcdefghi	12147 abdcef	474	711	2.4
16 Hutton	2.14	4 a	415 a	127	11	0.1
17 PAN723	2	3 a	285 a	99	9	0.1
18 PAN494	4.25	50 jkl	32965 efgh	93	1084	6.5
19 Dumela	5	109 qr	85369 kl	142	3989	17.0
20 Wilge	4.6	77 nop	64910 ijk	531	2491	12.9
21 Knap	4.56	74 no	65197 jk	300	2445	13.0
22 Nyala	3.05	12 abcd	1990 ab	163	70	0.3
23 Forrest	2.9	10 abc	7852 abcde	1202	394	1.5
24 Hutcheson	4.54	73 mno	57153 hij	834	1405	11.4
25 SNK60	2	3 a	7615 abcd	3489	271	1.5
26 Hennops	4.78	91 opq	74101 jkl	515	3027	14.8
27 Ibis	4.04	34 defghijk	13630 abcdef	1423	286	2.7
28 PAN790	4.06	36 efghijk	15033 abcdefg	448	420	3.0
29 Prima	5	108 qr	95440 l	555	3227	19.0
30 PAN812	2.39	6ab	5837 abcd	1112	141	1.1
31 Crawford	5	100 pqr	86293 kl	1626	2862	17.2
32 Tamboti	5	115 r	85599 kl	828	2436	17.1
33 PAN430	4.45	65 lmn	34394 fgh	1221	1293	6.8
34 Sonop	4.26	51 klin	39953 ghi	1752	1601	7.9
35 Wenner	3.85	28 efghijk	14588 abcdef	612	1086	2.9
36 Amstel	3.35	18 abcdef	13253 abcdef	1439	1093	2.6
37 Kiaat	4.17	44 ijkl	20329 abcdefg	2163	926	4.0
38 Mukwa	3.2	15 abcde	19031 abcdefghijk	4858	445	3.8

<sup>1</sup> Egg mass index: 0 = 0; 1 = 1; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; 5 ≥ 100

<sup>a</sup> Values in columns followed by different letters differ significantly (P < 0.05).

<sup>a</sup> *M. incognita*

### **5.6.1 ELF-indices**

#### **Category 1 : Resistant**

produced egg masses on all cultivars (Table 28), no one being resistant to this species (Table 33).

#### **Category 2 : Moderately resistant**

The following cultivars are classified as moderately resistant to *M. incognita* race 4 on the basis of ELF-indices ranging between 1.0 and 3.2 (Table 28): A5409, A7119, Hutton, PAN723, Nyala, Forrest, SNK60, PAN812 and Mukwa (Table 33).

#### **Category 3 : Susceptible**

The following cultivars are classified as susceptible to *M. incognita* race 4 on the basis of ELF-indices ranging between 3.3 and 3.7 (Table 28): Highveld Top, Zebra, CRN2233, Bamboes and Amstel (Table 33).

#### **Category 4 : Very susceptible**

The following cultivars are classified as very susceptible to *M. incognita* race 4 on the basis of ELF-indices ranging between 3.8 and 5 (Table 28): PAN581, Columbus, Impala, PAN717, Gazelle, Talana, A5308, Bakgat, SCS1, PAN494, Dumela, Wilge, Knap, Hutcheson, Hennops, Ibis, PAN790, Prima, Crawford, Tamboti, PAN430, Sonop, Wenner and Kiaat (Table 33).

## 5.6.2. RF-values

### Category 1 : Poor hosts

The following cultivars had RF-values smaller than 1 for *M.incognita* race 4 and are classified as poor hosts (Table 29) : CRN2233, A7119, Hutton, PAN723 and Nyala (Table 33).

These cultivars also supported in eggs/g root ranging between 9 for PAN723 to 160 for A7119 (Table 33).

### Category 2 : Good hosts

The following cultivars had RF-values between 1 and 5 and are classified as good hosts (Table 29) to *M. incognita* race 4: A5409, Zebra, Gazelle, Talana, A5308, SCS1, Bamboes, Forrest, SNK60, Ibis, PAN790, Wenner, Amstel, Kiaat and Mukwa (Table 33).

These cultivars supported in eggs/g root ranging between 141 for PAN812 to 926 for Kiaat (Table 33).

### Category 3 : Excellent hosts

Cultivars with RF-values greater than 5, being classified as excellent hosts (Table 29) for this species are : PAN581, Columbus, Impala, Highveld Top, PAN717, Bakgat, PAN494, Dumela, Wilge, Knap, Hutcheson, Hennops, Prima, Crawford, Tamboti, PAN430 and Sonop (Table 33).

These cultivars supported in eggs/g root ranging between 1017 for Bakgat to 3989 for Dumela (Table 33).

### 5.6.3. Eggs/egg mass

Low numbers of eggs/egg mass is an indication of antibiosis resistance exhibited by a specific cultivar. Numbers of eggs/egg mass ranged between 93 for PAN494 to 4858 for Mukwa (Table 33).

## 5.7. Discussion

There was substantial variation between the 38 soybean cultivars with regard to host suitability according to ELF-indexes and RF-values when screened to *M. javanica* and *M. incognita* race 1 and 4. Inoculation with *M. incognita* race 2, however, resulted in overall high ELF-indices and RF-values.

Some cultivars exhibited moderate resistance to *M. javanica* according to both ELF-indexes and RF-values, for example: A5409, Zebra, Gazelle, Talana, A7119, SCS1, Nyala, Forrest and PAN812. The above-mentioned criteria did not necessarily result in the same cultivars sharing resistance or being classified as poor hosts for a certain root-knot nematode species, e.g. Columbus and Impala were moderately resistant to *M. javanica* according to the ELF-index rating but could not be classified as poor hosts according to RF-values. This tendency was also applicable to *M. incognita* race 1 and 4.

Although ELF-index as well as other egg mass indices are commonly used as a criteria for expressing nematode resistance in soybean cultivars (Birchfield & Brister, 1969; Boquet *et al.*, 1975; Dropkin, 1959; Hirunsalee *et al.*, 1995; Hussey & Boerma, 1981; Ibrahim & Lewis, 1985 & 1986; Lim & Castillo, 1979; McDonald & De Waele, 1989; Wyatte & Fassuliotis, 1979), egg masses may not be an accurate reflection of the number of eggs according to Hirunsalee *et al.* (1995). Furthermore, although egg mass indices give an indication of nematode reproduction rate, the number of eggs per egg mass may vary, as indicated by these results and by Windham and Williams (1987 & 1988). Some cultivars had high ELF-indices but their nematode RF-values were smaller than 1. For example: PAN494 had an ELF-index of 3.4 but a RF-value of 0.7 for *M. javanica*. Conversely some cultivars had low ELF-indices but RF-values greater than 1, e.g. Columbus resulted in an ELF-index of 2.9 but RF-value of 1.1 for *M. javanica*.

According to Windham and Williams (1987 & 1988) egg production (number of eggs/root system) is a more reliable measure of crop resistance to *Meloidogyne* species than root-gall ratings or egg mass indices. Oostenbrink's RF-value, therefore, provides a basic measurement

of nematode reproductive capabilities and gives a good indication of the level of resistance present in a plant. Windham and Barker (1986) also used RF-value for screening soybean cultivars to *M. incognita* race 1, 2, 3 and 4.

Cultivars with RF-values smaller than 1 for a root-knot nematode species supported low numbers of eggs/g root compared to cultivars with high RF-values. CRN2233 was an exception, however, with high numbers of eggs/g root but a low RF-value when screened to *M. incognita* race 1. According to Windham & Williams (1987) calculation of number of eggs/g root is preferable when verifying resistance, especially with inbreds. This criterium was also used in screening soybean cultivars for resistance by Swanson and Van Gundy (1984) and Luzzi *et al.* (1987). Shepherd (1979) also found egg counts to be more appropriate than egg mass counts when screening cotton to root-knot nematodes and Bernard and Keyserling (1985) when screening sunflower for resistance to *Meloidogyne* species.

In this study A7119 was the only cultivar that was classified a poor host, based on RF-values for both *M. javanica* and *M. incognita* race 1 and 4, while CRN2233, A7119, Hutton and PAN723 were poor hosts to both *M. incognita* race 1 and 4. Forrest, classified as a poor host to *M. javanica* in this study, was also found resistant to this species by Ibrahim and El-Saedy (1982 & 1987), Kinloch and Hinson (1973) and Rodríguez-Kábana and Thurlow (1980). Hutton did not have a RF-value smaller than 1 for *M. incognita* race 2 but was the poorest host with a RF-value of 1.1.

There was variation in reproduction between root-knot nematode races on several cultivars, e.g. Talana supported a low RF-value when screened to *M. incognita* race 1 but supported high RF-values when screened to *M. incognita* race 2 and 4. Although Swanson and Van Gundy (1984) reported soybean resistance to *M. incognita* to be race specific, Windham and Barker (1986) suggested that soybean resistance appears to be population specific rather than race specific, based on the fact that soybean is not included in the North Carolina Differential Host Range Test.

Although RF-value is a reliable criterium for resistance, it cannot be used in the identification

of the mechanism of resistance present in a plant. Calculation of eggs/egg mass which can indicate antibiosis resistance, however, may supply valuable information about the resistance mechanism exhibited by a specific cultivar. This information is valuable for plant breeders because they may be able to trace the source of resistance in the soybean lines used for breeding for resistance against root-knot nematodes.

Based on its low RF-value for *M. javanica*, which indicates a negative population growth, SNK60 possibly have antibiosis resistance (low numbers of eggs/egg mass) to this nematode species. Cultivars SCS1, A5409, Talana and Nyala, which had RF-values smaller than 1 and an ELF-index below 3.2, show possible antixenosis resistance (low numbers of egg laying females/root system) for *M. javanica*. Ibis turned out to be the poorest host for *M. incognita* race 1, with possible antibiosis resistance based on a low-RF-value of 1.0 and low numbers of eggs/egg mass. Cultivars Forrest, SNK60, A7119 and PAN723 had both RF-values smaller than 1 and ELF-indices of less than 3.2, which indicates antixenosis resistance for this root-knot species. Most cultivars exhibited antibiosis resistance when evaluated to *M. incognita* race 2, based on low numbers of eggs/egg mass. PAN723 and Hutton had RF-values smaller than 1, low numbers of eggs/egg mass and ELF-indices below 3.2 for *M. incognita* race 4, which indicates both antibiosis and antixenosis resistance. A7119 also had a RF-value smaller than 1 and an ELF-index of less than 3.2, which indicates antixenosis. All cultivars with RF-values smaller than 1 and/or ELF-indices below 3.2 for a specific species do, however not necessarily have antibiosis or antixenosis resistance. These cultivars supported high numbers of eggs/egg mass and high numbers of egg lying females/root system.

Results of this study suggest that RF-values and eggs/egg mass or RF-values alone could be used as criteria for identification of poor nematode host suitability to different root-knot nematode species in soybean. Cultivars exhibiting both RF-values below 1 with antibiosis or antixenosis resistance or both can be recommended for inclusion in an IPM system and should suppress root-knot nematode population growth.

Inclusion of cultivars highly susceptible to root-knot nematodes in crop rotation systems will result in rapid build-up of nematode populations, which will make crop rotation an ineffective

management strategy. Yield losses due to increased nematode infestations may be encountered.

## 5.8. Conclusions

The root-knot nematode species that occur most frequently in South African agricultural soil is *M. javanica* and *M. incognita* race 2 or a combination of these two species (K.P.N. Kleynhans, 1997- personal communication). Cultivars, that are poor hosts to both these species are thus preferable when high population densities of these species are present in a field. Cultivar suitability for specific regions in South Africa should, however, be borne in mind when recommendations are made to commercial and small scale farmers. A7119 for example, showed low RF-values for both *M. javanica* and *M. incognita* race 2 and is also popular because of its high yield potential in the temperate region of South Africa.

Although Dumela, Wilge, Knap and Prima were found to be highly susceptible to all the root-knot nematode species evaluated in this study, these cultivars are very popular South Africa due to their high yield potentials. Further research, to find combinable germplasm sources of root-knot nematode resistance to cross with such cultivars is needed.

Greenhouse screening procedures have several advantages over field screening such as i) screenings can be accomplished rapidly and continuously throughout the year, ii) nematode inoculum can be applied uniformly to test plants (Hussey & Boerma, 1981) and iii) surface-sterilized eggs is easily obtainable. It is important, however, to evaluate cultivars in field experiments where natural infestations of root-knot nematodes occur. It is also important to include soybean yield as a parameter for distinguishing between resistance, hypersensitivity and tolerance in soybean cultivars.

## CHAPTER 6

### CONCLUSIONS

Twelve plant-parasitic nematode genera, including 15 species, were found associated with soybean in South Africa in this study. These were *P. zae*, *P. brachyurus*, *P. crenatus*, *P. teres*, *P. neglectus*, *P. thornei*, *M. javanica*, *M. incognita*, *M. hapla*, *M. ethiopica*, *D. africanus*, *Helicotylenchus* spp., *Scutellonema* spp., *Rotylenchus* spp. *Rotylenchulus* spp., *P. minor*, *Mesocriconema* spp., *Tylenchorhynchus* spp., *L. pisi*, *X. vanderlindei* and *X. elongatum*.

First reports of plant-parasitic genera and species on soybean in South Africa are *P. zae*, *P. crenatus*, *P. teres*, *P. neglectus*, *P. thornei*, *M. ethiopica*, *D. africanus*, *Rotylenchulus* spp., *P. minor*, *Mesocriconema* spp., *L. pisi*, *X. vanderlindei* and *X. elongatum*.

Free-living and predatory nematodes from the orders Rhabditida and Monochida were also present in soil samples surrounding soybean root systems.

Predominant endoparasitic nematodes from the rhizosphere of soybean root systems were *P. zae*, *P. crenatus* and *Meloidogyne* spp., while *Helicotylenchus* spp. and *Scutellonema* spp. were the predominant semi-ectoparasites.

Predominant endoparasitic nematodes in soybean roots were *P. zae* and *P. brachyurus* and *Scutellonema* spp. as the predominant semi-ectoparasites.

Combining nematode numbers from soil and root samples resulted in *Meloidogyne* spp., *P. zae* and *P. brachyurus* being the predominant endoparasitic nematodes while *Helicotylenchus* spp. and *Scutellonema* spp. were the predominant semi-ectoparasitic nematodes.

Ectoparasitic *P. minor* is associated with transmission of tobacco rattle virus in South Africa while *L. pisi*, *X. vanderlindei* and *X. elongatum* are not associated with virus transmission in

this country.

*Pratylenchus* spp. and *Meloidogyne* spp. were present in mixed populations at several localities. Mixed populations of *Pratylenchus* included *P. zae* and *P. teres* at Vaalharts, *P. zae* and *P. brachyurus* at Piet Retief, Viljoenskroon, Bethal, Ventersdorp and Oberholzer. *P. brachyurus*, *P. crenatus* and *P. thornei* occurred in combination at Beestekraal. *P. zae* population densities dominated over *P. brachyurus* at Piet Retief, Ventersdorp and Oberholzer. *P. teres* population densities dominated over *P. zae* at Vaalharts, while *P. brachyurus* population densities dominated over *P. zae* at Viljoenskroon and Bethal. *P. thornei* was the predominant lesion nematode species at Beestekraal, followed by *P. crenatus* and *P. brachyurus*.

Root-knot nematode species were identified at Badplaas [*M. incognita* (85%) and *M. javanica* (15%)], Greytown [*M. incognita* (52%) and *M. ethiopica* (48%)] and Vaalharts [*M. incognita* (57%) and *M. hapla* (43%)], Dundee (*M. javanica* - 100%), Hill Crest (*M. incognita* - 100%), Middelburg - Cape (*M. javanica* - 100%) and Beestekraal (*M. incognita* - 100%). *M. incognita* population densities dominated over other root-knot nematode species when present in mixed populations at localities sampled during this study.

Different nematode genera and species differed in prominence to different cultivars. *P. zae* and *P. brachyurus* the lowest prominence in roots of A5308 while *Scutellonema* spp., *Helicotylenchus* spp. and *D. africanus* had the lowest prominence in roots of Highveld Top. *Meloidogyne* spp. occurred with the lowest prominence on PAN812 and highest prominence on Prima. PAN812 was found to be a poor host, in greenhouse trials, for *M. javanica* and *M. incognita* race 1, while Prima was highly susceptible to *M. javanica* and *M. incognita* race 1, 2 and 4.

The following cultivars will not be recommended for inclusion in an IPM-system due to high numbers of *P. zae*: Forrest at Badplaas, PAN812 at Greytown, PAN723 at Dundee, PAN812 at Vaalharts, PAN717 at Hill Crest, Wenner at Groblersdal, CRN2233 at Bergville, Wenner at Ficksburg, Hutcheson at Piet Retief, Bakgat at Delmas, PAN430 at Viljoenskroon,

Tamboti at Bethlehem, PAN812 at Bethal, Crawford at Ventersdorp and PAN430 at Oberholzer.

Cultivars maintaining low numbers of *P. zea* that can be recommended for inclusion in an IPM-system were: Highveld Top at Badplaas, CRN2233 at Greytown, Dumela at Dundee, SCS1 at Vaalharts, Hutcheson, Highveld Top and PAN812 at Hill Crest, Gazelle at Groblersdal, Forrest at Bergville, A5308 at Ficksburg, Amstel at Piet Retief, Forrest at Delmas, Highveld Top at Viljoenskroon, PAN494 at Bethlehem, SNK60 at Bethal, SNK60 at Ventersdorp and Amstel at Oberholzer.

The following cultivars will not be recommended for inclusion in an IPM-system due to high numbers of *P. brachyurus*: Hutcheson at Piet Retief, PAN723 at Viljoenskroon, Forrest at Bethal, Nyala at Ventersdorp, Prima, PAN430 and Wilge at Beestekraal and PAN430 at Oberholzer.

Cultivars maintaining low numbers of *P. brachyurus* that can be recommended for inclusion in an IPM-system were: PAN790 at Piet Retief, A5308 at Viljoenskroon, Kiaat at Bethal, SNK60 at Ventersdorp, Bakgat, CRN2233, Sonop, Knap, A7119, PAN719, Mukwa, Nyala and Gazelle Beestekraal and Amstel at Oberholzer.

The following cultivars will not be recommended for inclusion in an IPM-system due to high numbers of *Meloidogyne* spp. : Prima at Badplaas (*M. javanica* and *M. incognita*), Prima at Greytown (*M. incognita* and *M. ethiopica*), Wenner at Dundee (*M. javanica*), Knap at Vaalharts (*M. incognita* and *M. hapla*), Sonop at Hill Crest (*M. incognita*), Hutcheson at Middelburg-Cape (*M. javanica*) and Crawford at Beestekraal (*M. incognita*).

Cultivars maintaining low numbers of *Meloidogyne* spp. that can be recommended for inclusion in an IPM-system were: PAN812 at Badplaas (*M. javanica* and *M. incognita*), Gazelle at Greytown (*M. incognita* and *M. ethiopica*), Tamboti, A5308, A5409, Sonop, PAN494, Amstel, Dumela, PAN790, PAN812, PAN723, Kiaat and Ibis at Dundee (*M. javanica*), Crawford and PAN723 at Vaalharts (*M. incognita* and *M. hapla*), A5409 and

PAN430 at Hill Crest (*M. incognita*), Knap and Gazelle at Middelburg-Cape (*M. javanica*) and Tamboti, CRN2233, PAN430, Hutcheson, Highveld Top, Knap, A7119, Forrest, PAN723, Kiaat, Ibis, Gazelle and SCS1 at Beestekraal (*M. incognita*).

Substantial variation was present in 38 commercial soybean cultivars with regard to host suitability for *M. javanica* and *M. incognita* race 1, 2 and 4 in greenhouse screenings.

Results from this study showed that, although egg mass indices are commonly used as criteria for expressing root-knot nematode resistance, RF-values is a more reliable criterium for identification of host suitability of soybean cultivars to different *Meloidogyne* species and races.

Several cultivars were identified as poor hosts for *M. javanica* and *M. incognita* race 1 and 4 with regard to RF-values. No cultivars had RF-values lower than 1 when screened to *M. incognita* race 2. A7119 was classified a poor host for both *M. javanica* and *M. incognita* race 1 and 4, while CRN2233, A7119, Hutton and PAN 723 were poor hosts for both *M. incognita* race 1 and 4.

The mechanism of resistance cannot be determined when RF-values are lower than 1. Low numbers of eggs/egg mass indicated antibiosis resistance, while low numbers of egg laying females/root system indicated antixenosis resistance. Cultivars exhibiting antibiosis/antixenosis resistance as well as RF-values below 1, are preferable for inclusion in an IPM-system.

Cultivars highly susceptible to *M. javanica* and *M. incognita* race 1, 2 and 4 included Dumela, Wilge, Knap, Hennops, Prima and Tamboti. Inclusion of these cultivars in crop rotation systems by commercial and small scale farmers will result in build-up of root-knot nematode populations. Crop rotation will thus be rendered ineffective, with potential yield losses to crops susceptible to these root-knot nematode species and races.

Screenings of cultivars indicated as poor hosts for different *Meloidogyne* species and races in

greenhouse trials have to be repeated in field experiments where yield can be implemented as an important parameter to distinguish between resistance, hypersensitivity and tolerance in soybean cultivars.

## CHAPTER 7

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