

**PLANT-PARASITIC NEMATODES ASSOCIATED WITH WEEDS IN
DEVELOPING AGRICULTURE WITH SPECIAL REFERENCE TO ROOT-
KNOT NEMATODES**

KEIKANTSEMANG NANCY NTIDI

**A dissertation submitted in partial fulfilment of the requirements for the
degree Master of Environmental Sciences (M. Env. Sci.) to the School of
Environmental Sciences and Development at the North-West University
(Potchefstroom Campus)**

June 2008

Promoter : Dr H. Fourie

Co-promoter: Prof. A. H. Mc Donald

TABLE OF CONTENTS

DECLARATION	I
ACKNOWLEDGMENTS	II
LIST OF TABLES	III
LIST OF FIGURES	VII
ABSTRACT	IX
OPSOMMING	XI

CHAPTER 1

Introduction

1.1 General introduction	1
1.2 Weeds	1
1.3 Definition and characteristics of weeds	1
1.4 Classification and life cycle of weeds	2
1.5 Economically important weeds that commonly occur in subsistence farming systems in South Africa	2
1.5.1 Classification	3
1.5.2 Origin, description, classification and control	3
1.5.2.1 <i>Amaranthus hybridus</i> L.	3
1.5.2.2 <i>Bidens bipinnata</i> L.	4
1.5.2.3 <i>Xanthium strumarium</i> L.	5
1.5.2.4 <i>Chenopodium carinatum</i> L.	6
1.5.2.5 <i>Argemone ochroleuca</i> L.	7
1.5.2.6 <i>Cynodon dactylon</i> L.	7
1.5.2.7 <i>Cyperus rotundus</i> L.	8
1.5.2.8 <i>Datura stramonium</i> L.	9
1.5.2.9 <i>Eleusine corocana</i> (L.) Gaertn.	10
1.5.2.10 <i>Hibiscus cannabinus</i> L. and <i>H. trionum</i> L.	11

1.5.2.11 <i>Ipomoea purpurea</i> L.	12
1.5.2.12 <i>Lepidium africanum</i> L.	13
1.5.2.13 <i>Tagetes minuta</i> L.	14
1.6 Diseases and pests associated with weeds	15
1.7 Plant-parasitic nematodes	16
1.7.1 Classification morphology and biology	16
1.7.2 Feeding habits of plant-parasitic nematodes	17
1.7.3 General	18
1.7.4 Nematode control	19
1.7.4.1 Chemical control	19
1.7.4.2 Cultural nematode control	19
1.8 Plant-parasitic nematodes associated with weeds	20
1.8.1 Root-knot nematodes	23
1.9 Root-knot nematodes associated with weeds	24
1.10 Objective of the study	26

CHAPTER 2

Nematode survey and identification of plant-parasitic nematodes associated with weeds

2.1 Introduction	27
2.2 Material and methods	27
2.2.1 Sampling of nematodes	27
2.2.2 Extraction of nematodes from root and soil samples of weed species	32
2.2.2.1 Root samples (5g)	32
2.2.2.2 The importance of kaolin	33
2.2.2.3 Soil samples	34
2.2.3. Counting of nematodes	35
2.2.4 Species identification	35
2.2.4.1 Transfer of nematodes to glycerin	35
2.2.4.2 Fixation of nematodes	36
2.2.4.3 Mounting of nematodes on slides	36

2.3 Data analyses	37
2.3.1 Nematodes	37
2.3.1.1 Population density of each nematode species	37
2.3.1.2 Frequency of occurrence of nematode species	37
2.3.1.3 Prominence value	38
2.3.2 Weeds	39
2.3.2.1 Frequency of occurrence of weed genera/species	39
2.4 Results	40
2.4.1 Weed species	40
2.4.2 Root samples (20-g): Root-knot nematodes	43
2.4.3 Five-gram (5-g) root samples: Plant-parasitic nematodes	47
2.4.4 Soil samples (200-ml): Plant-parasitic nematodes	62
2.5 Discussion	78

CHAPTER 3

Identification of *Meloidogyne* species SCAR-PCR assays

3.1. Introduction	83
3.2. Materials and methods	84
3.2.1. Rearing of <i>Meloidogyne</i> populations from different localities for root-knot nematodes identification	84
3.2.2 DNA extraction	84
3.2.3. SCAR amplification	85
3.2.4 Results	86
3.2.5 Discussion	91

CHAPTER 4

Recommendations and conclusions	93
---------------------------------	----

CHAPTER 5

References	95
------------	----

DECLARATION

I K. N Ntidi declare herewith that the dissertation entitled, 'Plant-parasitic nematodes associated with weeds in developing agriculture with special reference to root-knot nematodes, which I submitted to the North-West University as completion of the requirements set for the M. Env. Sci. degree, is my own work and has not already been submitted to any other university.

University no: 13161784

Date: 11/08/2008

Ntidi
Ntidi Keikantsemang Nancy

Fourie

Date: 11/08/2008

Supervisor: Dr H. Fourie

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the following people and Institute:

ARC-Grain Crops Institute for funding my studies.

My promoter, Dr H. Fourie for her scientific expertise, professional guidance, words of wisdom and helping me to stay sane through all the insanity while pursuing my master's degree.

Ms S. Steenkamp, Ms E. Venter, Ms R. Jantjies, Ms B. Matuli, Ms L. Bronkhorst, Mr A Tladi and Mr S. Kwenza for technical assistance throughout this study.

Professor A. H. McDonald for editing and reviewing the dissertation.

Mrs W. Du Rand for compiling maps and Mrs E. Van den Berg for assistance on locating the latitude and longitude of localities sampled.

Taxonomists of ARC-PPRI for identifying plant-parasitic nematode to species level.

Dr J Saayman, Weed scientist of ARC-GCI for identifying weeds to the species level.

The staff of the Biotechnology unit (ARC-GCI) for their assistance on the DNA extraction (molecular identification of root-knot nematodes). The staff of Maize Breeding unit (ARC-GCI) played a role on identifying some of the localities to be sampled.

My † father and mother for their love and guidance through my life. They taught me that knowledge is something that can never be taken away from me. My son, sisters and brother for their support and understanding during this study.

The Heavenly Father for giving me strength and a second chance in life.

LIST OF TABLES

Chapter 1

Table 1: Root-knot nematodes associated with weeds in South Africa (Keetch & Buckley, 1984).	25
--	----

Chapter 2

Table 1: Overall frequency of occurrence (%) of weeds sampled for identification of plant-parasitic nematodes over 44 sites in South Africa.	40
--	----

Table 2: Frequency of occurrence (%) of weeds sampled for identification of plant-parasitic nematodes in 34 sites located in the eastern and 10 sites in the western regions of South Africa in terms of rainfall patterns.	41
---	----

Table 3: Prominence value (PV), frequency of occurrence (%) and mean population density of <i>Meloidogyne</i> spp. eggs and J2 in roots of weeds sampled from 44 sites in South Africa.	43
---	----

Table 4: Prominence value (PV), frequency of occurrence (%) and mean population density of <i>Meloidogyne</i> spp. eggs and J2 per 20g roots of weeds sampled from 34 sites located in the eastern and 10 sites in the western regions of South Africa.	43
---	----

Table 5: Prominence value (PV), frequency of occurrence (%) and mean population density of <i>Meloidogyne</i> spp. in roots of weeds sampled from 44 sites in South Africa.	44
---	----

Table 6: Prominence value (PV), frequency of occurrence (%) and mean population density of <i>Meloidogyne</i> spp. per 20g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.	45
---	----

Table 7: Prominence value (PV), frequency of occurrence (%) and mean population density of plant-parasitic nematodes in roots of weeds sampled from 44 sites in South Africa. 47

Table 8: Prominence value (PV), frequency of occurrence (%) and mean population density of plant-parasitic nematodes per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 48

Table 9: Prominence value (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. in roots of weeds sampled from 44 sites in South Africa. 50

Table 10: Prominence value (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites western regions of South Africa. 51

Table 11: Prominence value (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zae* in 5g roots of weeds sampled from 44 sites in South Africa. 53

Table 12: Prominence value (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zae* per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 54

Table 13: Prominence value (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystera* in 5g roots of weeds collected from 44 sites in South Africa. 56

Table 14: Prominence value (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystera* per 5g roots of weeds

collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 57

Table 15: Prominence value (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* in 5g roots of weeds sampled from 44 sites in South Africa. 59

Table 16: Prominence value (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 60

Table 17: Prominence value (PV), frequency of occurrence (%) and mean population density of plant-parasitic, free-living and predatory nematodes in soil of weed sampled from 44 sites in South Africa. 62

Table 18: Prominence value (PV), frequency of occurrence (%) and mean population density of plant-parasitic, free-living and predatory nematodes for 200ml soil of weed collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 64

Table 19: Prominence value (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. per 200ml soil of weeds sampled from 44 sites in South Africa. 66

Table 20: Prominence value (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. per 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 67

Table 21: Prominence value (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zae* per 200ml soil of weeds sampled from 44 sites in South Africa. 69

Table 22: Prominence value (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zaeae* per 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 70

Table 23: Prominence value (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystrera* per 200ml soil of weeds sampled from 44 sites in South Africa. 72

Table 24: Prominence value (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystrera* per 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa. 74

Table 25: Prominence value (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* per 200ml soil of weeds sampled from 44 sites in South Africa. 76

Table 26: Prominence value (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* per 200ml soil of weeds collected from 34 sites located in the eastern and 10 localities western regions of South Africa. 76

Chapter 3

Table 1: Sequence of the Primers (Zijlstra, 2000 & Zijlstra *et al.*, 2000) 85

LIST OF FIGURES

Chapter 1

Figure 1.	<i>Amaranthus hybridus</i> (Botha, 2001)	3
Figure 2.	<i>Bidens bipinnata</i> (Botha, 2001)	4
Figure 3.	<i>Xanthium strumarium</i>	5
Figure 4.	<i>Chenopodium carinatum</i> (Bromilow, 2001)	6
Figure 5.	<i>Argemone ochroleuca</i> (Botha, 2001)	7
Figure 6.	<i>Cynodon dactylon</i> (Bromilow, 2001)	8
Figure 7.	<i>Cyperus rotundus</i> (Bromilow, 2001)	9
Figure 8.	<i>Datura stramonium</i> (Botha, 2001)	10
Figure 9.	<i>Eleusine corocana</i> (Botha, 2001)	11
Figure 10.	<i>Hibiscus cannabinus</i>	12
Figure 11.	<i>Hibiscus trionum</i>	12
Figure 12.	<i>Ipomoea purpurea</i> (Botha, 2001)	12
Figure 13.	<i>Lepidium africanum</i>	13
Figure 14.	<i>Tagetes minuta</i> (Botha, 2001)	14

Chapter 2

Figure 1. Rainfall distribution for the eastern and western regions of South Africa. 29

Figure 2A&B. Localities where weed species were sampled in the western (Northern Cape) and eastern (Limpopo and Mpumalanga) regions. 30

Figure 2C&D. Localities where weed species were sampled in the eastern (KwaZulu-Natal and Eastern Cape) region. 31

Chapter 3

Figure 1. Amplification products of PCR reactions using primers Fh and Rh for *M. hapla* template DNA of *Meloidogyne* juveniles. 88

Figure 2. Amplification products of PCR reactions using primers Fjav and Rjav for *M. javanica* and DNA of *Meloidogyne* juveniles. 88

Figure 3. Amplification products of PCR reactions using primers Finc and Rinc for *M. incognita* template DNA of *Meloidogyne* juveniles. 89

Figure 4. Amplification products of PCR reactions using primers Fa and Ra for *M. arenaria* template DNA of *Meloidogyne* juveniles. 90

Figure 5. Amplification products of PCR reactions using primers Fc and Rc for *M. chitwoodi* template DNA of *Meloidogyne* juveniles. 90

Figure 6. Amplification products of PCR reactions using primers Ff and Rf for *M. fallax* template DNA of *Meloidogyne* juveniles. 90

ABSTRACT

Plant-parasitic nematodes are ubiquitous, soil-borne pests that cause significant damage to a wide range of agricultural crops. A variety of weeds occurring in small-scale farming systems often serve as reservoirs for these parasites and compete with crop plants for light, water and nutrients. This study focussed on the association between plant-parasitic nematodes and weeds by identifying predominant nematode species or genera as well as weed species or genera that most frequently occur in small-scale farming systems in South Africa. A nematode survey was conducted at 44 sites located in the eastern (Eastern Cape, Limpopo, KwaZulu-Natal and Mpumalanga provinces) and western (Northern Cape province) resource-poor farming regions of South Africa. The sampled areas were divided into these regions based on the substantial difference in rainfall between east (wet) and west (dry). Thirty-seven weed species and 33 genera were identified as hosts of plant-parasitic nematodes during this survey which differed substantially with regard to their frequency of occurrence in the two regions. *Cynodon dactylon* had the highest frequency of occurrence for both the eastern and western regions. With regard to plant-parasitic nematodes, 20 species and 12 genera were reported for the first time in South Africa to parasitise weeds. Root-knot nematodes (*Meloidogyne* spp.), followed by *Pratylenchus zeae*, *Helicotylenchus dihystra* and *Rotylenchus unisex* were generally the predominant endo- and semi-endoparasites extracted both from root and soil samples in the two regions. *Meloidogyne* species identified by means of molecular techniques were *M. javanica* and *M. hapla*, with the latter species generally being predominant at some sites in the eastern region, but *M. javanica* was predominant at some sites located in both the eastern and western regions. In terms of the association between plant-parasitic nematodes and weeds, the four predominant nematode species or genera mentioned above had the highest frequency of occurrence in root as well as soil samples of *C. dactylon*. They, however, differed substantially in terms of their predominance on weeds for the two regions as well as when data were pooled over the two regions. Root-knot nematodes were predominant in the

predominant in the roots of *Chloris virgata* and *Flaveria bidentis* in the eastern and western regions, respectively, while the genus was predominant in roots of *Bidens bipinnata* when pooled over the two regions. *Pratylenchus zae* was predominant in roots of *Cyperus esculentus* in the eastern region and in roots of *Sonchus oleraceus* in the western areas. Although *H. dihystra* was predominant in roots of *Nicandra physalodes* and *S. oleraceus* in the eastern and western regions, respectively, it was predominant in roots of *Hibiscus. trionum* when data were pooled over the two regions. *Rotylenchus unisex* was predominant in roots of *N. physalodes* in the eastern region, while it was predominant in roots of *Bryophyllum* spp. occurring in the western region. Predominance of these plant-parasitic nematodes also differed with regard to numbers extracted from the soil samples for the weed species as well as for the two regions. Although this study focussed on the association of plant-parasitic nematodes with weeds, free-living and predatory members of the families Rhabditidae and Mononchidae were also extracted from these samples. Weeds identified during this study that maintain plant-parasitic nematodes, particularly root-knot nematodes, could have a negative impact on crop production when they are not eradicated timely and effectively. This problem is of particular significance in resource-poor, subsistence-farming systems where literacy and knowledge levels are low.

OPSOMMING

Plantparasitiese aalwurms is alomteenwoordige, grondgedraagde organismes wat wêreldwyd betekenisvolle oesverliese by 'n wye omvang landbougewasse tot gevolg het. Hierdie parasiete word deur 'n wye reeks onkruidspesies wat in kleinboerstelsels voorkom onderhou en ding met gewasse mee om beskikbare lig, grondwater en voedingstowwe. Hierdie studie fokus derhalwe op die verwantskap tussen plantparasitiese aalwurms en onkruid wat algemeen in landerye van plaaslike kleinboere voorkom. 'n Aalwurmopname wat 44 lokaliteite in die oostelike (Oos Kaap, Limpopo, KwaZulu-Natal en Mpumalanga provinsies) sowel as westelike gebiede (Noordkaap provinsie) ingesluit het, is onderneem. Die twee streke is ingedeel op grond van uiteenlopende verskille ten opsigte van reënval tussen die twee streke. Sewe-en-dertig onkruidspesies van 33 genusse is, tydens opname as gashere van plantparasitiese aalwurms geïdentifiseer. Die voorkoms van onkruid het betekenisvol verskil in van die oostelike en westelike gebiede maar *Cynodon dactylon* het die hoogste voorkoms getoon vir beide gebiede. Met betrekking tot plantparasitiese aalwurms is 20 spesies en 12 genusse vir die eerste keer gerapporteer as parasiete van onkruid in Suid-Afrika. Knopwortelaalwurms (*Meloidogyne* spp.), gevolg deur letselaalwurm, *Pratylenchus zaeae* en spiraalaalwurm *Helicotylenchus dihystra* en *Rotylenchus unisex* was die mees prominente endo- en ektoparasitiese aalwurm-groepe wat uit beide wortel- en grondmonsters geëkstraheer is in beide die oostelike en westelike gebiede. *Meloidogyne javanica* en *M. hapla* is gedurende hierdie studie deur middel van molekulêre tegnieke as die mees prominente knopwortelaalwurmspesies in die wortels van onkruid geïdentifiseer. *Meloidogyne javanica* was mees prominent in onkruid vanaf lokaliteite in beide die oostelike en westelike gebiede, terwyl *M. hapla* gedomineer het in die oostelike gebied. Ten opsigte van die verwantskap tussen plantparasitiese aalwurms en onkruid het die vier bogenoemde mees prominente spesies die hoogste voorkoms gehad in wortel- en grondmonsters van *C. dactylon*. Laasgenoemde verwantskap het egter wesenlik verskil tussen die twee gebiede, sowel as wanneer al 44 lokaliteite se data bymekaar-gevoeg is. Knopwortelaalwurms was mees prominent in wortels van *Chloris virgata* in die

oostelike en *Flaveria bidentis* in die westelike gebied. Wanneer die twee gebiede se data egter saamgevoeg is, was die prominensie van knopwortelaalwurms hoogste in die wortels van *Bidens bipinnata*. *Pratylenchus zae* was meer prominent in die wortels van *Cyperus esculentus* in die oostelike gebied, maar in *Sonchus oleraceus* in die westelike gebied. *Helicotylenchus dihystra* was meer prominent in wortels van *Nicandra physalodes* in die oostelike en *S. oleraceus* in westelike gebied. *Rotylenchus unisex* was meer prominent in wortels van *N. physallodes* in die oostelike en *Bryophulium* spp. in die westelike gebied. Dieselfde tendens het voorgekom vir hierdie vier plantparasitiese aalwurmspesies ten opsigte van grondmonsters wat die twee gebiede aanbetref. Alhoewel hierdie studie gefokus het op die verwantskap tussen plantparasitiese aalwurmspesies/genusse met onkruide, is vrylewende en predatoriese individue van die families Rhabditidae en Mononchidae ook geïdentifiseer. Onkruidspesies/genusse wat tydens hierdie studie geïdentifiseer is, kan 'n negatiewe invloed op die verbouing van landbougewasse hê indien dit nie betyds en effektief verwyder of beheer word nie. Dit geld veral in kleinboerestelsels waar geletterdheid en kennis wat plantparasitiese aalwurms aanbetref, onvoldoende is.

Chapter 1: Introduction

1.1 General introduction

This study focuses on the association between weed species and plant-parasitic nematodes, which both are omnipresent in resource-poor, subsistence-agricultural systems. In this introductory chapter the background, classification and control of relevant weeds will be discussed. Root-knot nematodes (*Meloidogyne* spp.), as a very important agricultural constraint will be elaborated on, with special reference to their lifecycle and interaction with weeds. Management of both plant-parasitic nematodes and weeds will be discussed in brief.

1.2 Weeds

To be considered a weed a plant species must have certain characteristics that distinguish it from other plants and allow it to invade, become established and persist in agricultural production systems (Monaco *et al.*, 2002). According to Bromilow (2001), weeds are usually vigorous growers, compete with crops for water, light, space and nutrients and can easily invade a wide range of ecological niches. Although weeds thrive under favourable conditions, they also have well developed survival mechanisms for stress conditions (Bridge, 1996).

1.3 Definition and characteristics of weeds

According to Bromilow (2001) a weed is a plant that grows in an undesirable place, for example in fields where horticultural or agricultural crops are produced. Weeds are also defined as plants that are able to establish themselves in cultivated habitats and have the potential to suppress or displace desirable plant populations that are cultivated deliberately. The latter are crops or plants that would be of ecological, industrial, life-supporting or aesthetic interest (Navas, 1991). Weeds, furthermore, have been described by different authors as colonisers or pioneer species in disturbed fields, for example plants growing in waste sites, along roadsides, or in potentially productive environments (Bridge, 1996; Bridges, 1994; Wyse, 1994).

1.4 Categorisation and life cycle of weeds

Weeds are placed in three categories, namely annuals, biennials and perennials (Baker, 1974). Each group contains broadleaf weeds (dicotyledon) and grasses (monocotyledon) as described by Rao (2000). The latter author, also placed annual weeds in two groups, namely summer and winter weeds.

Annual weeds complete their life cycle within a year or less, they are propagated by seeds and are normally considered easy to control (Freed, 1980). Summer annual weeds generally hamper the production of summer crops such as maize, sorghum, soybean, cotton, groundnut, tobacco and most vegetable crops (Monaco *et al.*, 2002).

Biennial weeds have a lifespan of at least two years (Bridges, 1994), while, perennial weeds usually grow actively for more than two years (Monaco *et al.*, 2002). Such weeds are propagated by seeds as well as vegetatively by underground organs such as rhizomes, stolons, bulbs or tubers (Monaco *et al.*, 2002).

1.5 Economically important weeds that commonly occur in subsistence-farming systems in South Africa

Weeds suppress crop production and increase the cost of agricultural products since they reduce crop yields, limit land use, result in higher costs for disease and pest control, adversely affect the optimal quality of produce and adversely affect use of available water (Monaco *et al.*, 2002). The majority of the abovementioned costs are related to the control of alien plants that have been introduced and became major weeds in local cropping systems (Pimentel *et al.*, 1999). Certain weeds may also reduce plant yield by releasing allelopathic compounds (Monaco *et al.*, 2002). Furthermore, weeds may maintain a range of pest and disease organisms, thus increasing opportunities for those organisms to reinfest agricultural crops in succeeding seasons (Zimdahl, 1999). A proper weed management programme should, therefore, include a well-planned approach that focuses on integration of control strategies with all other practices (Thill *et al.*, 1991). Weed control

strategies entail chemical, cultural and biological practices that could be integrated for efficient removal of these plants.

1.5.1 Classification

All weed species listed in this study belong to the Kingdom Plantae, Division Magnoliophyta and Class Magnosiopsida, except for *Cynodon dactylon* L., *Cyperus rotundus* L and *Eleusine corocana*, which belong to the class Liliopsida.

1.5.2 Origin, description and control

1.5.2.1 *Amaranthus hybridus* L.

Amaranthus hybridus L. (Fig. 1), generally known as 'common pigweed', (Bromilow, 2001), is an exotic, broadleaf weed originating from various parts of The United States of America and mostly occurs in temperate as well as tropical regions (Enama, 1994). *Amaranthus* species belong to the order Caryophyllales and the family Amaranthaceae. It is the most abundant and widely distributed broadleaf weed in South Africa (Bromilow, 2001) and is a popular food source for subsistence farmers (Enama, 1994).



Figure 1. *Amaranthus hybridus* (Botha, 2001)

Common pigweed competes with most agricultural crops for water, nutrients, light and space (Botha, 2001). *Amaranthus* species have also been reported as alternative hosts to *Verticillium* species that attacks several crops including potato, tomato and cotton (Bromilow, 2001). All species of *Amaranthus* are susceptible to the normal broadleaf-weed herbicides and are easy to remove manually when still immature.

1.5.2.2 *Bidens bipinnata* L.

Bidens bipinnata (Fig. 2) was introduced into South Africa from Eurasia (Bromilow, 2001), belongs to the order Asterales and family Asteraceae (Holm *et al.*, 1977). Seeds of this weed are known as 'blackjack' that stick to clothing, hair and animal hides. *Bidens* is a genus comprising of approximately 200 species (Enama, 1994).



Figure 2. *Bidens bipinnata* (Botha, 2001)

Bidens species are cosmopolitan in their distribution and are also alternative hosts to *Verticillium* fungus species that attack several agricultural crop plants (Bromilow, 2001). Due to the uniform and shallow germination of *Bidens* spp., they are relatively easy to control with post-emergence herbicides (Bromilow, 2001).

1.5.2.3 *Xanthium strumarium* L.

Xanthium strumarium 'cockbur' (Fig. 3) belongs to the order Asterales, family Asteraceae and originated from South America (Bromilow, 2001). It is often found along streams and rivers (Botha, 2001). It is a common and poisonous arable weed that contaminate wool of sheep (Bromilow, 2001).

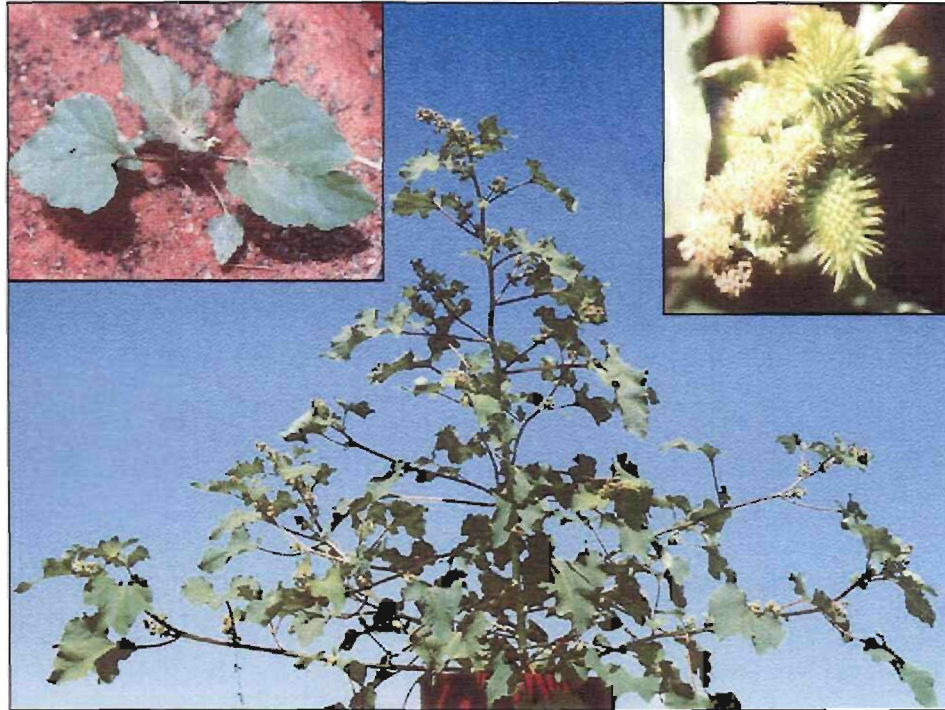


Figure 3. *Xanthium strumarium*

Xanthium strumarium species are usually successfully controlled with post-emergence herbicides and could also be easily controlled by shallow cultivation at the seedling stage of the weed (Bromilow, 2001).

1.5.2.4 *Chenopodium carinatum* L.

Chenopodium species (Fig. 4) originated from Australia, but are now widespread throughout South Africa (Bromilow, 2001). *Chenopodium* spp. belong to the order Caryophyllales and the family Chenopodiaceae (Holm *et al.*, 1977).



Figure 4. *Chenopodium carinatum* (Bromilow, 2001)

Chenopodium carinatum causes yield losses in most agricultural crops, especially vegetables in Mpumalanga, Limpopo, Western and Eastern Cape provinces (Bromilow, 2001). This weed species could be eradicated by most pre- and post-emergence herbicides and can also easily be controlled by cultivation during the seedling stage of the weed (Botha, 2001).

1.5.2.5 *Argemone ochroleuca* L.

Argemone ochroleuca belong to the order Papaverales and family Onagraceae (Holm *et al.*, 1977). It is commonly known as 'white-flower mexican poppy' (Chester *et al.*, 1997). This weed species (Fig. 5) is indigenous to South Africa (Holm *et al.*, 1977).



Figure 5. *Argemone ochroleuca* (Botha, 2001)

The seeds of *A. ochroleuca* contaminate wool of sheep (Bromilow, 2001). *Argemone* species are usually successfully controlled with post-emergence herbicides and could also be easily controlled by shallow cultivation at the seedling stage (Bromilow, 2001).

1.5.2.6 *Cynodon dactylon* L.

Cynodon is a genus comprising nine species of grasses, which are native to temperate warm and tropical regions (Botha, 2001). According to Bromilow (2001) this grass probably originated from tropical Africa or Asia but became widespread throughout the world. *Cynodon dactylon* ('couch grass') (Fig. 6) is a creeping perennial and spreads by means of stolons and underground rhizomes (Bromilow, 2001). *Cynodon* spp. belong to the order Poales and the family Poaceae (Holm *et al.*, 1977).

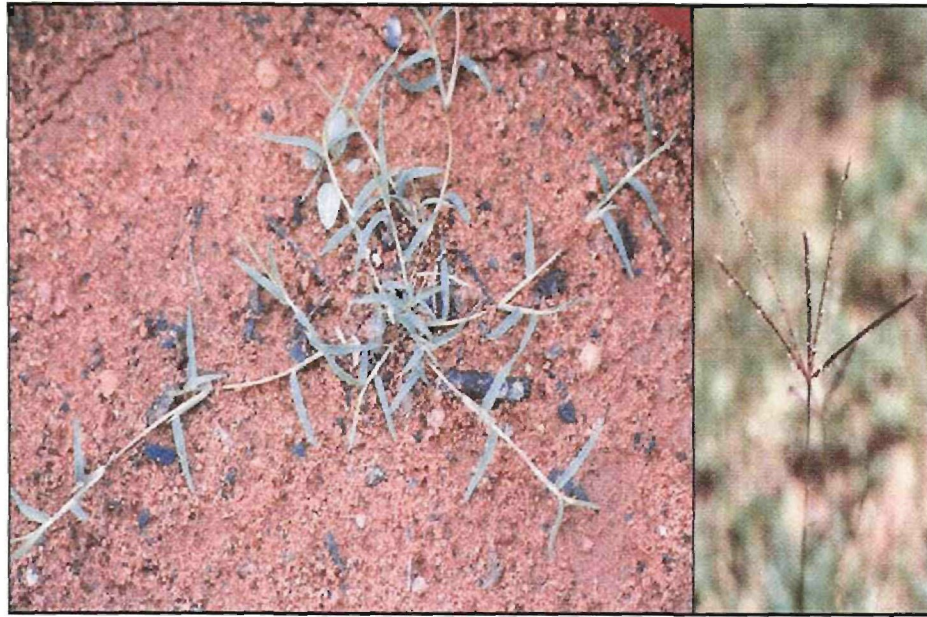


Figure 6. *Cynodon dactylon* (Bromilow, 2001)

Couch grass is a vigorous grower, particularly in sugarcane fields. Due to its tough growth habit it is valuable for combating erosion (Bromilow, 2001). This grass is, however, extremely difficult to eradicate with chemicals as well as manually due to its extensive underground root system (Botha, 2001).

1.5.2.7 *Cyperus rotundus* L.

Cyperus is a large genus consisting of approximately 600 species of sedges and is distributed worldwide throughout tropical to temperate regions of the world (Bromilow, 2001). *Cyperus rotundus* (Fig. 7) most probably originated from Africa, Europe or Asia (Botha, 2001). These species are annual or perennial plants, are mostly aquatic and grow in still or slow-moving water (Botha, 2001). *Cyperus* spp. belongs to the order Poales and the family Cyperaceae (Holm *et al.*, 1977).



Figure 7. *Cyperus rotundus* (Bromilow, 2001)

C. rotundus ('nutsedge') is one of the most aggressive weeds (Rao, 2000). Its presence in a field significantly reduces crop yield due to its competitiveness for water and nutrients and also because it is allelopathic to other plants (Hance & Holly, 1990). Nutsedge is difficult to control since it resists more selective herbicides (Botha, 2001). It is therefore crucial to identify the weed accurately before expensive control programmes are initiated (Bromilow, 2001).

1.5.2.8 *Datura stramonium* L.

Datura stramonium (Fig. 8) is native to North America and is a declared weed in South Africa (Holm *et al.*, 1977). This species is widespread and is economically important in most agricultural crops (Bromilow, 2001). *Datura stramonium* is cultivated in Central Europe and South America for the production of the antidote atropine (Bromilow, 2001) and belongs to the order Solanales and the family Solanaceae (Holm *et al.*, 1977).



Figure 8. *Datura stramonium* (Botha, 2001)

Datura species are declared weeds because of their poisonous properties and also due to their tall and aggressive growth habit (Uva *et al.*, 1997). Seed of these species contaminates maize grain and the plants are extremely difficult to control, both chemically and manually (Bromilow, 2001). Reliable control can, however, be achieved with post-emergence herbicides because seed of *Datura* spp. germinates deep down the soil profile. For this reason *Datura* spp. generally germinate after application of pre-emergence herbicides (Bromilow, 2001).

1.5.2.9 *Eleusine corocana* (L.) Gaertn.

Eleusine corocana ('African goosegrass') (Fig. 9) is an indigenous, annual grass weed competing with agricultural and horticultural crops (Holm *et al.*, 1977). African goosegrass is, however, the main food grain for most people in drought-stricken areas of India and Sri Lanka (Bogdan, 1977). *Eleusine corocana* belongs to the order Cyperales and the family Poaceae (Holm *et al.*, 1977).

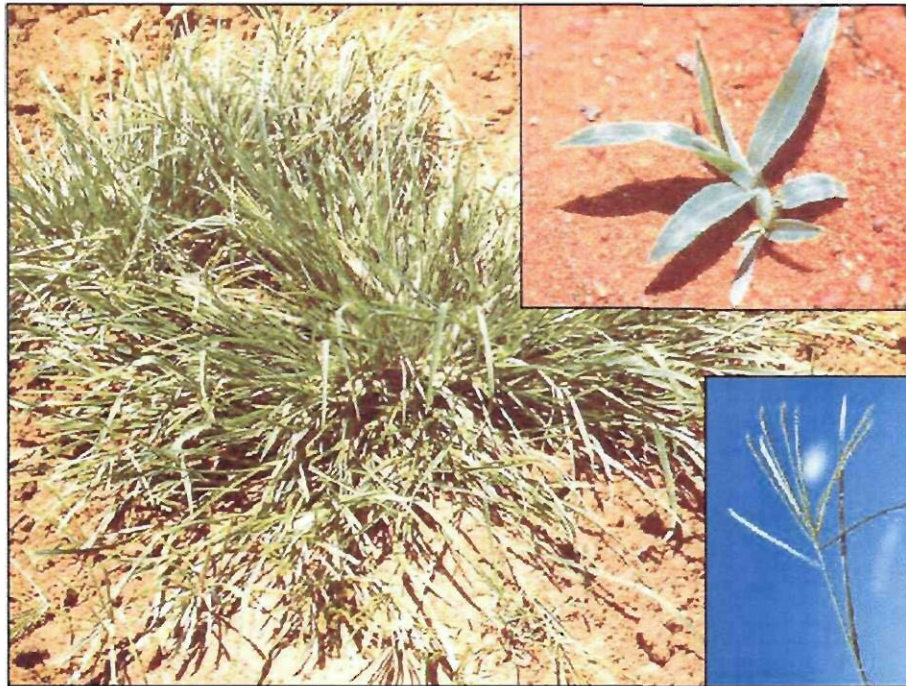


Figure 9. *Eleusine corocana* (Botha, 2001)

African goosegrass is difficult to weed by hand because of its tough root system (Bromilow, 2001). According to Botha (2001) the seeds of *E. corocana* are milled as a substitute for flour in times of food scarcity. Cultivation is not an effective control method for *E. corocana* since it continues to germinate throughout the summer growing season (Botha, 2001). However, it can be controlled by grass herbicides and by a range of pre-emergence broadleaf-weed herbicides (Bromilow, 2001).

1.5.2.10 *Hibiscus cannabinus* L. and *H. trionum* L.

Hibiscus species are members of the order Malvales and the family Malvaceae (Holm *et al.*, 1977). In terms of economic importance they are the third largest fibre crop after cotton and jute (Starr & Page, 1990). The group is indigenous to Africa and originated from Sudan (Holm *et al.*, 1977). Although the crop is commonly cultivated for both food and fiber in West Africa (Bromilow, 2001), it is a declared weed due to its omnipresence in crop fields. *Hibiscus cannabinus* (Fig. 10) and *H. trionum* (Fig. 11) are common throughout South Africa and many other warm parts of the world (Botha, 2001).



Figure 10. *Hibiscus cannabinus*

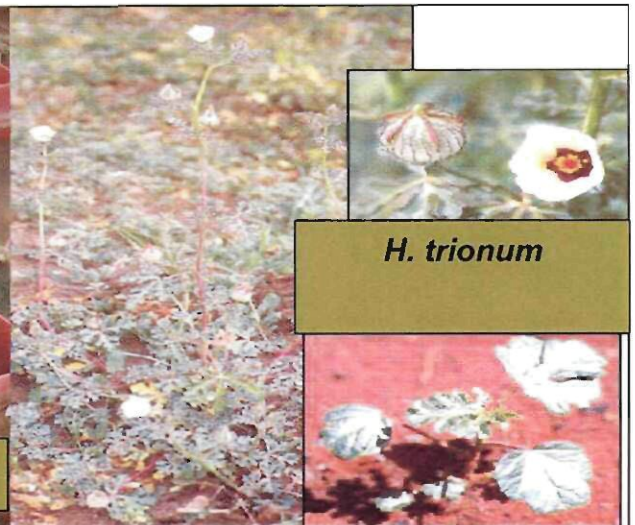


Figure 11. *Hibiscus trionum*

Hibiscus species can be competitive in annual crop production and interfere with the harvesting process (Bromilow, 2001). *Hibiscus* species can be controlled with post-emergence chemicals, but since its roots penetrate deep into the soil profile it is less susceptible to pre-emergence herbicides (Bromilow, 2001).

1.5.2.11 *Ipomoea purpurea* L.

Ipomoea purpurea (Fig. 12), commonly known as 'common morning glory' is native to Mexico and Central America (Bromilow, 2001). This weed can grow in a wide range of soil types (Uva *et al.*, 1997) and belong to the order Solanales and the family Convolvulaceae (Holm *et al.*, 1977).



Figure 12. *Ipomoea purpurea* (Botha, 2001)

Maize grain consignments are downgraded even when only a small number of *Ipomoea* seeds are present (Botha, 2001). The climbing habits of common morning glory interfere with the harvesting of crops since plants are stringed together (Bromilow, 2001). Common morning glory is an extremely difficult weed to control since it escapes most pre- and post-emergence herbicide treatments (Bromilow, 2001). However, common morning glory is very sensitive to the hormone-type herbicides (Bromilow, 2001).

1.5.2.12 *Lepidium africanum* L.

Lepidium halepense (Fig. 13.), commonly known as 'pepper weed' originated from South America (Bromilow, 2001). Pepper weed belongs to the order Capparales and the family Brassicaceae (Holm *et al.*, 1977).



Figure 13. *Lepidium africanum*

Lepidium species are common throughout South Africa and are resistant to frost. Therefore, this weed often causes problems in winter cropping systems (Holm *et al.*, 1977). Pepper weed is susceptible to most of the conventional registered herbicides (Bromilow, 2001).

1.5.2.13 *Tagetes minuta* L.

The genus *Tagetes* comprises approximately 60 species including annual and perennial herbaceous plants from the daisy family (Holm *et al.*, 1977). *Tagetes* species belong to the order Asterales and the family Asteraceae (Holm *et al.*, 1977). *Tagetes* species are native to the southwestern United States, Mexico and South America (Motsinger *et al.*, 1977). It is commonly known as tall khakiweed, is widespread and a serious weed of many crops (Bromilow, 2001). Maize grain consignments containing tall khakiweed seeds are usually downgraded (Bromilow, 2001). Cultivation may be effective to control *Tagetes* seedlings, although it may result in the spread of seed on the field surface where it can subsequently germinate. Tall khakiweed (Fig. 14) is susceptible to most pre-emergence herbicides since it is dependent on sunlight for germination (Bromilow, 2001). Other important *Tagetes* spp. classified as weeds are *T. erecta*, *T. patula* and *T. tenuifolia*.

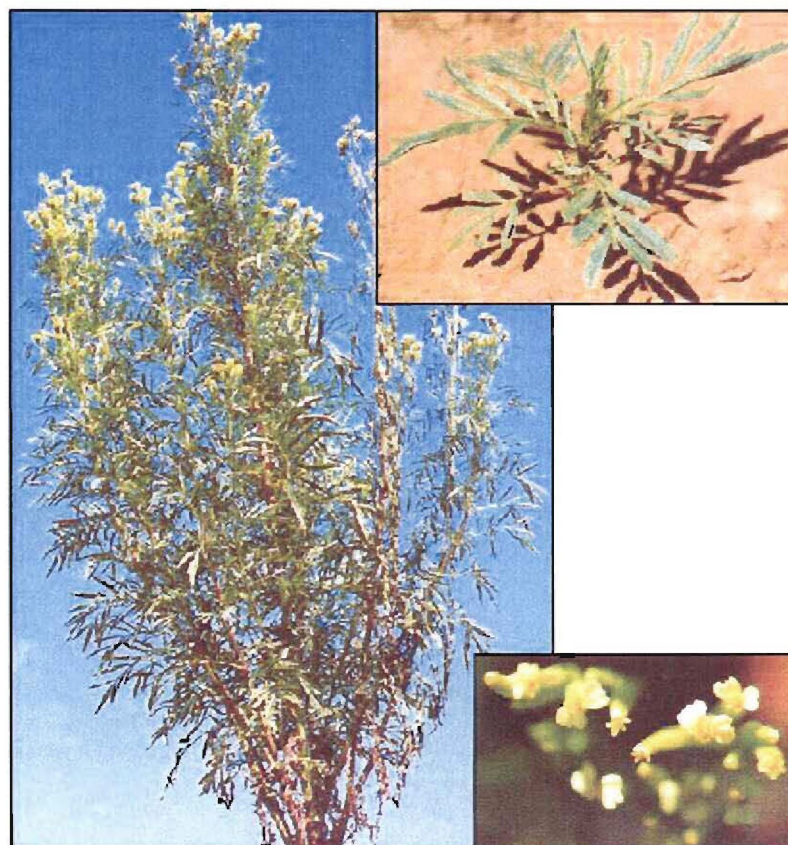


Figure 14. *Tagetes minuta* (Botha, 2001)

1.6 Diseases and pests associated with weeds

Weeds that grow within as well as on the edges of crop fields, serve as hosts and as a source of infestation for a variety of pests and diseases (Zimdahl, 1999), including plant-parasitic nematodes (Bélair & Benoit, 1996; Keetch & Buckley, 1984; Coyne *et al.*, 1999). According to Agrios (1997) weeds may act as alternative hosts for some cotton pathogens and enable the survival of these pathogens between subsequent cotton crops. Cooper and Harrison (1973) observed that potato blackleg disease caused by *Erwinia carotovora* var. *atroseptica* and potato soft rot caused by *E. carotovora* var. *carotovora* are maintained by common lambsquarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*) and black nightshade (*Solanum ptycanthum*). Furthermore, volunteer wheat is a primary host of wheat streak mosaic virus (Zimdahl, 1999). With regard to vegetables, particularly sugarbeet and tomato, Russian thistle (*Salsola kali*) is an alternative host for the curly-top virus (Young, 1991) and the beet leafhopper (*Circulifer tenellus* spp.). Leafhoppers and curly-top virus of sugarbeet infest weeds and use them as a breeding host to increase their inoculum density and infect subsequent crops (Piemiesel, 1954).

In some cases plant species may be regarded as weeds because they are alternate hosts in the life cycles of some plant pathogens (Hance & Holly, 1990). An example is a plant species belonging to the family Cruciferae that host the pathogen *Plasmodiophora brassicae* (clubroot) that infects Brassicas (Hance & Holly, 1990). King (1966) reported that the stem rust fungus (*Puccinia graminis* var. *tritici*) uses the weed 'European barberry' (*Berberis vulgaris*) as an alternate host, which resulted in estimated wheat yield losses of more than 600 million bushels per year during the early 1960s as a result of this fungal disease. Legumes and members of the Chenopodiaceae also act as hosts for aphids that attack field and broad beans (Hance & Holly, 1990).

Numerous authors reported that a wide range of weed species are parasitised by plant-parasitic nematodes and thus act as secondary hosts to these parasites, which finally infect other follow-up agricultural crops (De Waele *et al.*, 1990; Hance & Holly, 1990; Keetch, 1989). An example is bladder ketmia

(*H. trionum*), which maintain plant-parasitic nematodes (Keetch, 1989) and may act as an alternative host for the pathogens that causes Verticillium wilt, Fusarium wilt and Alternaria leaf spot of cotton.

Since this study focuses on the association between weeds and plant-parasitic nematodes, the following section will elaborate on these parasites, in particular root-knot nematodes.

1.7 Plant-parasitic nematodes

Plant-parasitic nematodes often become a limiting factor in crop production, especially when crop or disease management practices are employed that favour population build-up of a particular plant-parasitic nematode species (Kinloch, 1998).

Crop yield and quality is limited worldwide due to infection by plant-parasitic nematode species, including root-knot nematodes (Sasser & Freckman, 1987). Yield losses attributed to plant-parasitic nematodes globally in life-sustaining crops such as grains, legumes, cassava, potato, sweet potato, banana, coconut, sugar cane and sugar beet are estimated at approximately 11 % (Agrios, 1997). In South Africa plant-parasitic nematodes account for variable yield losses to staple food crops such as maize, wheat, groundnut and banana (Keetch, 1989).

1.7.1 Classification morphology and biology

Nematodes belong to the animal kingdom Animalia and comprise a large phylum (Nematoda) that includes plant, animal, human and free-living species. Parasitism of plants by nematodes is restricted to two classes, namely the Adenophora and Secernentea. Adenophorean parasites are confined to the two families Longidoridae and Trichodoridae within the order Dorylaimida (Maggenti, 1981).

Plant-parasitic nematodes are obligate, biotrophic organisms that obtain nutrients only from the cytoplasm of living plant cells (Agrios, 1997). The stylet distinguishes plant-parasitic nematodes from free-living nematodes (Luc *et al.*,

2005). In the order Tylenchida (including the Tylenchina and Aphelenchina) the stylet is known as a stomato stylet. In the Longidoridae it is an odontostylet and an onchiostylet in the Trichodoridae.

Although nematodes are generally wormlike in shape, mature females of some genera, namely *Meloidogyne*, *Heterodera*, *Nacobbus* have swollen, saccate-like bodies (Agrios, 1997). Nematodes have no appendages and are barely visible to the naked eye (Agrios, 1997) but are, however, easily observed under a microscope (Agrios, 1997). The nematode body is generally transparent and covered by a colourless, striated cuticle. The digestive system comprises a hollow tube, stretching from the mouth through the oesophagus, intestine, rectum and terminates in the anus (Agrios, 1997). The male reproductive system consists of sexual organs, namely the testis, seminal vesicle and *vas deferens*, which are attached to an ejaculatory duct that opens into the rectum. In females the ovaries are followed by an oviduct, uterus and vagina, which terminate in a vulva (Ferraz & Brown, 2002). The most common mode of reproduction is either amphimixis (cross-fertilization) or parthenogenesis (obligatory or facultative) (Luc *et al.*, 1993). The life cycles of different nematode genera are basically similar and generally advance through four moults, with the first mold usually being within the egg (Agrios, 1997).

1.7.2 Feeding habits of plant-parasitic nematodes

Plant-parasitic nematodes are often separated into two major groups according to their feeding habits, namely ectoparasites and endoparasites, which can both be migratory or sedentary (Boerma & Hussey, 1992). Most plant-parasitic nematodes have a hollow stylet, which serve to inject enzymatic secretions in pierced plant cells for subsequent ingestion of the cytoplasmic content (Ferraz & Brown, 2002). Ectoparasitic nematodes generally remain outside their host's tissue and feed on outer cell layers, (Boerma & Hussey, 1992). Migratory ectoparasites remain vermiform throughout their life cycle and feed at selected sites for only a limited period (Boerma & Hussey, 1992). Endoparasitic nematodes maintain an intimate relationship with their host and for the completion of their life cycle they

depend on nutrients withdrawn from specialised feeding cells that are induced inside the roots of susceptible plants (Sasser & Freckman, 1987). For example, syncytia are formed as a result of feeding by cyst nematodes while giant cells are initiated for feeding by root-knot nematodes (Huang, 1985).

1.7.3 General

Root-knot nematodes are obligate, sedentary endoparasites (Kleyhans, 1991) that complete most of their life cycle within the roots of a host plant. The nematodes survive in soil in the egg phase and also as second-stage juveniles (J2). *Meloidogyne javanica* and *M. incognita* reproduce by mitotic parthenogenesis (Xu *et al.*, 2001), with a strong reproductive potential of multiple generations per season (Trudgill, 1997). The life cycle of both root-knot nematode species is generally the same, requiring only 21-25 days at 26°C for completion of their life cycle (Taylor & Sasser, 1978).

The vermiform, infective J2 hatch from eggs. This infective, motile J2 migrates through soil and penetrates root tips of suitable host plants where it establishes a permanent feeding site by thrusting its stylet into plant cells surrounding the nematode's head (Dropkin, 1980). This feeding activity induces the formation of giant cells in which nutrients are available as a source of food for the nematode. Feeding causes the vermiform J2 to enlarge and undergo morphological changes to become sedentary and an adult female after three more molts (Sikora & Fernandez, 2005). The mature female is embedded inside the root and deposits several hundred eggs into a gelatinous matrix, called an egg sac or egg mass. Continuous feeding by nematodes adversely affects the normal physiological processes of the host within the root system, which is phenotypically manifested in the formation of root galls (Xu *et al.*, 2001). Root galls vary in size and shape, depending on the type of plant, nematode population levels and species of root-knot nematode present in the soil (Sikora & Fernandez, 2005). Plant roots infected with root-knot nematodes are seriously hampered in their main functions to take up and transport water and nutrients to other parts of the plant (Sikora & Fernandez, 2005). When susceptible plants are infected at the seedling stage, losses are usually severe. It may even result in complete destruction of the

crop (Agrios, 1997). Below-ground symptoms usually include galled root systems and/or disfigured tubers (potato, carrot and beetroot), with subsequent reduced market value (Agrios, 1997). Above-ground symptoms due to infection by plant-parasitic nematodes may include stunted growth, wilt despite sufficient irrigation, yellowing of leaves, reduced yields and premature death of plants (Sikora & Fernandez, 2005).

The duration of the parasitic phase of the nematode within the host plant is affected by genetic qualities of the host plant (Johnson & Fassuliotis, 1984), environmental conditions, soil temperature, host suitability and soil type (Abawi & Chen, 1998). These factors influence the relationship between nematode and plant, constituting a total host-nematode inter-relationship complex (Johnson & Fassuliotis, 1984).

1.7.4 Nematode control

Nematode control strategies can be categorised into two major groups, namely chemical and cultural control (Bridge, 1996).

1.7.4.1 Chemical nematode control

Nematicides (fumigants and non-fumigants) have been used extensively since the early 1900's (Ferraz & Brown, 2002) in order to reduce nematode populations in high-value crops such as flowers, vegetables (Netscher & Sikora, 1993), tobacco (Shepherd & Barker, 1993), legumes (Sikora & Greco, 1993), cereals (Swarup & Sosa-Moss, 1993) and a range of other agricultural and horticultural crops (Luc *et al.*, 1993).

Due to increased pressure from environmentalists, the use of nematicides is declining globally (Ferraz & Brown, 2002). Nematicides will, however, continue to play a major role in the reduction of nematode populations for a variety of crops, as well as for use in regulatory and quarantine procedures (Johnson, 1985).

1.7.4.2 Cultural nematode control

Plant-parasitic nematodes can be controlled in both commercial and subsistence agricultural systems by integrating different farming practices. This could be achieved by preventing the spread of nematodes using nematode-free planting material (Bridge, 1996). Methods to achieve this include applying quarantine measures by using healthy, uninfected crop tubers (vegetable, yam, potato, etc.) (Luc *et al.*, 1993), heat treatments hot-water dipping and soil solarisation (Luc *et al.*, 1993). Direct, non-chemical, cultural and physical control methods (Bridge, 1996) could also be used. The most popular and cost-effective nematode control strategies in both commercial and subsistence agriculture are host plant resistance (Bridge, 1996), crop rotation (Sasser, 1980) and the use of trap crops (Ferraz & Brown, 2002). According to Ploeg (2002), *Tagetes* species produce derivatives of chemicals that are toxic to root-knot nematodes and are used to control these parasites. Regular and timely removal of weeds is also important to minimize the effect of root-knot nematodes on crop production (Bromilow, 2001).

1.8 Plant-parasitic nematodes, other than root-knot nematodes, associated with weeds

Weeds play a significant role in the ecology of many plant-parasitic nematodes that infect a range of agricultural and horticultural crops (Hooper & Stone, 1981). Weeds are, however, often neglected from the perspective of plant-parasitic nematode management and can, therefore, reduce the efficacy of crop rotation aimed at the management of plant-parasitic nematodes (Bélair & Benoit, 1996). Hollis (1977) reported that weed management has an impact on the population dynamics of plant-parasitic nematodes. However, according to Coyne *et al.* (1999) the impact of weeding on plant-parasitic nematode population densities depend on the level of interspecific competition between a specific weed and the crop, the mechanism of nematode parasitism (migratory or sedentary) and the host status of the relevant weeds.

According to Salawu *et al.* (1991), weed species that commonly occur in a wide range of cropping systems have not always been evaluated for host

suitability to plant-parasitic nematodes. Most weeds that are, however, susceptible to plant-parasitic nematodes may maintain nematode populations between and during crop growing seasons (Quénéhervé *et al.*, 1995). Such weeds are an important reservoir for nematodes during periods when crop hosts are absent (Salawu *et al.*, 1991). Weeds that may act as hosts for plant-parasitic nematodes are often deliberately not weeded. For example, on *Amaranthus* species that are used as a food source nematode populations may build-up to unacceptably high levels. Furthermore, the weed species *Gynandra gynandropsis* and *Solanum nigrum* are often semi-cultivated together with the main crop in order to harvest it for food (Martin, 1959). Such weed species may, however, serve as indicator hosts for the presence of plant-parasitic nematodes before susceptible crops are planted (Hogger & Bird 1976).

The interaction between weeds and plant-parasitic nematodes generally adversely affects crop production, not only by reducing the potential benefit of crop rotations (Bélair & Benoit, 1996) but also by negatively affecting the use of nematode-resistant crops (Wong & Tylka, 1994). The abundance of weeds in a given field and the rate of nematode reproduction on specific weeds determine the magnitude of the effect that the weed has on plant-parasitic nematode population densities (McSorley, 1996). Although information on the distribution, occurrence and prominence of plant-parasitic nematodes on weeds is limited, a number of reports have been published on surveys or host-suitability aspects (Davis & Webster, 2005; Martin, 1959; Quénéhervé *et al.*, 2006; Quénéhervé *et al.*, 2000; Ishaq, 1992; Salawu *et al.*, 1991).

A nematode survey conducted by Ishaq (1992) and De Waele *et al.* (1990) showed that a variety of weeds maintained a wide range of plant-parasitic nematode genera and species. These include *Ditylenchus dispaci*, *D. destructor*, *Pratylenchus penentrans* and *Paratylenchus bukowinensis*. A greenhouse study by Kaplan and MacGowan (1982) indicated that ornamental weeds such as *Chamaedorea elegans*, *C. varigatum* and *Schinus terebinthifolius* were found to be suitable hosts to the lesion nematode *P. coffeae*. A study in the United States by Timper *et al.* (2004) showed that *C.*

dactylon was a poor host for *P. zaeae*, while a cover- crop sequence study in southern United States by Brodie *et al.* (1969) showed that *Panicum ramosum* supported high numbers of *P. brachyurus*. *Cynodon dactylon*, however, to a lesser extent maintained *P. brachyurus* and *Trichodorus christiei*, while *Indigofera hirsute* and *Crotalaria spectabilis* supported high numbers of *P. brachyurus*. *Tagetes minuta* did, however, not favour development of any parasitic nematode species present at that locality (Brodie *et al.*, 1969).

With regard to cyst nematodes, a wide range of weeds from the families Chenopodiaceae and Cruciferae were identified as hosts to these parasites, including, *Caladium bicolor*, *Commelina diffusa*, *Echinochloa colona* and *Phenax sonneratii* (Quénéhervé, 2000). Griffin (1982) observed differences in the host suitability responses of certain weeds to *Heterodera schachtii* in different localities in the USA and reported that weed host suitability is dependent on genetic differences in the nematode population as well as on weed biotypes.

Results from a nematode survey conducted in banana fields in Martinique where 41 weed species were sampled to extract plant-parasitic nematodes, indicated that all 24 weed species maintained adults and second-stage juveniles (J2) of *Rotylenchus reniformis*, 23 species hosted *Helicotylenchus* spp., 13 species hosted *Pratylenchus* spp. and *Hoplolaimus seinhorsti*, while *Rotylenchus reniformis* was consistently extracted from weed roots of the three plant families Euphorbiaceae, Poaceae and the Solaneaceae (Quénéhervé *et al.*, 2006). Results from the latter survey also revealed that nematode infection levels in some weeds such as *Caladium bicolor*, *Commelina diffusa*, *Echinochloa colona* and *Phenax sonneratii* equaled or exceeded the level of nematode infection in roots of banana plants that grew in the same field (Quénéhervé *et al.*, 2006). According to Webster and MacDonald (2001) *Desmodium tortuosum* is a predominant weed in peanut production in the southeastern USA that was identified as a good host for *R. reniformis*.

Populations of the ectoparasitic nematode, *Belonolaimus longicaudatus* is sufficiently suppressed by *Tagetes minuta*, *Crotalaria spectabilis* and *Indigofera hirsute*, while weeds such as *Panicum ramosum*, *Desmodium tortuosum* and *C. dactylon* stimulated populations of this nematode species (Brodie *et al.*, 1969). Furthermore, a study on cover crop sequences in the south of the USA indicated that the weed species *Panicum ramosum* maintained a rapid increase of *B. longicaudatus*, while *C. dactylon* also supported reproduction of the sting nematode.

A study by McSorley and Campbell (1980) showed that avocado roots maintained higher nematode populations in cropping systems where weeds occur as opposed to weed-free areas. *Trichodorus obtusus* caused more damage than *Paratrichodorus minor* to both *Agrostis palustris* and *C. dactylon* in a study done by Crow and Welch (2004) in the southern part of the USA. Sikora *et al.* (2001) showed that a wide variety of ecto- and endoparasitic nematode genera, namely *Helicotylenchus* spp., *Mesocriconema* spp., *Tylenchorhynchus* spp., *Hoplolaimus* spp. and *Meloidogyne* spp. were also associated with *C. dactylon* in golf courses in Alabama in the USA.

1.8.1 Root-knot nematodes

Root-knot nematodes are globally considered the economically most important plant-parasitic nematode group that infects nearly every crop (Sasser & Freckman, 1987). Their worldwide distribution, extensive host ranges and interaction with other plant pathogens in disease complexes rank them among the major plant pathogens, significantly affecting world food supply (Sasser, 1980).

According to De Waele and Elsen (2007) 92 nominal *Meloidogyne* spp. had been described by 2006. Among the root-knot nematodes species *Meloidogyne incognita* and *M. javanica* are the most widely distributed in Africa (Sasser, 1980) and South Africa (Kleynhans, 1991), particularly predominating in the warmer areas (Kleynhans, 1991; Whitehead, 1969). Furthermore, *M. chitwoodi* and *M. fallax* are important quarantine parasites of potato on Europe (Castagnone-Sereno *et al.*, 1999) and have also been

identified on tomato, potato (*M. chitwoodi* and *M. fallax*) and groundnut in South Africa (Fourie & Mc Donald, 2001).

Temperature has a major effect on the expression of resistance by crops to *Meloidogyne* species and the losses caused by root-knot nematodes depend on the growing conditions and population levels of these parasites (Mullin *et al.*, 1991). This study focuses on root-knot nematodes because this genus account for most of the crop losses (Xu *et al.*, 2001) and because it is predominant in South African agricultural soils (Fourie *et al.*, 2001; Kleynhans, 1991).

1.9 Root-knot nematodes associated with weeds

The wide host ranges of root-knot nematodes include most common weed species that occur in crop fields (Robinson *et al.*, 1997). Comprehensive surveys conducted in Zimbabwe revealed that most weed species were invaded by *Meloidogyne* species. Five out of 22 weed species, namely, *Ageratum conyzoides*, *Crotalaria* spp., *Crotalaria incanum*, *Galinsoga parviflora* and *N. physaloides* were identified as good hosts for *M. javanica* (Martin, 1959; Salawu *et al.*, 1991). A wide range of weeds (Table. 1) have been identified as hosts of root-knot nematodes in South Africa (Keetch & Buckley, 1984).

Table 1. Root-knot nematodes associated with weeds in South Africa (Keetch & Buckley, 1984).

Weed genus/ species	Root-knot nematode spp.
<i>Amaranthus paniculatus</i>	<i>Meloidogyne</i> spp.
<i>Atriplex</i> spp.	<i>Meloidogyne</i> spp.
<i>Bidens pilosa</i>	<i>M. hapla</i>
<i>Bromus inermis</i>	<i>M. arenaria</i>
	<i>M. hapla</i>
	<i>M. incognita</i>
	<i>M. javanica</i>
<i>Chenopodium ambrosioides</i>	<i>Meloidogyne</i> spp.
<i>Citrullus lanatus</i>	<i>M. arenaria</i>
<i>Crotalaria lanceolata</i>	<i>M. arenaria</i>
	<i>M. hapla</i>
	<i>M. incognita</i>
	<i>M. javanica</i>
<i>Crotalaria sphaerocarpa</i>	<i>M. incognita</i>
<i>Datura</i> spp.	<i>Meloidogyne</i> spp.
<i>Eleusine indica</i>	<i>M. incognita</i>
	<i>M. javanica</i>
<i>Hibiscus cannabinus</i>	<i>M. arenaria</i>
	<i>M. hapla</i>
	<i>M. incognita</i>
	<i>M. javanica</i>
<i>Ipomoea</i> spp.	<i>Meloidogyne</i> spp.
<i>Malva parviflora</i>	<i>Meloidogyne</i> spp.
<i>Medicago sativa</i>	<i>M. hapla</i>
	<i>M. javanica</i>
<i>Melilotus alba</i>	<i>M. incognita</i>
	<i>M. javanica</i>
<i>Paspalum dilatatum</i>	<i>M. incognita</i>
<i>Phalaris tuberosa</i>	<i>M. incognita</i>
<i>Sesbania</i> spp.	<i>M. javanica</i>
<i>Solanum nigrum</i>	<i>Meloidogyne</i> spp.
<i>Tagetes erecta</i>	<i>M. javanica</i>

2. Objective of the study

Since information on the effect of the association of weeds and plant-parasitic nematodes on crops is limited in South Africa (De Waele *et al.*, 1990; Keetch & Buckley, 1984) this study focused on the (i) identification of plant-parasitic nematodes on economically important and widely distributed weeds in small-scale farming systems in South Africa, (ii) frequency of occurrence, population densities and prominence values were calculated for plant-parasitic nematodes associated with weeds and (iii) molecular identification of root-knot nematodes from all 44 localities sampled. This research will be useful to farmers, particularly with regard to minimising production costs, but also by optimising crop yields and stabilizing household income and food security.

Chapter 2: Survey and identification of plant-parasitic nematodes associated with weeds

2.1 Introduction

Although an earlier survey (Fourie & Mc Donald, 2001) of home, community and small-scale farming systems indicated that plant-parasitic nematodes, particularly root-knot nematodes parasitised agricultural and horticultural crops at the majority of the localities sampled, limited information is available on the association of these parasites with common weeds (Keetch & Buckley, 1984). The characterisation of nematode communities is important for research, disease diagnosis, advisory and regulatory purposes (Barker, 1985). The relationship of nematode population density and frequency of occurrence of a species or genus is expressed as prominence value (PV) in terms of a specific area (De Waele & Jordaan, 1988). The PV is thus an index of categorising nematode genera or species that were identified during surveys (De Waele & Jordaan, 1988). High prominence values, therefore, in this study indicate which nematode genera or species are predominant on a specific weed in a specific locality, while low prominence values indicate nematode genera/species of minor importance to a specific weed in a specific area.

The objectives of this study were to (i) identify plant-parasitic nematodes associated with economically important and widely distributed weeds that occur in small-scale farming systems in South Africa and to (ii) determine the PV, frequency of occurrence (%) and population density of the relevant nematode genera or species that occur on weeds in specific areas.

2.2 Materials and methods

2.2.1 Sampling of nematodes

From 2004 to 2006 a survey was conducted at 44 sites located in the eastern and western regions of South Africa in the provinces of the Eastern Cape, Limpopo, Mpumalanga, Northern Cape and Kwazulu-Natal. These are the regions and communities where most traditional and active small-scale crop production enterprises are located. The eastern and western regions of South

Africa are characterised by distinct differences in particularly precipitation and vegetation (Schulze, 1997; Fig. 1). Areas in the eastern region generally has higher rainfall compared to areas in the western region. For this reason a rainfall gradient exists from east to west. Nematode sampling was thus done over such a gradient. Selected farmers at the sites were requested to rank the top three weed species that occurred on their respective fields. These weed species were randomly sampled for nematode assessment. Generally between one and three weed plants per species occurring in farmer's fields were sampled, depending on its availability and abundance. The root system with adhering soil from each sampled plant was removed with a spade and placed in labeled plastic bags. These samples were kept in cooler bags and transported to the laboratory of the Nematology Unit of the Agricultural Research Council-Grain Crops Institute (ARC-GCI) in Potchefstroom. The samples were stored at 8°C - 10°C until nematode extraction, but not for longer than two weeks (Barker & Nusbaum, 1971). Sampling of weeds were done during the summer growing season.

The weed species sampled from the different sites were identified in consultation with weed scientists of the ARC-GCI. Plant-parasitic and free-living nematodes associated with the sampled weeds were counted and identified in collaboration with nematode taxonomists of the ARC-Plant-Protection Research Institute (PPRI) in Pretoria, South Africa.

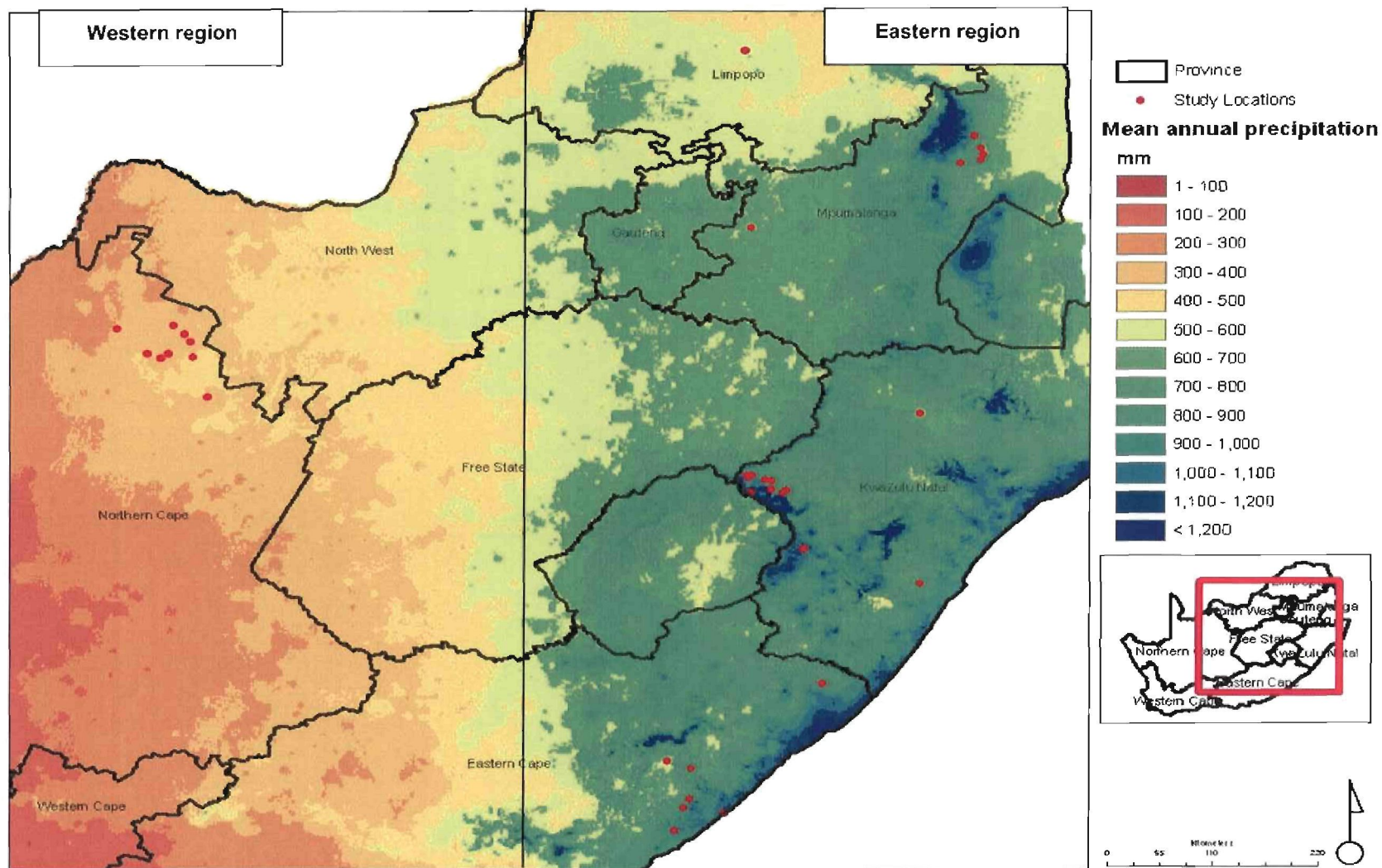
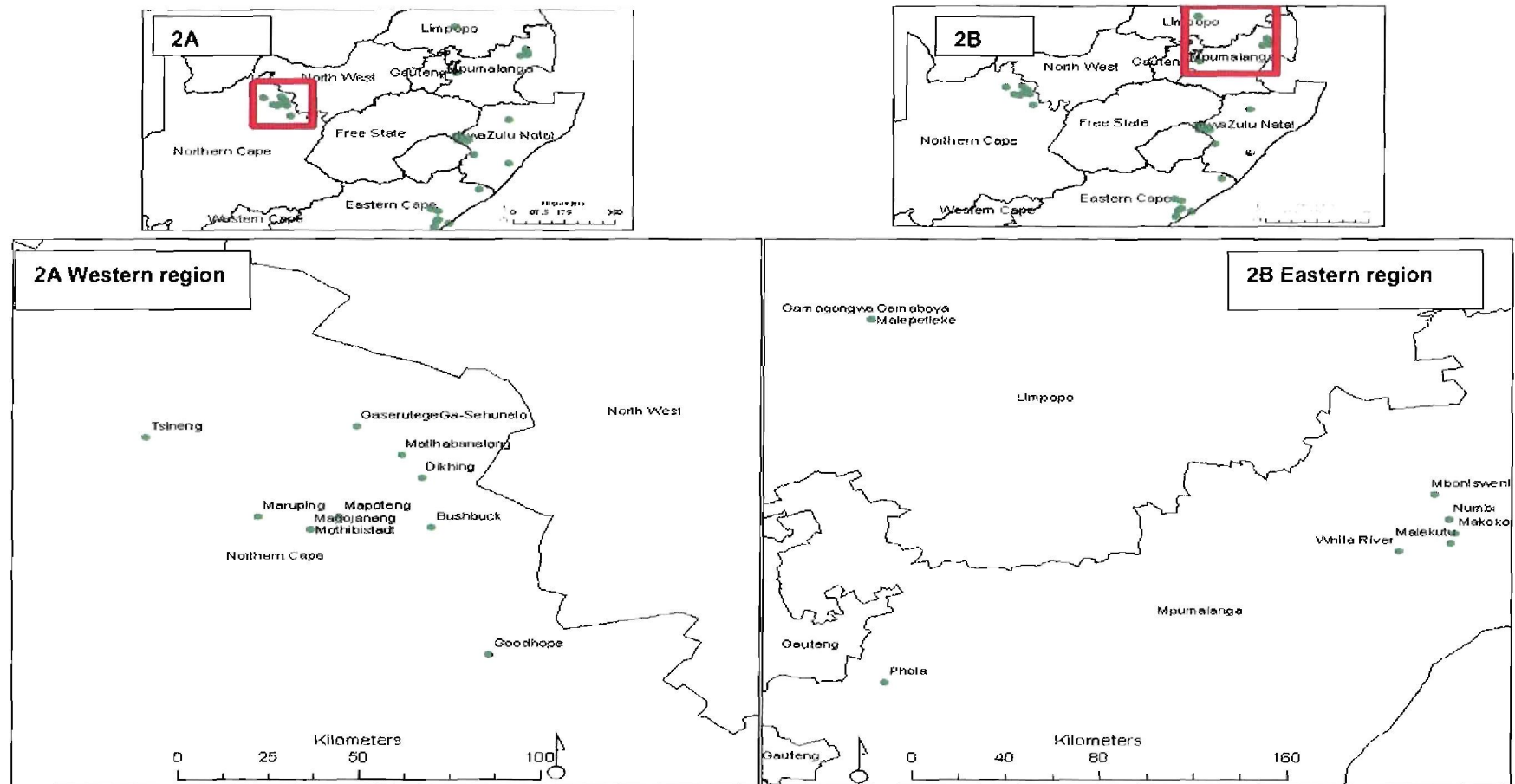
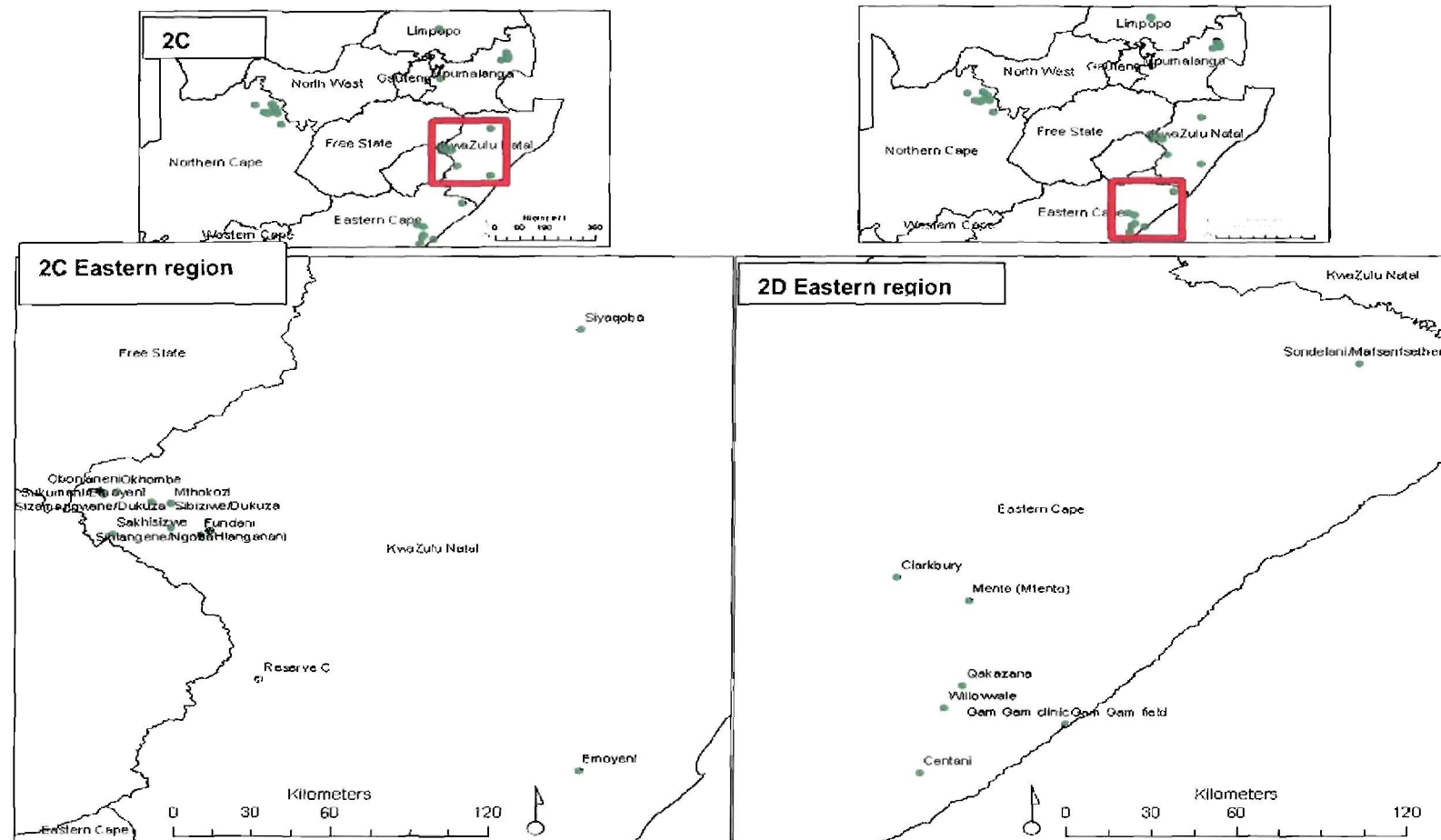


Figure 1. Rainfall distribution for the eastern and western regions of South Africa.



Figures. 2A & B. Sites where weed species were sampled in the western (Northern Cape province, 2A) and eastern region (Limpopo and Mpumalanga provinces, 2B) of South Africa for identification of plant-parasitic nematodes.



Figures. 2C & D. Sites where weed species were sampled in the eastern region [KwaZulu Natal; (2C) and Eastern Cape provinces; (2D)] of South Africa for identification of plant-parasitic nematodes.

2.2.2 Extraction of nematodes from root and soil samples of weed species.

2.2.2.1 Root samples

Extraction of root-knot nematode eggs and J2 from 20-g root samples was done using the adapted NaOCl method (Riekert, 1995), while a wide range of plant-parasitic nematodes were extracted from 5g roots using the sugar centrifugal-flotation method (Coolen & D'Herde, 1972)

Adapted NaOCl-method

A 20-g subsample was taken from the root systems of each weed species, cut into 1-cm pieces and the pieces were thoroughly mixed. Each root subsample was subsequently shaken for four minutes in 400ml of a 1-% NaOCl solution. This weak bleach solution dissolves the gelatinous matrix surrounding root-knot nematode eggs and J2 and releases them into the solution (Riekert, 1995). The mixture containing eggs and J2 was then decanted through a range of 710- μ m, 250- μ m, 75- μ m, 25- μ m and a 10- μ m mesh nested (from top to bottom) sieves. This ensures less clogging of unwanted material on the bottom sieve. A vacuum pump was connected to a filtering pipe at the bottom sieve (10- μ m mesh) to apply suction and enhance passage of the suspension containing the nematode eggs and J2 through the range of sieves (Riekert, 1995). Rinsing proceeded for approximately four minutes. Root-knot nematode eggs and J2 were collected from the 10- μ m and 25- μ m-mesh sieves by washing into a sample bottle by a gentle stream of tap water guided through a short hose.

Centrifugal-flotation extraction method

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

A 5-g subsample was taken from each of the root system of the individual weed species sampled at each locality and were cut into 1-cm pieces. Each

root sample was then macerated in 250-ml tap water at high blade speed in a domestic blender for 90 seconds to release plant-parasitic nematodes from the root tissue. The suspension containing nematodes and root fragments were decanted on a 710- μm -mesh sieve which was nested on a 25- μm -mesh sieve. Root pieces on the 710- μm -mesh sieve were rinsed thoroughly with running tap water and the residue on the 25- μm -mesh sieve containing the nematodes was washed into a 50-ml centrifuge tube.

Kaolin (2cm^3) was added to the tube and stirred well. The kaolin-water suspension containing the nematodes was then centrifuged at 1800g for one minute to ensure settlement of the nematodes at the bottom of each tube. The supernatant was subsequently decanted and each tube was filled with a sucrose solution (specific gravity = $1.15\text{g}/\text{cm}^3$). The mixture was stirred well and centrifuged at 1800g for 1 minute. The supernatant was decanted onto a 25- μm -mesh sieve and rinsed well with tap water to remove the sucrose. The residue containing the nematodes was collected in a sample bottle for examination and counting of the nematodes.

2.2.2.2 The importance of kaolin

Kaolin is a clay mineral with a specific gravity of 2.6 and consists of particles, which are 2-3 μm in size. Although the density of kaolin is greater than that of the nematodes, kaolin particles are small and flat and sink to the bottom of the centrifuge tube more slowly than the nematodes. This way kaolin forms a layer over the loose sediment and nematodes 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 and bring the nematodes into suspension in the sugar solution. Another advantage of kaolin is that it precipitates during the second centrifugation, thus preventing re-mixing of the sedimented debris when the sugar solution is decanted. The final result is a suspension of nematodes in clear water (Coolen & D'Herde, 1972).

2.2.2.3 Soil samples

Nematodes were extracted from soil samples using the decanting and sieving method (Cobb, 1918), followed by the sugar centrifugal-flotation method (Caveness & Jensen, 1955).

Decanting and sieving method

According to Cobb (1918), this method is based upon the density and size of nematodes. Approximately 20% of the nematodes originally present in soil samples will be lost during this extraction procedure (Cobb, 1918).

Two hundred ml of soil from the rhizosphere of each weed root system was soaked in tap water in a 1-l beaker. Soil particles with a diameter of more than 1mm were removed by passing the sample through a 710- μ m mesh sieve nested on a 5-l bucket. The residue on the sieve was washed through with tap water for about 2 minutes and then discarded. The bucket containing the nematodes was filled up to 5l with tap water, whereafter the soil sample was thoroughly mixed and the mixture allowed to settle for about 30 seconds. The mixture was then decanted through a 25- μ m mesh sieve, leaving behind the sediment that had settled at the bottom of the bucket. The procedure was repeated once. The nematodes and fine soil particles from each sample retained on the 25- μ m mesh sieve were washed into 50-ml centrifuge tubes. The fine soil and nematode mixture in the centrifuge tubes was subsequently centrifuged for 5 minutes with a Relative Centrifugal Force (RCF) of 1800g. After centrifugation the supernatant was carefully decanted and discarded. Nematodes were subsequently collected at the bottom of the centrifuge tubes. The sugar centrifugal-flotation method subsequently followed to complete the nematode extraction process.

Sugar centrifugal-flotation method

This method is based on the specific gravity of nematodes. Terrestrial nematodes have a specific gravity of approximately 1.08. After centrifugation in water, only organic materials with a specific gravity lower than 1.0 will remain in suspension and could be discarded. When centrifuged in a solution with higher specific gravity than the density of the nematodes, the nematodes

will remain in suspension and can be separated from soil particles with a specific gravity larger than 1.15.

A sucrose solution with a gravity of 1.15 was therefore added to the centrifuge tubes containing the nematodes. This solution was prepared by adding 624g sugar to 1000ml water. The sucrose solution and the sediment containing the nematodes were thoroughly stirred with a spatula and centrifuged for one minute at a RCF of 1800g. When centrifugation was completed, the supernatant containing the nematodes was decanted on a 250- μ m-mesh sieve and gently rinsed with tap water to remove the sucrose as quickly as possible. The nematodes were washed into a sample bottle for counting and identification. Since the sucrose solution is osmotically active the nematodes suspended in it can plasmolyse. It was therefore critical that the time the nematodes were kept in the sucrose solution was limited, not exceeding a total of 4 minutes.

2.2.3. Counting of nematodes

Nematode population levels were determined in a counting dish under a stereo microscope and expressed either as the number of nematodes per 20g roots, 5g roots or 200ml soil. Root-knot nematodes eggs and J2 from 20-g root samples were counted without fixation, while plant-parasitic nematodes per 5g roots and 200ml soil were fixed after counting for morphological species identification.

2.2.4 Species identification

2.2.4.1 Transfer of nematodes to glycerin

To study finer morphological structures of plant-parasitic nematode species under a light microscope, nematodes were mounted in glycerin. Glycerin has a refraction index of nearly the same as that of glass. However, when living or fixed nematodes are suddenly placed in pure glycerin they will plasmolyse. Therefore, transferal of nematodes to glycerin must be done gradually, as described by De Gisse (1965).

2.2.4.2 Fixation of nematodes (De Grisse, 1965).

Nematodes suspended in water after extraction from root (5-g) and soil (200-ml) samples, were individually picked with a needle and transferred to an eye glass (syracuse dish) containing 5ml distilled water. Five millilitre of a heated ($\pm 60-70^{\circ}\text{C}$) formalin, propionic acid and glycerine solution (F.P.G solution: 100ml of a 40 % formalin solution, 10ml propionic acid, 890ml distilled water, 10ml glycerine and 1mg of picric acid for staining) were added to the nematode suspension. The eye glass was transferred to a dessicator filled with F.P.G solution and kept in an incubator at 40°C for 72 hours.

Nematodes were transferred from the F.P.G solution to an eye glass containing a glycerin I solution (200ml of a 95 % ethanol solution, 10ml glycerin and 790ml distilled water). The eye glass with the nematodes suspended in the glycerin I solution was then placed in a dessicator containing a 95-% ethanol solution to saturate the atmosphere inside the dessicator.

The dessicator containing the eye glass with nematodes was subsequently placed in an oven at $38-40^{\circ}\text{C}$ for 12 hours. The eye glass containing the nematodes was then removed from the dessicator, the nematodes were transferred to an eye glass containing a glycerin II solution (950ml of a 95-% ethanol solution and 5ml glycerin) and placed back in an oven at $38-40^{\circ}\text{C}$. The alcohol slowly evaporated so that the nematodes remained in pure glycerin after 12 to 24 hours. The nematodes were then mounted on slides in anhydrous glycerin for morphological species identification.

2.2.4.3 Mounting of nematodes on slides

A small drop ($\pm 2\text{mm}$ in diameter) of glycerin was placed in the center of a glass slide with three fiberglass threads arranged around the edges of the glycerin drop. Nematodes were individually picked with a needle and transferred to the middle of the glycerin drop on the slide. They were gently pressed down onto the surface of the slide and sealed off by slipping a cover

glass over the glycerin drop. Finally the outside edge of the cover glass was sealed off with colourless, acrylic nail hardener (Cutex).

2.3 Data analyses

2.3.1 Nematodes

2.3.1.1 Population density of each nematode species

Population density for each nematode species on the 44 sites sampled was calculated as follows:

$$\frac{\text{total number of nematodes present per genus/species}}{\text{number of sites on which the nematode species occurred}}$$

Population density of each nematode species for each region was calculated using the following formula:

$$\frac{\text{total number of a nematode genus/species present at each site for each region}}{\text{number of sites on which the nematode genus/species occurred/region}}$$

Population density of each nematode genus/species on each weed species per was calculated using the formula:

$$\frac{\text{total number of each nematode genus/species present on each weed species/region}}{\text{number of sites on which the species occurred on each weed species/region}}$$

2.3.1.2 Frequency of occurrence of nematode

Frequency of occurrence for each nematode genus/species from the 44 sites is expressed as a percentage and was calculated using the formula:

$$\frac{\text{number of sites on which the nematode genus/species occurred} \times 100}{\text{number of sites sampled}}$$

Frequency of occurrence of each nematode genus/species for each region is expressed as a percentage and was calculated using the formula:

$$\frac{\text{number of weeds on which the nematode genus/species occurred/region} \times 100}{\text{number of sites sampled/region}}$$

Frequency of occurrence of each nematode genus/species on each weed species is expressed as a percentage and was calculated using the formula:

$$\frac{\text{number of times the nematode genus/species occurred on each weed/region}}{\text{number of sites sampled/region}}$$

2.3.1.3 Prominence value

Prominence values were calculated for plant-parasitic, free-living and predatory nematode populations in root (20g and 5g) and soil (200ml) samples (De Waele & Jordaan, 1988) according to the following formula:

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

2.3.2 Weeds

2.3.2.1 Frequency of occurrence of weed genera/species

Only the frequency of occurrence of weed species could be calculated since the number of weed species per site or per region was not determined. The number of root and soil samples taken per weed species per site differed because it depended upon the availability of each species. As some weed species such *Amaranthus* are used as food for farmers, only one root sample could be taken per locality. Frequency of occurrence for each weed species from 44 sites was expressed as a percentage and was calculated using the formula:

number of sites on which the weed species occurred x 100

number of sites sampled

2.4 Results

2.4.1 Weed species

Table 1. Overall frequency of occurrence (%) of weeds sampled for identification of plant-parasitic nematodes over 44 sites in South Africa.

Weed genera/spp.	Frequency of occurrence (%)
<i>Cynodon dactylon</i>	34
<i>Bidens bipinnata</i>	30
<i>Amaranthus hybridus</i>	23
<i>Xanthium strumarium</i>	16
<i>Tagetes minuta</i>	16
<i>Senecio latifolius</i>	14
<i>Datura stramonium</i>	11
<i>Commelina benghalensis</i>	11
<i>Eleusine corocana</i>	9
<i>Nothoscordum gracile</i>	9
<i>Argemone ochroleuca</i>	9
<i>Lepidium africanum</i>	9
<i>Richardia brasiliensis</i>	7
<i>Hibiscus trionum</i>	7
<i>Chenopodium carinatum</i>	7
<i>Emex australis</i>	7
<i>Cyperus rotundus</i>	7
<i>Ipomoea purpurea</i>	5
<i>Physalis viscosa</i>	2
<i>Coronopus didymus</i>	2
<i>Conyza canadensis</i>	2
<i>Boerhavia diffusa</i>	2
<i>Portulaca oleracea</i>	2
<i>Tribulus terrestris</i>	2
<i>Oxalis latifolia</i>	2
<i>Malva parvifolia</i>	2
<i>Chloris virgata</i>	2
<i>Nicandra physalodes</i>	2
<i>Chenopodium murale</i>	2
<i>Flaveria bidentis</i>	2
<i>Sparganium</i> spp.	2
<i>Chamaesyce inaequilatera</i>	2
<i>Datura ferox</i>	2
<i>Hibiscus cannabinus</i>	2
<i>Amaranthus deflexus</i>	2
<i>Bryophyllum</i> spp.	2
<i>Sonchus oleraceus</i>	2
<i>Sida cordifolia</i>	2
<i>Cyperus esculentus</i>	2

Thirty nine weed species and 35 genera from the 44 sites located in the eastern and western regions of South Africa were identified. Frequency of occurrence ranged between 2 % and 34 % (Table 1), the highest frequency of occurrence being for *C. dactylon*.

Table 2. Frequency of occurrence (%) of weeds sampled for identification of plant-parasitic nematodes in 34 sites located in the eastern and 10 sites in the western regions of South Africa in terms of rainfall patterns.

Region	Weed genera/spp.	Frequency of occurrence (%)
Eastern region	<i>Cynodon dactylon</i>	53
	<i>Bidens bipinnata</i>	38
	<i>Amaranthus hybridus</i>	29
	<i>Tagetes minuta</i>	21
	<i>Senecio latifolius</i>	18
	<i>Xanthium strumarium</i>	18
	<i>Commelina benghalensis</i>	15
	<i>Nothoscordum gracile</i>	12
	<i>Eleusine corocana</i>	12
	<i>Hibiscus trionum</i>	9
	<i>Datura stramonium</i>	9
	<i>Emex australis</i>	9
	<i>Cyperus rotundus</i>	9
	<i>Richardia brasiliensis</i>	9
	<i>Ipomoea purpurea</i>	6
	<i>Amaranthus deflexus</i>	3
	<i>Physalis viscosa</i>	3
	<i>Chenopodium carinatum</i>	3
	<i>Datura ferox</i>	3
	<i>Hibiscus cannabinus</i>	3
	<i>Cyperus esculentus</i>	3
	<i>Boerhavia diffusa</i>	3
	<i>Portulaca oleracea</i>	3
	<i>Tribulus terrestris</i>	3
	<i>Oxalis latifolia</i>	3
	<i>Malva parvifolia</i>	3
	<i>Chloris virgata</i>	3
	<i>Nicandra physalodes</i>	3
	<i>Chenopodium murale</i>	3
Western region	<i>Cynodon dactylon</i>	50
	<i>Lepidium africanum</i>	40
	<i>Argemone ochroleuca</i>	40
	<i>Chenopodium carinatum</i>	20
	<i>Datura stramonium</i>	20
	<i>Coronopus didymus</i>	10
	<i>Conyza canadensis</i>	10
	<i>Flaveria bidentis</i>	10
	<i>Chamaesyce inaequilatera</i>	10
	<i>Sonchus oleraceus</i>	10
	<i>Sparganium</i> spp.	10
	<i>Bryophyllum</i> spp.	10
	<i>Sida cordifolia</i>	10
	<i>Xanthium strumarium</i>	10

Twenty-nine weed species and 24 genera were identified in the eastern region, while 14 weed species and 14 genera were identified from sites in the western region of South Africa (Table 2). Frequency of occurrence ranged

between 3 % and 53% in the eastern region, while it ranged between 10 % and 50% in the western region. The highest frequency of occurrence being for *C. dactylon* in both regions.

2.4.2 Twenty-gram (20-g) root samples: Root-knot nematodes

Table 3. Prominence values (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. eggs and second-stage juveniles in roots of weeds sampled from 44 sites in South Africa.

Nematode genera	PV	Frequency of occurrence (%)	Mean population density/ 20g roots
<i>Meloidogyne</i> spp.	5 190	100	5 190

Root-knot nematodes occurred at all sites sampled, with a high PV and mean population density (Table 3).

Table 4. Prominence values (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. eggs and second-stage juveniles per 20g roots of weeds sampled from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	PV	Frequency of occurrence (%)	Mean population density/ 20g roots
Eastern region	6 283	100	6 283
Western region	1 472	100	1 472

PV and mean population densities of root-knot nematodes were substantially higher at sites located in the eastern region compared to those in the western region. Root-knot nematodes occurred at all sites sampled in the eastern as well as western region, with the highest PV and population density of 6 283 in the eastern region.

Table 5. Prominence values (PV), frequency of occurrence and (%) and mean population density of *Meloidogyne* spp. in roots of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/ 20g roots
<i>Bidens bipinnata</i>	30 142	30	55 032
<i>Cynodon dactylon</i>	14 832	34	25 437
<i>Amaranthus hybridus</i>	12 003	23	25 029
<i>Chloris virgata</i>	7 156	2	50 604
<i>Datura stramonium</i>	4 625	11	13 945
<i>Nicandra physalodes</i>	3 139	2	22 197
<i>Chenopodium murale</i>	2 786	2	19 701
<i>Xanthium strumarium</i>	1 117	16	2 792
<i>Eleusine corocana</i>	980	9	3 268
<i>Flaveria bidentis</i>	967	2	6 841
<i>Ipomoea purpurea</i>	612	5	2 739
<i>Sparganium</i> spp.	319	2	2 256
<i>Commelina benghalensis</i>	310	11	934
<i>Chamaesyce inaequilatera</i>	272	2	1 926
<i>Datura ferox</i>	218	2	1 543
<i>Hibiscus cannabinus</i>	213	2	1 509
<i>Hibiscus trionum</i>	214	7	807
<i>Chenopodium carinatum</i>	188	7	709
<i>Amaranthus deflexus</i>	145	2	1 025
<i>Tagetes minuta</i>	64	16	161
<i>Sonchus oleraceus</i>	53	2	375
<i>Emex australis</i>	27	7	103
<i>Bryophyllum</i> spp.	15	2	106
<i>Cyperus rotundus</i>	13	7	48
<i>Lepidium africanum</i>	9	9	29
<i>Senecio latifolis</i>	7	14	18
<i>Richardia brasiliensis</i>	4	7	5
<i>Sida cordifolia</i>	2	2	15
<i>Cyperus esculentus</i>	1	2	8
<i>Argemone ochroleuca</i>	1	9	3
<i>Physalis viscosa</i>	0	0	0
<i>Coronopus didymus</i>	0	0	0
<i>Conyza canadensis</i>	0	0	0
<i>Boerhavia diffusa</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Tribulus terrestris</i>	0	0	0
<i>Oxalis latifolia</i>	0	0	0
<i>Nothoscordum gracile</i>	0	0	0
<i>Malva parvifolia</i>	0	0	0

Root-knot nematodes occurred on all 37 weed species and 35 genera sampled (Table 5). The highest PV for root-knot nematodes of 30 142 was obtained in roots of *B. bipinnata*, followed by *C. dactylon* (14 832) and *A. hybridus* (12 003). The lowest PV of zero was calculated for nine weed species. Frequency of occurrence (%) ranged between 2 % and 34 %, while population density ranged from 0 to 55 032 (*B. bipinnata*).

Table 6. Prominence values (PV), frequency of occurrence and (%) and mean population density of *Meloidogyne* spp. per 20g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/20g roots
Eastern region	<i>Chloris virgata</i>	8 765	3	50 604
	<i>Amaranthus hybridus</i>	5 213	29	9 681
	<i>Nicandra physalodes</i>	3 845	3	22 197
	<i>Bidens bipinnata</i>	4 805	38	7 794
	<i>Chenopodium murale</i>	3 412	3	19 701
	<i>Cynodon dactylon</i>	2 983	53	4 098
	<i>Datura stramonium</i>	1 391	9	4 637
	<i>Xanthium strumarium</i>	1 359	18	3 204
	<i>Eleusine corocana</i>	1 132	12	3 268
	<i>Ipomoea purpurea</i>	671	6	2 739
	<i>Commelina benghalensis</i>	362	15	934
	<i>Chenopodium carinatum</i>	301	3	1 735
	<i>Datura ferox</i>	267	3	1 543
	<i>Hibiscus cannabinus</i>	261	3	1 509
	<i>Amaranthus deflexus</i>	178	3	1 025
	<i>Tagetes minuta</i>	74	21	161
	<i>Senecio latifolius</i>	46	18	108
	<i>Hibiscus trionum</i>	33	9	109
	<i>Emex australis</i>	31	9	103
	<i>Cyperus rotundus</i>	14	9	48
	<i>Richardia brasiliensis</i>	4	9	14
	<i>Cyperus esculentus</i>	1	3	8
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Boerhavia diffusa</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Tribulus terrestris</i>	0	0	0
	<i>Oxalis latifolia</i>	0	0	0
	<i>Malva parvifolia</i>	0	0	0
	<i>Physalis viscosa</i>	0	0	0
Western region	<i>Flaveria bidentis</i>	2 163	10	6 841
	<i>Sparganium</i> spp.	713	10	2 256
	<i>Chamaesyce inaequilatera</i>	609	10	1 926
	<i>Lepidium africanum</i>	183	40	29
	<i>Cynodon dactylon</i>	181	50	256
	<i>Sonchus oleraceus</i>	119	10	375
	<i>Chenopodium carinatum</i>	81	20	181
	<i>Datura stramonium</i>	76	20	170
	<i>Bryophyllum</i> spp.	34	10	106
	<i>Xanthium strumarium</i>	7	10	21
	<i>Sida cordifolia</i>	5	10	15
	<i>Argemone ochroleuca</i>	2	40	3
	<i>Coronopus didymus</i>	0	0	0
	<i>Conyza canadensis</i>	0	0	0

In the eastern region root-knot nematodes were present in roots of all 29 weed species and 24 genera sampled (Table 6). The three predominant weed species maintaining root-knot nematodes were *C. virgata*, *A. hybridus* and *N. physalodes*, while root-knot nematodes occurred in roots of *C. dactylon*, *B. bipinnata* and *A. hybridus* at most of the sites. The PV ranged between 8 765 for *C. virgata* and 0 for six weed species. Frequency of occurrence ranged

between 3 % and 53 % (*C. dactylon*), while mean population density ranged between from 0 to 50 604 (*C. virgata*).

In the western region root-knot nematodes were present in roots of all 14 weed species and genera sampled (Table 6). The three predominant weed species maintaining root-knot nematodes were *F. bidentis*, *Sparganium* spp. and *C. inaequilatera*, while these parasites occurred in roots of *C. dactylon*, *L. africanum*, *C. carinatum* and *D. stramonium* occurred at most of the sites. The PV ranged between 0 for *C. didymus* and *C. canadensis* and 2 163 for *F. bidentis*. Frequency of occurrence ranged between 10 % and 50 % (*C. dactylon*), while population density ranged between 0 and 6 841 (*F. bidentis*).

2.4.3 Five-gram (5-g) root samples: Plant-parasitic nematodes

Table 7. Prominence values (PV), frequency of occurrence (%) and mean population densities of plant-parasitic nematodes in roots of weeds sampled from 44 sites in South Africa.

Nematode genera/spp.	PV	Frequency of occurrence (%)	Mean population density/5g roots
<i>Meloidogyne</i> spp.	59	100	59
<i>Helicotylenchus dihystra</i>	19	86	20
<i>Pratylenchus zeae</i>	16	97	16
<i>Rotylenchus unisex</i>	8	89	9
<i>Scutellonema brachyurus</i>	6	93	6
<i>Pratylenchus scribneri</i>	4	18	9
<i>Tylenchorhynchus capitatus</i>	4	43	6
<i>Aphelenchoides</i> spp.	3	25	5
<i>Tylenchorhynchus brevilineatus</i>	2	59	3
<i>Xiphinema</i> spp.	2	68	2
<i>Rotylenchus brevicaudatus</i>	1	2	6
<i>Criconemoides obtusicaudatus</i>	1	9	4
<i>Helicotylenchus digonicus</i>	1	5	3
<i>Rotylenchus karooensis</i>	1	9	3
<i>Radopholus similis</i>	1	7	3
<i>Rotylenchulus parvus</i>	1	55	2
<i>Helicotylenchus parapatyurus</i>	1	30	1
<i>Tylenchorhynchus mashhoodi</i>	1	25	1
<i>Aphelenchus avenae</i>	1	23	1
<i>Rotylenchulus borealis</i>	0.3	11	1
<i>Rotylenchus incultus</i>	0.2	5	1
<i>Scutellonema dreyeri</i>	0.1	2	1
<i>Scutellonema truncatum</i>	0.3	14	1
<i>Ditylenchus</i> spp.	0.1	2	1
<i>Geocenamus brevelineatus</i>	0.1	2	1

Twenty-one plant-parasitic nematode species and 14 genera have been identified from 5g weed roots sampled from 44 sites located in the eastern and western regions of South Africa (Table 7). *Meloidogyne* spp. was the predominant group with a PV of 59, followed by *H. dihystra* (19) and *P. zeae* (16). *Meloidogyne* spp. was present in the roots of all weeds sampled at all the sites with a frequency of occurrence of 100%, followed by *P. zeae* (97%) and *S. brachyurus* (93%). Population density ranged between 1 and 59 for *Meloidogyne* spp.

Table 8. Prominence values (PV), frequency of occurrence (%) and mean population density of plant-parasitic nematodes per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Nematode species	PV	Frequency of occurrence %	Mean population density/5g roots
Eastern region	<i>Meloidogyne</i> spp.	1 228	100	1 228
	<i>Helicotylenchus dihystra</i>	528	82	583
	<i>Pratylenchus zae</i>	524	97	532
	<i>Rotylenchus unisex</i>	247	88	263
	<i>Scutellonema brachyurus</i>	188	94	194
	<i>Tylenchorhynchus capitatus</i>	56	38	91
	<i>Xiphinema</i> spp.	41	44	62
	<i>Rotylenchulus parvus</i>	29	47	42
	<i>Scutellonema truncatum</i>	26	18	63
	<i>Tylenchorhynchus brevilineatus</i>	7	56	10
	<i>Pratylenchus scribneri</i>	5	15	14
	<i>Helicotylenchus paraplatus</i>	5	29	9
	<i>Tylenchorhynchus mashhoodi</i>	4	29	7
	<i>Aphelenchus avenae</i>	3	21	7
	<i>Rotylenchus brevicaudatus</i>	1	3	6
	<i>Helicotylenchus digonicus</i>	1	6	6
	<i>Radopholus similis</i>	1	9	3
	<i>Scutellonema dreyeri</i>	0.1	3	1
	<i>Geocenamus brevelineatus</i>	0.1	3	1
	<i>Ditylenchus</i> spp.	0.1	3	1
Western region	<i>Meloidogyne</i> spp.	43	100	43
	<i>Pratylenchus zae</i>	10	100	10
	<i>Helicotylenchus dihystra</i>	9	100	9
	<i>Rotylenchus unisex</i>	7	90	7
	<i>Scutellonema brachyurus</i>	4	90	4
	<i>Tylenchorhynchus capitatus</i>	2	60	2
	<i>Aphelenchoides</i> spp.	1	40	2
	<i>Criconeoides obtusicaudatus</i>	1	40	2
	<i>Xiphinema</i> spp.	1	90	1
	<i>Rotylenchulus parvus</i>	1	80	1
	<i>Tylenchorhynchus brevilineatus</i>	1	70	1
	<i>Rotylenchulus borealis</i>	1	50	1
	<i>Rotylenchus karoensis</i>	1	40	1
	<i>Aphelenchus avenae</i>	1	30	1
	<i>Pratylenchus scribneri</i>	1	30	1
	<i>Pratylenchus brachyurus</i>	0.4	20	1
	<i>Tylenchorhynchus mashhoodi</i>	0.3	10	1

PV and population densities of plant-parasitic nematodes were substantially higher in the eastern region than those for the western region. In the eastern region 18 plant-parasitic nematode species and 12 genera were identified from 5-g root samples (Table 8) of the 29 weed species and 24 genera (Table 5) sampled for *Meloidogyne* spp.

Meloidogyne spp. was predominant with a PV of 1 228, followed by *H. dihystra* (528), *P. zeae* (524) and *R. unisex* (247). Frequency of occurrence ranged between 3 % and 100 %. Population density ranged between 1 228 for *Meloidogyne* species and 1 for four other species.

In the western region 14 plant-parasitic nematode species and 10 genera were identified from 5g roots (Table 8) of the 14 weed species and genera (Table 5) sampled. *Meloidogyne* spp. was predominant with PV of 43, followed by *P. zeae* (10) and *H. dihystra* (9). Frequency of occurrence ranged between 10 % and 100 % and population density ranged between 1 and 43.

Table 9. Prominence values (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. in roots of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
<i>Hibiscus trionum</i>	52	7	195
<i>Eleusine corocana</i>	18	9	59
<i>Cynodon dactylon</i>	17	52	24
<i>Bryophyllum</i> spp.	16	2	116
<i>Ipomoea purpurea</i>	15	5	67
<i>Chamaesyce inaequilatera</i>	14	2	102
<i>Sparganium</i> spp.	11	2	76
<i>Chenopodium carinatum</i>	11	5	50
<i>Amaranthus deflexus</i>	10	2	69
<i>Nicandra physalodes</i>	9	2	66
<i>Sonchus oleraceus</i>	9	2	61
<i>Amaranthus hybridus</i>	8	20	18
<i>Xanthium strumarium</i>	7	14	35
<i>Commelina benghalensis</i>	6	11	17
<i>Bidens bipinnata</i>	6	30	11
<i>Chloris virgata</i>	5	2	33
<i>Chenopodium murale</i>	4	2	27
<i>Cyperus esculentus</i>	4	2	26
<i>Datura ferox</i>	3	2	21
<i>Cyperus rotundus</i>	3	2	21
<i>Lepidium africanum</i>	2	7	6
<i>Datura stramonium</i>	1	9	3
<i>Hibiscus cannabinus</i>	1	2	5
<i>Emex australis</i>	1	7	4
<i>Richardia brasiliensis</i>	1	7	2
<i>Argemone ochroleuca</i>	1	9	0
<i>Oxalis latifolia</i>	1	2	5
<i>Senecio latifolius</i>	0.3	14	1
<i>Sida cordifolia</i>	0.2	2	1
<i>Flaveria bidentis</i>	0.1	2	1
<i>Malva parvifolia</i>	0.1	2	2
<i>Tagetes minuta</i>	0	0	0
<i>Nothoscordum gracile</i>	0	0	0
<i>Physalis viscosa</i>	0	0	0
<i>Coronopus didymus</i>	0	0	0
<i>Conyza canadensis</i>	0	0	0
<i>Boerhavia diffusa</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Tribulus terrestris</i>	0	0	0

Meloidogyne spp. occurred in roots of 30 weed species and 28 genera sampled over the 44 sites (Table 9). *Meloidogyne* spp. was predominant in the roots of *H. trionum* (56), *E. corocana* (18) and *C. dactylon* (17). Frequency of occurrence ranged between 0 and 52 % (*C. dactylon*), while population density ranged from 0 to 195 (*H. trionum*).

Table 10. Prominence values (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
Eastern region	<i>Cyperus esculentus</i>	46	3	266
	<i>Cynodon dactylon</i>	20	53	28
	<i>Eleusine corocana</i>	20	12	58
	<i>Hibiscus trionum</i>	20	9	65
	<i>Ipomoea purpurea</i>	16	6	67
	<i>Amaranthus deflexus</i>	12	3	69
	<i>Nicandra physalodes</i>	11	3	66
	<i>Bidens bipinnata</i>	7	38	11
	<i>Xanthium strumarium</i>	7	12	2
	<i>Commelina benghalensis</i>	7	15	17
	<i>Cyperus rotundus</i>	6	9	21
	<i>Chloris virgata</i>	6	3	33
	<i>Amaranthus hybridus</i>	5	29	17
	<i>Chenopodium murale</i>	5	3	27
	<i>Datura ferox</i>	4	3	21
	<i>Emex australis</i>	4	9	12
	<i>Physalis viscosa</i>	2	3	10
	<i>Richardia brasiliensis</i>	1	9	2
	<i>Tagetes minuta</i>	1	21	2
	<i>Hibiscus cannabinus</i>	1	3	5
	<i>Oxalis latifolia</i>	1	3	5
	<i>Senecio latifolius</i>	0.4	18	1
	<i>Datura stramonium</i>	0.3	3	2
	<i>Malva parvifolia</i>	0.1	3	1
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Boerhavia diffusa</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Tribulus terrestris</i>	0	0	0
Western region	<i>Bryophyllum</i> spp.	116	100	116
	<i>Chamaesyce inaequilatera</i>	102	100	102
	<i>Sparganium</i> spp.	76	100	0
	<i>Sonchus oleraceus</i>	61	100	61
	<i>Cynodon dactylon</i>	9	100	9
	<i>Lepidium africanum</i>	6	100	6
	<i>Datura stramonium</i>	3	100	3
	<i>Flaveria bidentis</i>	1	100	1
	<i>Coronopus didymus</i>	0	0	0
	<i>Conyza canadensis</i>	0	0	0
	<i>Chenopodium carinatum</i>	0	0	0
	<i>Sida cordifolia</i>	0	0	0
	<i>Argemone ochroleuca</i>	0	0	0
	<i>Xanthium strumarium</i>	0	0	0

In the eastern region root-knot nematodes occurred in 24 of the weed species and 21 genera sampled (Table 10). Root-knot nematodes were predominant according to the PV in the roots of *C. esculentus* (46), followed by *C. dactylon*

(20), *E. corocana* (20) and *H. trionum* (20). Frequency of occurrence for these parasites ranged from 0 to 53 %, while population density ranged between 0 and 226 (*C. dactylon*).

Root-knot nematodes occurred in roots of six of the weed species and eight genera sampled in the western region (Table 10). The prominence value for *Meloidogyne* spp. (116) was highest in the roots of *Bryophyllum* spp., followed by *C. inaequilatera* (102) and *Sparganium* spp. (76). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged from 0 to 116 (*Bryophyllum* spp.).

Table 11. Prominence values (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zeae* in roots of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
<i>Sonchus oleraceus</i>	9	2	63
<i>Xanthium strumarium</i>	7	7	27
<i>Cynodon dactylon</i>	6	39	9
<i>Nicandra physalodes</i>	6	2	40
<i>Eleusine corocana</i>	4	9	12
<i>Hibiscus cannabinus</i>	4	2	28
<i>Hibiscus trionum</i>	4	7	15
<i>Ipomoea purpurea</i>	3	5	12
<i>Commelina benghalensi</i>	3	11	9
<i>Emex australis</i>	3	7	11
<i>Bryophyllum</i> spp.	2	2	17
<i>Richardia brasiliensis</i>	2	5	8
<i>Amaranthus deflexus</i>	2	2	16
<i>Bidens bipinnata</i>	2	27	4
<i>Amaranthus hybridus</i>	2	18	5
<i>Chloris virgata</i>	2	2	16
<i>Sparganium</i> spp.	2	2	13
<i>Chenopodium murale</i>	1	2	6
<i>Chenopodium carinatum</i>	1	2	8
<i>Cyperus esculentus</i>	1	2	9
<i>Cyperus rotundus</i>	1	7	3
<i>Datura ferox</i>	0.4	2	3
<i>Senecio latifolis</i>	0.3	9	1
<i>Datura stramonium</i>	0.3	9	1
<i>Chamaesyce inaequilatera</i>	0.1	2	1
<i>Flaveria bidentis</i>	0.1	2	1
<i>Oxalis latifolia</i>	0.1	2	1
<i>Nothoscordum gracile</i>	0	0	0
<i>Malva parvifolia</i>	0	0	0
<i>Argemone ochroleuca</i>	0	0	0
<i>Physalis viscosa</i>	0	0	0
<i>Coronopus didymus</i>	0	0	0
<i>Conyza canadensis</i>	0	0	0
<i>Boerhavia diffusa</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Tribulus terrestris</i>	0	0	0
<i>Sida cordifolia</i>	0	0	0
<i>Lepidium africanum</i>	0	0	0
<i>Tagetes minuta</i>	0	0	0

Pratylenchus zeae occurred in roots of 25 weed species and genera when sampled over 44 sites (Table 11). *Pratylenchus zeae* was predominant in the roots of *S. oleraceus* (9), *X. strumarium* (7), *C. dactylon* (6) and *N. physalodes*. Frequency of occurrence ranged between 0 and 39 % (*C. dactylon*), while population density ranged from 0 and 63 (*S. oleraceus*).

Table 12. Prominence values (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zae* per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
Eastern region	<i>Cyperus esculentus</i>	9	3	52
	<i>Xanthium strumarium</i>	8	9	27
	<i>Nicandra physalodes</i>	7	3	40
	<i>Bidens bipinnata</i>	6	35	5
	<i>Eleusine corocana</i>	4	12	12
	<i>Cynodon dactylon</i>	3	53	4
	<i>Chloris virgata</i>	3	3	16
	<i>Ipomoea purpurea</i>	3	6	12
	<i>Commelina benghalensis</i>	3	15	9
	<i>Amaranthus deflexus</i>	3	3	16
	<i>Emex australis</i>	3	9	11
	<i>Oxalis latifolia</i>	2	3	9
	<i>Amaranthus hybridus</i>	2	24	5
	<i>Richardia brasiliensis</i>	2	6	8
	<i>Hibiscus trionum</i>	1	3	5
	<i>Chenopodium murale</i>	1	3	6
	<i>Cyperus rotundus</i>	1	3	3
	<i>Senecio latifolius</i>	1	3	6
	<i>Datura stramonium</i>	0.3	3	2
	<i>Hibiscus cannabinus</i>	0.1	3	1
	<i>Tagetes minuta</i>	0	0	0
	<i>Datura ferox</i>	0	0	0
	<i>Physalis viscosa</i>	0	0	0
	<i>Boerhavia diffusa</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Tribulus terrestris</i>	0	0	0
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Malva parvifolia</i>	0	0	0
Western region	<i>Sonchus oleraceus</i>	63	100	63
	<i>Bryophyllum</i> spp.	17	100	17
	<i>Sparganium</i> spp.	13	100	0
	<i>Cynodon dactylon</i>	4	100	4
	<i>Chamaesyce inaequilatera</i>	1	100	1
	<i>Flaveria bidentis</i>	1	100	1
	<i>Coronopus didymus</i>	0	0	0
	<i>Conyza canadensis</i>	0	0	0
	<i>Lepidium africanum</i>	0	0	0
	<i>Chenopodium carinatum</i>	0	0	0
	<i>Sida cordifolia</i>	0	0	0
	<i>Argemone ochroleuca</i>	0	0	0
	<i>Xanthium strumarium</i>	0	0	0
	<i>Datura stramonium</i>	0	0	0

Pratylenchus zae occurred in roots of 20 species and 18 genera of weeds sampled (Table 12). *Pratylenchus zae* were predominant in roots of *C. esculentus* (9), followed by *X. strumarium* (8) and *N. physalodes* (7).

Frequency of occurrence for these parasites ranged from 0 to 53 % (*C. dactylon*), while population density ranged between 0 and 52 (*C. esculentus*).

In the western region *P. zae* occurred in roots of four weed species and six genera of weeds sampled (Table 12). The prominence value for *P. zae* (63) was highest in the roots of *S. oleraceus*, followed by *Bryophyllum* spp. (17) and *C. dactylon* (4). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged from 0 to 63 (*S. oleraceus*).

Table 13. Prominence values (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystra* in 5g roots of weeds sampled 44 sites in of South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
<i>Hibiscus trionum</i>	36	7	135
<i>Bidens bipinnata</i>	35	27	13
<i>Nicandra physalodes</i>	16	2	112
<i>Amaranthus deflexus</i>	13	2	92
<i>Cynodon dactylon</i>	11	39	17
<i>Eleusine corocana</i>	10	9	33
<i>Amaranthus hybridus</i>	8	23	16
<i>Datura stramonium</i>	7	5	5
<i>Ipomoea purpurea</i>	5	5	23
<i>Chloris virgata</i>	5	2	31
<i>Sonchus oleraceus</i>	4	2	31
<i>Sparganium</i> spp.	4	2	25
<i>Chenopodium murale</i>	3	2	16
<i>Xanthium strumarium</i>	2	11	6
<i>Commelina benghalensis</i>	2	7	8
<i>Tagetes minuta</i>	1	16	2
<i>Chenopodium carinatum</i>	1	2	5
<i>Chamaesyce inaequilatera</i>	1	2	8
<i>Hibiscus cannabinus</i>	1	2	9
<i>Flaveria bidentis</i>	1	2	10
<i>Cyperus rotundus</i>	1	7	5
<i>Lepidium africanum</i>	1	9	3
<i>Argemone ochroleuca</i>	1	2	4
<i>Bryophyllum</i> spp.	0.4	2	3
<i>Datura ferox</i>	0.2	2	2
<i>Senecio latifolis</i>	0	0	0
<i>Richardia brasiliensis</i>	0	0	0
<i>Sida cordifolia</i>	0	0	0
<i>Cyperus esculentus</i>	0	0	0
<i>Emex australis</i>	0	0	0
<i>Physalis viscosa</i>	0	0	0
<i>Coronopus didymus</i>	0	0	0
<i>Conyza canadensis</i>	0	0	0
<i>Boerhavia diffusa</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Tribulus terrestris</i>	0	0	0
<i>Oxalis latifolia</i>	0	0	0
<i>Nothoscordum gracile</i>	0	0	0
<i>Malva parvifolia</i>	0	0	0

Helicotylenchus dihystra occurred in roots of 23 species and 21 genera of weed sampled (Table 13). *Helicotylenchus dihystra* was predominant in the roots of *H. trionum* (36), *B. bipinnata* (35) and *N. physalodes* (16). Frequency of occurrence ranged between 0 and 39 % (*C. dactylon*), while population density ranged between 0 and 135 (*H. trionum*).

Table 14. Prominence values (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystra* per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
Eastern region	<i>Nicandra physalodes</i>	19	3	112
	<i>Amaranthus deflexus</i>	16	3	92
	<i>Cynodon dactylon</i>	14	35	23
	<i>Hibiscus trionum</i>	14	9	45
	<i>Eleusine corocana</i>	11	12	33
	<i>Xanthium strumarium</i>	10	15	25
	<i>Amaranthus hybridus</i>	9	29	16
	<i>Bidens bipinnata</i>	8	35	13
	<i>Chloris virgata</i>	6	3	32
	<i>Ipomoea purpurea</i>	6	6	23
	<i>Tagetes minuta</i>	5	21	10
	<i>Chenopodium murale</i>	3	3	16
	<i>Cyperus rotundus</i>	2	9	5
	<i>Commelina benghalensis</i>	2	15	6
	<i>Hibiscus cannabinus</i>	2	3	9
	<i>Physalis viscosa</i>	2	3	10
	<i>Datura stramonium</i>	1	3	6
	<i>Tribulus terrestris</i>	0.3	3	2
	<i>Oxalis latifolia</i>	0	0	0
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Malva parvifolia</i>	0	0	0
	<i>Boerhavia diffusa</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Senecio latifolius</i>	0	0	0
	<i>Richardia brasiliensis</i>	0	0	0
	<i>Cyperus esculentus</i>	0	0	0
	<i>Emex australis</i>	0	0	0
	<i>Datura ferox</i>	0	0	0
Western region	<i>Sonchus oleraceus</i>	31	100	31
	<i>Sparganium</i> spp.	25	100	0
	<i>Flaveria bidentis</i>	10	100	10
	<i>Chamaesyce inaequilatera</i>	8	100	8
	<i>Bryophyllum</i> spp.	3	100	3
	<i>Cynodon dactylon</i>	3	100	3
	<i>Lepidium africanum</i>	3	100	3
	<i>Datura stramonium</i>	2	100	2
	<i>Chenopodium carinatum</i>	0	0	0
	<i>Coronopus didymus</i>	0	0	0
	<i>Conyza canadensis</i>	0	0	0
	<i>Sida cordifolia</i>	0	0	0
	<i>Argemone ochroleuca</i>	0	0	0
	<i>Xanthium strumarium</i>	0	0	0

In the eastern region *H. dihystra* occurred in roots of 18 species and 16 genera of weed sampled (Table 14). *Helicotylenchus dihystra* was predominant in the roots of *N. physalodes* (19), followed by *A. deflexus* (16),

C. dactylon (14) and *H. trionum* (14). Frequency of occurrence for these parasites ranged from 0 to 35 % (*C. dactylon*) and *B. bipinnata* while population density ranged between 0 and 112 (*N. physalodes*).

Helicotylenchus dihystra occurred in roots of six species and eight genera of weed sampled (Table 14). The prominence value for *H. dihystra* (31) was highest in the roots of *S. oleraceus*, followed by *Sparganium* spp. (25) and *F. bidentis* (10). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged between 0 and 31 (*S. oleraceus*).

Table 15. Prominence values (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* in 5g roots of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
<i>Nicandra physalodes</i>	7	2	49
<i>Cynodon dactylon</i>	6	48	9
<i>Amaranthus hybridus</i>	6	18	13
<i>Xanthium strumarium</i>	5	27	10
<i>Eleusine corocana</i>	5	7	19
<i>Ipomoea purpurea</i>	4	2	26
<i>Hibiscus trionum</i>	4	7	15
<i>Amaranthus deflexus</i>	4	2	31
<i>Bryophyllum</i> spp.	4	2	28
<i>Bidens bipinnata</i>	3	27	5
<i>Datura stramonium</i>	3	5	3
<i>Chloris virgata</i>	2	2	14
<i>Sonchus oleraceus</i>	2	2	8
<i>Oxalis latifolia</i>	1	3	9
<i>Commelina benghalensis</i>	1	11	3
<i>Chenopodium murale</i>	1	2	8
<i>Cyperus rotundus</i>	1	7	3
<i>Tagetes minuta</i>	0.4	16	1
<i>Hibiscus cannabinus</i>	0.4	2	3
<i>Flaveria bidentis</i>	0.4	2	3
<i>Sparganium</i> spp.	0.2	2	2
<i>Emex australis</i>	0.2	5	1
<i>Chamaesyce inaequilatera</i>	0.2	2	2
<i>Richardia brasiliensis</i>	0.2	7	0
<i>Cyperus esculentus</i>	0.2	2	2
<i>Datura ferox</i>	0	0	0
<i>Argemone ochroleuca</i>	0	0	0
<i>Physalis viscosa</i>	0	0	0
<i>Coronopus didymus</i>	0	0	0
<i>Conyza canadensis</i>	0	0	0
<i>Boerhavia diffusa</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Tribulus terrestris</i>	0	0	0
<i>Nothoscordum gracile</i>	0	0	0
<i>Malva parvifolia</i>	0	0	0
<i>Sida cordifolia</i>	0	0	0
<i>Lepidium africanum</i>	0	0	0
<i>Senecio latifolius</i>	0	0	0
<i>Chenopodium carinatum</i>	0	0	0

Rotylenchus unisex occurred in roots of 25 species and genera of weed sampled (Table 15). PV for *R. unisex* was highest in the roots of *N. physalodes* (7), followed by *C. dactylon* (6) and *A. hybridus* (6). Frequency of occurrence ranged between 0 and 48 % (*C. dactylon*), while population density ranged between 0 and 49 (*N. physalodes*).

Table 16. Prominence values (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* per 5g roots of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/5g roots
Eastern region	<i>Nicandra physalodes</i>	8	3	49
	<i>Cynodon dactylon</i>	7	47	10
	<i>Eleusine corocana</i>	7	12	19
	<i>Amaranthus hybridus</i>	6	24	13
	<i>Amaranthus deflexus</i>	5	3	31
	<i>Bidens bipinnata</i>	3	35	5
	<i>Ipomoea purpurea</i>	2	3	13
	<i>Xanthium strumarium</i>	2	3	9
	<i>Chloris virgata</i>	2	3	14
	<i>Hibiscus trionum</i>	2	9	5
	<i>Hibiscus cannabinus</i>	1	3	3
	<i>Commelina benghalensis</i>	1	15	3
	<i>Chenopodium murale</i>	1	3	8
	<i>Cyperus rotundus</i>	1	9	3
	<i>Datura stramonium</i>	1	3	3
	<i>Physalis viscosa</i>	1	3	4
	<i>Cyperus esculentus</i>	0.4	3	2
	<i>Richardia brasiliensis</i>	0.3	9	1
	<i>Emex australis</i>	0.2	6	1
	<i>Oxalis latifolia</i>	0.1	3	1
	<i>Tagetes minuta</i>	0.1	21	1
	<i>Senecio latifolius</i>	0	0	0
	<i>Datura ferox</i>	0	0	0
	<i>Boerhavia diffusa</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Tribulus terrestris</i>	0	0	0
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Malva parvifolia</i>	0	0	0
Western region	<i>Bryophyllum</i> spp.	28	100	28
	<i>Sparganium</i> spp.	27	100	0
	<i>Flaveria bidentis</i>	13	100	3
	<i>Sonchus oleraceus</i>	8	100	8
	<i>Cynodon dactylon</i>	5	100	5
	<i>Chamaesyce inaequilatera</i>	2	100	2
	<i>Coronopus didymus</i>	0	0	0
	<i>Conyza canadensis</i>	0	0	0
	<i>Lepidium africanum</i>	0	0	0
	<i>Chenopodium carinatum</i>	0	0	0
	<i>Argemone ochroleuca</i>	0	0	0
	<i>Xanthium strumarium</i>	0	0	0
	<i>Datura stramonium</i>	0	0	0
	<i>Sida cordifolia</i>	0	0	0

Rotylenchus unisex occurred in 21 species and 19 genera of weed sampled (Table 16). *Rotylenchus unisex* was predominant in the roots of *N. physalodes* (8), followed by *C. dactylon* (7) and *E. corocana* (7). Frequency of occurrence

for these parasites ranged from 0 to 47 % (*C. dactylon*), while population density ranged from 0 to 49 (*N. physalodes*).

In the western region *R. unisex* occurred in roots of four weed species and six of the genera sampled (Tables 16). The prominence value for *R. unisex* was highest in the roots of *Bryophyllum* spp. (28), followed by *Sparganium* (27) and *F. bidentis* (13). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged from 0 to 28 (*Bryophyllum* spp.).

2.4.4. Soil samples (200ml): Plant-parasitic, free-living and predatory nematodes

Table 17. Prominence values (PV), frequency of occurrence (%) and mean population density of plant-parasitic, free-living and predatory nematodes in soil of weeds sampled from 44 sites in South Africa.

Nematode families, genera/spp.	PV	Frequency of occurrence (%)	Mean population density/200ml soil
<i>Meloidogyne</i> spp.	966	100	966
<i>Helicotylenchus dihystra</i>	780	100	780
<i>Rotylenchus unisex</i>	659	89	699
Rhabditidae	641	100	641
Mononchidae	638	100	638
<i>Scutellonema brachyurus</i>	209	93	217
<i>Pratylenchus zeae</i>	133	98	134
<i>Helicotylenchus parapatyurus</i>	116	32	205
<i>Rotylenchulus parvus</i>	30	55	40
<i>Tylenchorhynchus brevilineatus</i>	25	45	37
<i>Xiphinema</i> spp.	21	68	26
<i>Tylenchorhynchus capitatus</i>	17	45	25
<i>Scutellonema truncatum</i>	12	14	32
<i>Helicotylenchus digonicus</i>	8	5	38
<i>Aphelenchoides</i> spp.	5	25	9
<i>Rotylenchulus borealis</i>	4	11	13
<i>Aphelenchus avenae</i>	4	25	7
<i>Pratylenchus scribneri</i>	3	18	6
<i>Tylenchorhynchus mashhoodi</i>	2	18	5
<i>Rotylenchus karooensis</i>	2	9	8
<i>Scutellonema dreyeri</i>	1	2	6
<i>Rotylenchus incultus</i>	1	7	5
<i>Ditylenchus</i> spp.	1	5	4
<i>Ditylenchus africanus</i>	1	5	3
<i>Criconemoides obtusicaudatus</i>	1	9	3
<i>Radopholus similis</i>	1	7	2
<i>Criconemoides sphaerocephalus</i>	0.1	2	3
<i>Rotylenchus brevicaudatus</i>	0.1	2	2
<i>Geocenamus brevelineatus</i>	0.1	2	2
<i>Histotylenchus histoides</i>	0.1	2	2
<i>Pratylenchus brachyurus</i>	0.1	2	2
<i>Criconema corbeti</i>	0.1	2	1
<i>Heterodera</i> spp.	0.1	2	1
<i>Tylenchorhynchus ventralis</i>	0.1	2	1

Twenty-seven plant-parasitic nematode species and 17 genera as well as individuals from saprophytic (Rhabditidae) and predatory (Mononchida) families were identified from soil samples from 44 sites (Table 17). *Meloidogyne* spp. were predominant with a PV of 966, followed by *H. dihystra* (780) and *R. unisex* (659). Root-knot nematodes and *H. dihystra* occurred (100 %) in all soil samples. Population densities ranged between 1 and 966 *Meloidogyne* spp. Nematode individuals from the families Rhabditidae and Mononchidae were present in all soil samples from all sites, with PV and population densities of 641 and 638, respectively.

Table 18. Prominence values (PV), frequency of occurrence (%) and mean population density of plant-parasitic, free-living and predatory nematodes for 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Nematode families, genera/spp.	PV	Frequency of occurrence %	Mean population density/200ml soil
Eastern region	Rhabditidae	6 015	100	6 015
	<i>Meloidogyne</i> spp.	5 194	100	5 194
	Mononchidae	5 350	100	5 350
	<i>Helicotylenchus dihystra</i>	3 513	82	3 880
	<i>Rotylenchus unisex</i>	3 110	88	3 315
	<i>Helicotylenchus paraplaturus</i>	1 848	41	2 886
	<i>Pratylenchus zae</i>	1 380	97	1 401
	<i>Scutellonema brachyurus</i>	1 242	94	1 281
	<i>Tylenchorhynchus capitatus</i>	290	41	453
	<i>Xiphinema</i> spp.	134	81	149
	<i>Tylenchorhynchus brevilineatus</i>	61	91	64
	<i>Rotylenchulus parvus</i>	26	85	28
	<i>Scutellonema truncatum</i>	15	23	32
	<i>Helicotylenchus digonicus</i>	9	6	38
	<i>Tylenchorhynchus mashhoodi</i>	8	21	17
	<i>Aphelenchoides</i> spp.	5	21	10
	<i>Aphelenchus avenae</i>	5	24	11
	<i>Pratylenchus scribneri</i>	3	15	7
	<i>Rotylenchus incultus</i>	2	6	7
	<i>Radopholus similis</i>	1	9	2
	<i>Ditylenchus</i> spp.	1	3	6
	<i>Scutellonema dreyeri</i>	1	3	6
	<i>Geocenamus brevilineatus</i>	0.3	3	2
	<i>Rotylenchus brevicaudatus</i>	0.3	3	2
	<i>Tylenchorhynchus ventralis</i>	0.1	3	1
	<i>Criconema corbeti</i>	0.1	3	1
Western region	Mononchidae	450	100	450
	<i>Meloidogyne</i> spp.	284	100	284
	Rhabditidae	202	100	202
	<i>Rotylenchus unisex</i>	179	90	189
	<i>Helicotylenchus dihystra</i>	137	100	137
	<i>Pratylenchus zae</i>	89	100	89
	<i>Scutellonema brachyurus</i>	57	90	60
	<i>Xiphinema</i> spp.	21	90	22
	<i>Rotylenchulus parvus</i>	18	80	20
	<i>Tylenchorhynchus brevilineatus</i>	13	70	15
	<i>Rotylenchulus borealis</i>	9	50	13
	<i>Rotylenchulus karoensis</i>	5	40	8
	<i>Aphelenchoides</i> spp.	4	40	7
	<i>Tylenchorhynchus capitatus</i>	4	60	5
	<i>Criconemoides obtusicaudatus</i>	2	40	3
	<i>Pratylenchus scribneri</i>	2	30	3
	<i>Tylenchorhynchus mashhoodi</i>	1	10	3
	<i>Criconemoides sphaerocephalus</i>	1	10	3
	<i>Aphelenchus avenae</i>	1	30	2
	<i>Histotylenchus histoides</i>	1	10	2
	<i>Pratylenchus brachyurus</i>	1	20	2
	<i>Heterodera</i> spp.	0.3	10	1
	<i>Ditylenchus africanus</i>	0.3	10	1

PV and population densities for plant-parasitic, free-living and predatory nematodes were substantially higher in the eastern region compared to those

in the western region. Nineteen plant-parasitic nematode species and 14 genera were identified from 200-ml soil samples obtained from sites in the eastern region (Table 18). The predominant plant-parasitic nematode species according to PV were *Meloidogyne* species (5 194), followed by *H. dihystra* (3 513) and *R. unisex* (3 110). Frequency of occurrence ranged between 3 % and 100 %. Population density ranged between 1 and 5 194 (*Meloidogyne* spp.).

Seventeen plant-parasitic nematode species and 14 genera were identified from 200-ml soil samples obtained from sites in the western region (Table 18). Predominant plant-parasitic nematode species according to PV were *Meloidogyne* spp. (284), followed by *R. unisex* (179) and *H. dihystra* (137). Frequency of occurrence values ranged between 10 % and 100 %, while population density ranged between 1 and 284 (*Meloidogyne* spp.).

Nematodes from the family Rhabditidae were predominant in 200-ml soil samples from the eastern region, while these from the family Mononchidae were predominant in soil samples from the western region (Table 18). PV and population densities for the Rhabditidae family were 6 015 and 202 for soil samples from the eastern and western regions, respectively, while for the Mononchidae family PV were 5 350 and 450, respectively, for the eastern and western regions. Both Rhabditidae and Mononchidae occurred at all sites sampled (100 %) in the eastern as well as western regions of South Africa.

Table 19. Prominence values (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. per 200ml soil of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/200ml soil
<i>Hibiscus cannabinus</i>	698	7	2 637
<i>Cyperus rotundus</i>	362	7	1 370
<i>Hibiscus trionum</i>	266	2	1 881
<i>Sparganium</i> spp.	163	2	1 156
<i>Senecio latifolius</i>	161	14	431
<i>Commelina benghalensis</i>	79	11	238
<i>Datura stramonium</i>	75	9	250
<i>Cynodon dactylon</i>	74	52	102
<i>Bidens bipinnata</i>	61	52	85
<i>Xanthium strumarium</i>	43	16	108
<i>Sida cordifolia</i>	38	2	266
<i>Tagetes minuta</i>	36	16	91
<i>Eleusine corocana</i>	33	9	110
<i>Ipomoea purpurea</i>	30	5	133
<i>Tribulus terrestris</i>	28	2	39
<i>Amaranthus hybridus</i>	27	23	56
<i>Amaranthus deflexus</i>	22	2	157
<i>Sonchus oleraceus</i>	18	2	124
<i>Nicandra physalodes</i>	18	2	127
<i>Malva parvifolia</i>	13	2	89
<i>Bryophyllum</i> spp.	11	2	79
<i>Argemone ochroleuca</i>	11	9	36
<i>Chenopodium murale</i>	10	2	70
<i>Chenopodium carinatum</i>	9	7	34
<i>Boerhavia diffusa</i>	8	2	55
<i>Datura ferox</i>	8	2	55
<i>Flaveria bidentis</i>	8	2	59
<i>Emex australis</i>	7	7	26
<i>Richardia brasiliensis</i>	6	7	23
<i>Chloris virgata</i>	6	2	41
<i>Cyperus esculentus</i>	5	3	30
<i>Conyza canadensis</i>	4	2	30
<i>Physalis viscosa</i>	2	2	11
<i>Chamaesyce inaequilatera</i>	2	2	12
<i>Oxalis latifolia</i>	1	2	8
<i>Coronopus didymus</i>	1	2	7
<i>Lepidium africanum</i>	0.3	9	1
<i>Nothoscordum gracile</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0

Meloidogyne spp. occurred in the soil samples from the rhizosphere of 35 weed species and 32 genera sampled (Table 19). *Meloidogyne* spp. was predominant in the soil of *H. cannabinus* (698), followed by *C. rotundus* (362) and *H. trionum* (266). Frequency of occurrence ranged between 0 and 52 % (*C. dactylon* & *B. bipinnata*), while population density ranged between 0 and 2 637 (*H. cannabinus*).

Table 20. Prominence values (PV), frequency of occurrence (%) and mean population density of *Meloidogyne* spp. per 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/200ml soil
Eastern region	<i>Hibiscus trionum</i>	791	9	2 637
	<i>Cyperus rotundus</i>	411	9	1 370
	<i>Hibiscus cannabinus</i>	325	3	1 881
	<i>Senecio latifolis</i>	183	18	431
	<i>Datura stramonium</i>	101	6	411
	<i>Commelina benghalensis</i>	92	15	238
	<i>Bidens bipinnata</i>	92	38	150
	<i>Cynodon dactylon</i>	71	53	98
	<i>Xanthium strumarium</i>	49	21	108
	<i>Tagetes minuta</i>	42	21	91
	<i>Eleusine corocana</i>	38	12	110
	<i>Amaranthus hybridus</i>	30	29	56
	<i>Ipomoea purpurea</i>	30	5	133
	<i>Amaranthus deflexus</i>	27	3	157
	<i>Nicandra physalodes</i>	22	3	125
	<i>Malva parvifolia</i>	15	3	89
	<i>Chenopodium murale</i>	12	3	70
	<i>Datura ferox</i>	10	3	55
	<i>Emex australis</i>	8	9	26
	<i>Chloris virgata</i>	7	3	41
	<i>Richardia brasiliensis</i>	7	9	23
	<i>Boerhavia diffusa</i>	7	3	41
	<i>Tribulus terrestris</i>	7	3	39
	<i>Cyperus esculentus</i>	5	3	30
	<i>Physalis viscosa</i>	2	3	11
	<i>Oxalis latifolia</i>	1	3	8
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
Western region	<i>Sparganium</i> spp.	1 156	100	1 156
	<i>Sida cordifolia</i>	266	100	266
	<i>Datura stramonium</i>	178	100	178
	<i>Sonchus oleraceus</i>	124	100	124
	<i>Cynodon dactylon</i>	115	100	115
	<i>Bryophyllum</i> spp.	79	100	79
	<i>Flaveria bidentis</i>	59	100	59
	<i>Argemone ochroleuca</i>	36	100	36
	<i>Xanthium strumarium</i>	35	100	35
	<i>Chenopodium carinatum</i>	34	100	34
	<i>Conyza canadensis</i>	30	100	30
	<i>Chamaesyce inaequilatera</i>	12	100	12
	<i>Coronopus didymus</i>	7	100	7
	<i>Lepidium africanum</i>	1	100	1

Root-knot nematodes occurred in soil samples from the rhizosphere of 26 species and 23 genera of weeds sampled at localities in the eastern region (Table 20). These parasites were predominant in the soil samples of *H. trionum* (791), followed by *C. rotundus* (411) and *H. cannabinus* (325). Frequency of occurrence for these parasites ranged from 0 to 53 % (*C. dactylon*), while population density ranged from 0 to 2 637 (*H. trionum*).

In the western region, root-knot nematodes occurred in soil samples from the rhizosphere of 12 species and 14 genera of the weeds sampled (Table 20). The prominence value for *Meloidogyne* spp. (1 156) was highest in the soil of *Sparganium* spp., followed by *S. cordifolia* (266) and *D. stramonium* (178). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged from 1 to 1156 (*Sparganium* spp.).

Table 21. Prominence values (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zae* in soil of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/200ml soil
<i>Cyperus rotundus</i>	68	7	258
<i>Cynodon dactylon</i>	32	39	52
<i>Commelina benghalensis</i>	15	11	46
<i>Xanthium strumarium</i>	10	16	24
<i>Tagetes minuta</i>	9	11	27
<i>Emex australis</i>	8	7	30
<i>Eleusine corocana</i>	8	9	27
<i>Datura stramonium</i>	7	9	23
<i>Bidens bipinnata</i>	7	30	3
<i>Richardia brasiliensis</i>	7	7	26
<i>Amaranthus hybridus</i>	6	20	13
<i>Cyperus esculentus</i>	6	3	34
<i>Lepidium africanum</i>	5	9	17
<i>Malva parvifolia</i>	5	2	34
<i>Bryophyllum</i> spp.	5	2	33
<i>Ipomoea purpurea</i>	4	5	18
<i>Nicandra physalodes</i>	3	2	23
<i>Hibiscus cannabinus</i>	3	7	13
<i>Amaranthus deflexus</i>	3	2	19
<i>Chenopodium carinatum</i>	2	7	7
<i>Oxalis latifolia</i>	2	2	11
<i>Chenopodium murale</i>	1	2	9
<i>Chloris virgata</i>	1	2	6
<i>Boerhavia diffusa</i>	1	2	9
<i>Tribulus terrestris</i>	1	2	8
<i>Datura ferox</i>	1	2	9
<i>Physalis viscosa</i>	0.4	2	3
<i>Senecio latifolius</i>	0.3	9	1
<i>Sonchus oleraceus</i>	0	0	0
<i>Hibiscus trionum</i>	0	0	0
<i>Sida cordifolia</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Argemone ochroleuca</i>	0	0	0
<i>Nothoscordum gracile</i>	0	0	0
<i>Coronopus didymus</i>	0	0	0
<i>Conyza canadensis</i>	0	0	0
<i>Flaveria bidentis</i>	0	0	0
<i>Sparganium</i> spp.	0	0	0
<i>Chamaesyce inaequilatera</i>	0	0	0

Pratylenchus zae occurred in soil samples from the rhizosphere of 27 species and 25 genera of the weeds sampled (Table 21). *Pratylenchus zae* was predominant in the soil of *C. rotundus* (68), followed by *C. dactylon* (32) and *C. benghalensis* (15). Frequency of occurrence ranged between 0 and 39 % (*C. dactylon*), while population density ranged from 0 to 258 (*C. dactylon*).

Table 22. Prominence values (PV), frequency of occurrence (%) and mean population density of *Pratylenchus zae* per 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/200ml soil
Eastern region	<i>Cyperus rotundus</i>	77	9	258
	<i>Cynodon dactylon</i>	33	53	46
	<i>Commelina benghalensis</i>	33	15	84
	<i>Eleusine corocana</i>	27	12	79
	<i>Xanthium strumarium</i>	11	21	24
	<i>Tagetes minuta</i>	10	15	27
	<i>Datura stramonium</i>	9	6	36
	<i>Emex australis</i>	9	9	30
	<i>Bidens bipinnata</i>	8	38	13
	<i>Richardia brasiliensis</i>	8	9	26
	<i>Malva parvifolia</i>	6	3	34
	<i>Amaranthus hybridus</i>	6	29	11
	<i>Cyperus esculentus</i>	6	3	34
	<i>Ipomoea purpurea</i>	4	5	18
	<i>Hibiscus trionum</i>	4	9	13
	<i>Nicandra physalodes</i>	3	3	20
	<i>Chenopodium murale</i>	2	3	9
	<i>Amaranthus deflexus</i>	3	3	19
	<i>Oxalis latifolia</i>	2	3	11
	<i>Datura ferox</i>	2	3	9
	<i>Tribulus terrestris</i>	1	3	8
	<i>Chloris virgata</i>	1	3	6
	<i>Physalis viscosa</i>	1	3	3
	<i>Boerhavia diffusa</i>	1	3	6
	<i>Senecio latifolius</i>	0.3	12	1
	<i>Hibiscus cannabinus</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Nothoscordum gracile</i>	0	0	0
Western region	<i>Sparganium</i> spp.	169	100	169
	<i>Cynodon dactylon</i>	87	100	67
	<i>Sida cordifolia</i>	67	100	67
	<i>Sonchus oleraceus</i>	62	100	62
	<i>Bryophyllum</i> spp.	33	100	33
	<i>Xanthium strumarium</i>	32	100	32
	<i>Lepidium africanum</i>	17	100	17
	<i>Datura stramonium</i>	10	100	10
	<i>Chenopodium carinatum</i>	5	50	7
	<i>Flaveria bidentis</i>	3	100	3
	<i>Coronopus didymus</i>	0	0	0
	<i>Conyza canadensis</i>	0	0	0
	<i>Chamaesyce inaequilatera</i>	0	0	0
	<i>Argemone ochroleuca</i>	0	0	0

Pratylenchus zae occurred in soil samples from the rhizosphere of 28 species and 24 genera of the weeds sampled at sites in the eastern region (Table 22). Individuals of *P. zae* were predominant in the soil samples of *C.*

rotundus (77), followed by *C. dactylon* (33) and *C. benghalensis* (33). Frequency of occurrence for these parasites ranged from 0 to 53 % (*C. dactylon*), while population density ranged from 0 to 258 (*C. rotundus*).

In the western region, *P. zae* individuals occurred in soil samples from the rhizosphere of 12 species and 14 genera of weed sampled (Table 22). The prominence value for *P. zae* (169) was highest in the soil of *Sparganium* spp., followed by *C. dactylon* (87) and *S. cordifolia* (67). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged from 0 to 169 (*Sparganium* spp.).

Table 23. Prominence values (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystra* in soil of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/200ml soil
<i>Hibiscus trionum</i>	366	2	2 590
<i>Cyperus rotundus</i>	297	7	1 121
<i>Commelina benghalensis</i>	284	9	948
<i>Datura stramonium</i>	73	9	243
<i>Bidens bipinnata</i>	70	27	134
<i>Eleusine corocana</i>	59	9	196
<i>Amaranthus hybridus</i>	54	20	120
<i>Cynodon dactylon</i>	51	39	81
<i>Hibiscus cannabinus</i>	40	7	151
<i>Malva parvifolia</i>	35	2	250
<i>Nicandra physalodes</i>	28	2	200
<i>Ipomoea purpurea</i>	27	5	122
<i>Tagetes minuta</i>	25	16	70
<i>Sparganium</i> spp.	24	2	169
<i>Xanthium strumarium</i>	21	12	60
<i>Amaranthus deflexus</i>	19	2	135
<i>Senecio latifolius</i>	13	14	35
<i>Sonchus oleraceus</i>	8	2	62
<i>Chloris virgata</i>	7	2	50
<i>Chenopodium murale</i>	2	2	13
<i>Physalis viscosa</i>	2	2	12
<i>Boerhavia diffusa</i>	2	2	14
<i>Tribulus terrestris</i>	1	2	7
<i>Conyza canadensis</i>	0.4	2	3
<i>Chamaesyce inaequilatera</i>	0	0	0
<i>Datura ferox</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Lepidium africanum</i>	0	0	0
<i>Flaveria bidentis</i>	0	0	0
<i>Oxalis latifolia</i>	0	0	0
<i>Nothoscordum gracile</i>	0	0	0
<i>Coronopus didymus</i>	0	0	0
<i>Richardia brasiliensis</i>	0	0	0
<i>Sida cordifolia</i>	0	0	0
<i>Cyperus esculentus</i>	0	0	0
<i>Argemone ochroleuca</i>	0	0	0
<i>Emex australis</i>	0	0	0
<i>Bryophyllum</i> spp.	0	0	0
<i>Chenopodium carinatum</i>	0	0	0

Helicotylenchus dihystra occurred in soil samples from the rhizosphere of 23 species and genera of weed sampled (Table 23). This parasite was predominant in the soil of *H. trionum* (366), followed by *C. rotundus* (297) and *C. benghalensis* (284). Frequency of occurrence ranged between 0 and 39 % (*C. dactylon*), while population density ranged between 0 and 2 590 (*H. trionum*).

Table 24. Prominence values (PV), frequency of occurrence (%) and mean population density of *Helicotylenchus dihystra* per 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence(%)	Mean population density/200ml soil
Eastern region	<i>Cyperus rotundus</i>	336	9	1 211
	<i>Datura stramonium</i>	113	6	463
	<i>Commelina benghalensis</i>	92	15	237
	<i>Bidens bipinnata</i>	79	35	134
	<i>ibiscus cannabinus</i>	78	3	449
	<i>Amaranthus hybridus</i>	65	29	120
	<i>Cynodon dactylon</i>	51	53	172
	<i>Hibiscus trionum</i>	45	9	151
	<i>Malva parvifolia</i>	43	3	250
	<i>Nicandra physalodes</i>	35	3	200
	<i>Tagetes minuta</i>	32	21	70
	<i>Xanthium strumarium</i>	28	15	72
	<i>Eleusine corocana</i>	27	12	78
	<i>Ipomoea purpurea</i>	27	5	122
	<i>Amaranthus deflexus</i>	23	3	135
	<i>Senecio latifolis</i>	15	18	35
	<i>Chloris virgata</i>	9	3	50
	<i>Chenopodium murale</i>	2	3	13
	<i>Physalis viscosa</i>	2	3	12
	<i>Boerhavia diffusa</i>	2	3	14
	<i>Tribulus terrestris</i>	1	3	7
	<i>Oxalis latifolia</i>	0	0	0
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Richardia brasiliensis</i>	0	0	0
	<i>Cyperus esculentus</i>	0	0	0
	<i>Emex australis</i>	0	0	0
	<i>Datura ferox</i>	0	0	0
Western region	<i>Sida cordifolia</i>	11	100	11
	<i>Conyza canadensis</i>	3	100	0
	<i>Cynodon dactylon</i>	1	100	1
	<i>Sparganium</i> spp.	0	0	0
	<i>Flaveria bidentis</i>	0	0	0
	<i>Bryophyllum</i> spp.	0	0	0
	<i>Sonchus oleraceus</i>	0	0	0
	<i>Argemone ochroleuca</i>	0	0	0
	<i>Xanthium strumarium</i>	0	0	0
	<i>Datura stramonium</i>	0	0	0
	<i>Coronopus didymus</i>	0	0	0
	<i>Lepidium africanum</i>	0	0	0
	<i>Chenopodium carinatum</i>	0	0	0
	<i>Chamaesyce inaequilatera</i>	0	0	0

Helicotylenchus dihystra individuals were present in soil samples from the rhizosphere of 19 species and 20 genera of the weeds sampled at sites in the eastern region (Table 24). Individuals of this parasite were predominant in the

soil of *C. rotundus* (336), followed by *D. stramonium* (113) and *C. benghalensis* (92). Frequency of occurrence for these parasites ranged from 0 to 53 % (*C. dactylon*), while population density ranged from 0 to 1 211 (*C. rotundus*).

In the western region, *H. dihystra* individuals occurred in soil samples of three species and genera of weed sampled (Table 24). PV for *H. dihystra* (11) was highest in the soil of *S. cordifolia*, followed by *C. Canadensis* (3) and *C. dactylon* (1). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged from 0 to 11 (*S. cordifolia*).

Table 25. Prominence values (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* in soil of weeds sampled from 44 sites in South Africa.

Weed species	PV	Frequency of occurrence (%)	Mean population density/200ml soil
<i>Cyperus rotundus</i>	328	7	1 240
<i>Hibiscus trionum</i>	320	2	2 261
<i>Sparganium</i> spp.	284	2	2 011
<i>Commelina benghalensis</i>	60	11	180
<i>Amaranthus hybridus</i>	47	20	104
<i>Cynodon dactylon</i>	46	48	67
<i>Eleusine corocana</i>	44	9	146
<i>Bidens bipinnata</i>	38	27	73
<i>Tagetes minuta</i>	25	16	63
<i>Xanthium strumarium</i>	20	12	59
<i>Nothoscordum gracile</i>	19	0	0
<i>Datura stramonium</i>	17	9	55
<i>Malva parvifolia</i>	16	2	111
<i>Emex australis</i>	16	7	60
<i>Bryophyllum</i> spp.	13	2	94
<i>Sonchus oleraceus</i>	13	2	89
<i>Cyperus esculentus</i>	11	3	65
<i>Hibiscus cannabinus</i>	9	7	35
<i>Senecio latifolius</i>	7	14	19
<i>Chenopodium carinatum</i>	6	5	22
<i>Amaranthus deflexus</i>	6	2	40
<i>Ipomoea purpurea</i>	4	5	18
<i>Richardia brasiliensis</i>	4	7	15
<i>Nicandra physalodes</i>	4	2	30
<i>Chenopodium murale</i>	3	2	19
<i>Chloris virgata</i>	3	2	21
<i>Conyza canadensis</i>	3	2	18
<i>Flaveria bidentis</i>	2	2	17
<i>Argemone ochroleuca</i>	2	9	5
<i>Sida cordifolia</i>	2	2	11
<i>Lepidium africanum</i>	2	9	7
<i>Oxalis latifolia</i>	1	2	9
<i>Physalis viscosa</i>	1	2	9
<i>Coronopus didymus</i>	1	2	4
<i>Boerhavia diffusa</i>	0	0	0
<i>Portulaca oleracea</i>	0	0	0
<i>Tribulus terrestris</i>	0	0	0
<i>Chamaesyce inaequilatera</i>	0	0	0
<i>Datura ferox</i>	0	0	0

Rotylenchus unisex occurred in soil samples of 32 species and 30 genera of weed sampled (Table 25). *Rotylenchus unisex* was predominant in the soil of *C. rotundus* (328), followed by *H. trionum* (320) and *Sparganium* spp. (284). Frequency of occurrence ranged between 0 and 48 % (*C. dactylon*), while population density ranged from 0 to 1 240 (*C. rotundus*).

Table 26. Prominence values (PV), frequency of occurrence (%) and mean population density of *Rotylenchus unisex* per 200ml soil of weeds collected from 34 sites located in the eastern and 10 sites in the western regions of South Africa.

Region	Weed species	PV	Frequency of occurrence (%)	Mean population density/200ml soil
Eastern region	<i>Cyperus rotundus</i>	372	9	1 240
	<i>Commelina benghalensis</i>	70	15	180
	<i>Hibiscus cannabinus</i>	68	3	392
	<i>Amaranthus hybridus</i>	59	26	116
	<i>Cynodon dactylon</i>	56	53	79
	<i>Bidens bipinnata</i>	43	35	73
	<i>Tagetes minuta</i>	29	21	63
	<i>Xanthium strumarium</i>	25	18	59
	<i>Datura stramonium</i>	24	6	100
	<i>Eleusine corocana</i>	20	12	59
	<i>Malva parvifolia</i>	19	3	111
	<i>Ipomoea purpurea</i>	13	5	59
	<i>Cyperus esculentus</i>	11	3	65
	<i>Hibiscus trionum</i>	11	9	35
	<i>Senecio latifolius</i>	8	18	19
	<i>Amaranthus deflexus</i>	7	3	40
	<i>Emex australis</i>	6	9	20
	<i>Richardia brasiliensis</i>	5	9	15
	<i>Nicandra physalodes</i>	5	3	30
	<i>Chloris virgata</i>	4	3	21
	<i>Chenopodium murale</i>	3	3	19
	<i>Oxalis latifolia</i>	2	3	9
	<i>Physalis viscosa</i>	2	3	9
	<i>Boerhavia diffusa</i>	0	0	0
	<i>Portulaca oleracea</i>	0	0	0
	<i>Tribulus terrestris</i>	0	0	0
	<i>Nothoscordum gracile</i>	0	0	0
	<i>Datura ferox</i>	0	0	0
Western region	<i>Sparganium</i> spp.	2 011	100	2 011
	<i>Bryophyllum</i> spp.	94	100	94
	<i>Sonchus oleraceus</i>	89	100	89
	<i>Xanthium strumarium</i>	64	100	64
	<i>Datura stramonium</i>	22	100	22
	<i>Conyza canadensis</i>	18	100	18
	<i>Flaveria bidentis</i>	17	100	17
	<i>Cynodon dactylon</i>	16	100	16
	<i>Lepidium africanum</i>	7	100	7
	<i>Argemone ochroleuca</i>	5	100	5
	<i>Chenopodium carinatum</i>	4	100	4
	<i>Coronopus didymus</i>	4	100	4
	<i>Chamaesyce inaequilatera</i>	0	0	0
	<i>Sida cordifolia</i>	0	0	0

Rotylenchus unisex individuals occurred in soil samples of 23 species and 20 genera of weeds sampled at sites in the eastern region (Table 26). *R. unisex* was predominant in the soil of *C. rotundus* (372), followed by *C. benghalensis*

(70) and *H. cannabinus* (68). Frequency of occurrence for these parasites ranged from 0 to 53 % (*C. dactylon*), while population density ranged from 0 to 1 240 (*C. rotundus*).

In the western region, *R. unisex* individuals occurred in soil samples of 10 species and 12 genera of weeds sampled (Table 26). PV for *R. unisex* (2 011) was highest in the soil of *Sparganium* spp., followed by *Bryophyllum* spp. (94) and *S. oleraceus* (89). Frequency of occurrence for these parasites ranged from 0 to 100 %, while population density ranged from 0 to 2 011 (*Sparganium* spp.).

2.5 Discussion

Twenty plant-parasitic nematode species and five genera were reported in association with weeds for the first time in South Africa as a result of this study. These species and genera are *Criconema corbeti*, *Criconemoides obtusicaudatus*, *C. sphaerocephalus*, *Geocenamus brevilineatus*, *Helicotylenchus paraplatus*, *H. digonicus*, *Histotylenchus histodes*, *Pratylenchus zaei*, *P. scribneri*, *Rotylenchulus parvus*, *R. borealis*, *R. incultus*, *R. brevicaudatus*, *R. karoensis*, *Scutellonema truncatum*, *S. dreyeri*, *Tylenchorhynchus mashhoodi*, *T. ventralis*, *T. capitatus* and *T. brevilineatus*. From the 21 plant-parasitic nematode species and 16 genera that were reported during 1984 from root and soil samples of a wide range of weed species sampled by Keetch and Buckley (1984), *Ditylenchus dipsaci*, *Helicotylenchus multicinctus*, *Merlinius brevidens*, *Pratylenchus thornei*, *P. pratensis*, *Rotylenchus capensis*, *Scutellonema paramaximus*, *Trichodorus* spp., and *Xiphinema mampara* were not recorded in our survey. Since this study will, however, be extended to other sites in the eastern and western regions of South Africa, some of the latter nematode genera or species may still be added to our list in the near future.

Plant-parasitic nematode genera and species that were predominant over all sites were generally the same for the root and soil samples, namely *Meloidogyne* spp., *P. zaei*, *H. dihystra* and *R. unisex*. The endoparasitic *Meloidogyne* spp. was the predominant plant-parasitic nematode genus

identified from both root and soil samples of all weed samples from all 44 sites. This is in agreement with a report by Fourie and Mc Donald (2001). The latter authors also recorded the predominance of *Meloidogyne* species at 49 out of 51 localities in root and soil samples of a variety of crops. However, substantially lower PV and population density values for *Meloidogyne* spp. as found in the western region in this study, may most probably be ascribed to distinct differences in environmental conditions, farming practices and cropping systems. The latter tendency was also applicable to the other predominant plant-parasitic nematode genera and species identified.

The occurrence of endoparasitic lesion nematodes, particularly *P. zae* as another predominant species in roots of weeds during our study in both regions, is also supported by reports from Keetch and Buckley (1984). Although the latter authors did not record *P. zae* during their study, they identified *P. thornei*, *P. pratensis* and *P. brachyurus* in association with local weeds. In our study the latter species as well as *P. scribneri* were identified as parasites of weeds. The latter species was not predominant, however. Keetch and Buckley (1984), furthermore identified a number of *Pratylenchus* spp. associated with weeds in other southern African countries such as Malawi, Zimbabwe and Mozambique, namely *P. crenatus*, *P. pratensis* and *P. thornei*. In addition, several other authors listed lesion nematodes as parasites of weeds in other parts of the world with similar climates to South Africa (Brodie *et al.*, 1969; Ishaq, 1992; Kaplan & Mac Gowan 1982; Quénéhervé *et al.*, 2006).

The semi-endoparasitic spiral nematodes *H. dihystra*, *R. unisex* and *S. brachyurus*, which were particularly predominant in soil samples but also in root samples were also recorded by Keetch and Buckley (1984) on weeds locally and in other southern African countries. Other species from these genera that were associated with weeds in southern Africa by the latter authors include *H. multicinctus*, *S. multistriatum* and *R. capensis*.

Although not predominant, other endo- and ectoparasitic plant-parasitic nematode genera identified during our study, for example *Aphelenchoides*

spp., *D. africanus* and an un-identified *Ditylenchus* spp., *Heterodera* spp., *Radopholus similis*, *Criconemoides* spp., *G. brevelineatus*, *Tylenchorhynchus* spp., *Xiphinema* spp. and *Aphelenchus avenae* have also been reported in association with weeds by other authors worldwide (Crow & Welch, 2004; De Waele & Jordaan, 1988; Ishaqe, 1992; Keetch & Buckley, 1984; Sikora *et al.*, 2001). These parasites often cause substantial yield losses in agricultural and horticultural crops (Heyns, 1971). *Heterodera* spp. for example have previously also been identified from root and soil samples of weeds that occur in agricultural fields in South Africa (Keetch & Buckley, 1984), as well as from countries beyond the borders of South Africa (Keetch & Buckley, 1984; Quénéhervé, 2000). Another example is the presence of *Ditylenchus* spp. that was also associated with weeds in our study. Keetch and Buckley (1984) and De Waele and Jordaan (1988) previously reported that the presence of *D. dipsaci* and *D. africanus* (previously *D. destructor*), respectively, on several weeds in South Africa. Furthermore, *Aphelenchoides arachidis* (Lesufi *et al.*, 2006), an economically important endoparasite of groundnut has recently been identified locally on groundnut on the Vaalharts Irrigation Scheme. The presence of such economically important nematodes on weeds in South Africa may have a negative impact on farming and related industries when crops that are susceptible to these parasites are planted. For this reason, farmers need to be informed about plant-parasitic nematodes that infect weeds occurring in their crop systems.

Although the aim of this study was to identify plant-parasitic nematodes, free-living as well as predatory nematode families were also recorded. Those families were Rhabditidae and Mononchidae, which are common in South African soils and are usually abundant in the presence of decomposing organic material in soil (Heyns, 1971).

It was evident from this study that *Cynodon dactylon* overall occurred at most of the sites sampled as well as in both the eastern and western regions. Furthermore, all four predominant nematode genera/species namely *Meloidogyne* spp., *P. zeae*, *H. dihystra* and *R. unisex* identified during this study also had the highest frequency of occurrence in both root and soil

samples from *C. dactylon*. Therefore this weed could be used as an indicator for establishing whether economically important plant-parasitic nematodes are present in farmer's fields or whether such species does not occur there. Subsequently, recommendations for pro-active strategies to prevent build up of such nematode populations could be made by experts and implemented by the farmers. This will include that farmers should remove *C. dactylon* as soon as it appears in their fields. Except for its high frequency of occurrence, *C. dactylon* was also one of the predominant weed species that has generally been parasitised by the former four plant-parasitic nematode species. Conversely, the weed *Chenopodium murale* that occurred only at a few sites sampled during this survey, had a relatively high PV particularly for root-knot nematodes. Therefore, despite the low frequency of occurrence of *C. murale* it may also result in a build-up of root-knot nematode populations. This has to be taken into account by farmers. While farmers in the eastern region must take cognisance that *Chloris virgata*, *Amaranthus hybridus* and *Nicandra physalodis* are most prone to attack by root-knot nematodes, the same is true for *Flaveria bidentis*, *Sparganium* spp. and *Chamaesyce inaequilatra* in the western region. The situation is further complicated since other weed species as those mentioned for root-knot nematodes are generally parasitised by the other three predominant plant-parasitic nematodes, namely *P. zaeae*, *H. dihystra* and *R. unisex*. Examples of such weeds are *Sonchus oleraceus* for *P. zaeae* and *H. dihystra* and *Bryophyllum* spp. for *R. unisex*. It is thus crucial that farmers should aim their weed management strategies to eradicate these weeds also.

A number of weed species or genera that had low or even zero prominence values for the various predominant plant-parasitic nematodes could be considered as relatively poor hosts of these parasites. The predominance of weeds species with regard to parasitism by the four most abundant plant-parasitic nematode genera/species identified during this study, differed substantially. These weeds also differed considerably between the eastern and western regions with regard to their maintenance of the predominant plant-parasitic nematodes. *Tagetes minuta* which is reported by Bromilow (2001) to be a non- or very poor host for plant-parasitic nematodes, had

relatively high prominence values, particularly for root-knot nematodes in the eastern region. Therefore, this weed species should also be eradicated in time.

Results from this study confirm the significant role that weeds can play in maintaining or in the building-up of plant-parasitic nematode populations, particularly root-knot nematodes. For this reason weeds need to be eradicated timely and on a continuous basis to protect crops from nematode parasitism and subsequent yield and quality losses. In conclusion it could be summarized that results from this study indicates that weeds may have an adverse effect on crop rotation programmes. This implies that root-knot nematodes that are maintained by certain weeds that are left to grow together or in the absence of a crop may build-up in numbers that could be damaging to the current and/or follow-up crop. This could particularly true for those which are aimed at reducing plant-parasitic nematodes in both small-scale and commercial agricultural systems. If weeds are neglected with regard to their ability to host plant-parasitic nematodes, nematode-related crop losses may escalate.

Chapter 3: Molecular identification of *Meloidogyne* species using sequence characterised amplified regions-polymerase chain reaction (SCAR-PCR) assays

3.1. Introduction

Timely detection and accurate identification of economically important plant-parasitic nematode species, particularly root-knot nematodes, are crucial for proper research on nematode resistance and management studies (Zijlstra *et al.*, 2000; Pottie *et al.*, 1992). Until recently, identification methods available for root-knot nematodes were generally based on morphological characters (Jepson, 1987), required highly skilled taxonomists and were often inconclusive due to considerable variation within populations (Blok *et al.*, 1997; Zijlstra *et al.*, 2000). Therefore, molecular methods to identify *Meloidogyne* spp. has become more popular over the last decade and are currently generally used on a routine basis (Blok *et al.*, 1997; Zijlstra *et al.*, 2000).

Deoxyribonucleic acid (DNA)-based diagnostics provide attractive, alternative solutions for reliable identification of root-knot nematodes (Zijlstra *et al.*, 2000) and have been successfully used in the past by several researchers (Zijlstra *et al.*, 2000; Castagnone-Sereno *et al.*, 1991 & 1993; Cenis *et al.*, 1992; Curran *et al.*, 1985 & 1986; Fargette *et al.*, 1996; Fourie *et al.*, 2001; Garate *et al.*, 1991; Pottie *et al.*, 1992; Powers *et al.*, 1986; Xue *et al.*, 1992). The reason for this is because molecular identification techniques do not rely on the expressed products of the nematode genome, they are independent of environmental influences as well as the developmental stage of the nematode and are potentially extremely discriminating (McLain *et al.*, 1987; Zijlstra *et al.*, 2000). Subsequently, root-knot nematode sampled during our survey (see Chapter 2) was subjected to SCAR-PCR analysis to enable accurate identification of species present at the sites included.

3.2. Materials and methods

3.2.1. Rearing of *Meloidogyne* populations from different localities for root-knot nematode identification

Forty-four 4 litre pots were filled with a methyl bromide-fumigated (1,1620g a.i./2m³ soil) and steam-pasteurised soil (4.5 % clay, 92.8 % sand, 2.4 % silt and 0.3 % organic matter content). The soil pH (H₂O) was 4.35. Nutrients were added according to a soil nutrient analyses, namely 24g dolomitic lime, 12g zinc (Zn), 1.68g 2:3:2 nitrogen-phosphorus-potassium (NPK), 0.78g potassium chloride (KCl) and 14.04g super phosphate (10 % phosphorus).

Meloidogyne species eggs and J2 extracted from 20-g root samples of each weed species obtained from the different localities were used to inoculate individual, susceptible tomato plants (cv. Moneymaker) in a greenhouse. One moneymaker seedling was planted in each pot to attract root-knot nematode J2 and establish root-knot nematode populations from each site separately. Infected tomato roots were removed 60 days later for extraction of DNA from *Meloidogyne* species J2 using molecular (SCAR-PCR) techniques.

3.2.2. DNA extraction

Ten root-knot nematode J2 from each of the sites sampled during the survey were randomly selected from the respective counting dishes after extraction of 50g tomato roots using the adapted NaOCl method (Riekert, 1995). The former method was specifically developed for the extraction of root-knot nematode eggs and J2 from plant roots. For reference populations, ten juveniles of *M. incognita* and *M. javanica*, from greenhouse *in vivo* reared colonies that were verified using the SCAR-PCR method as well as perineal pattern morphology, were obtained. These J2 were reared in tomato roots (cv. Rodade) in separate greenhouses for inoculation purposes. No reference populations were, however, available for *M. arenaria*, *M. chitwoodi* and *M. fallax*.

DNA was extracted using the method of Stanton *et al.* (1998). Ten root-knot nematode J2 from each locality were suspended in 5 µl distilled water in a 0.5-ml microcentrifuge tube, whereafter twenty microlitre of a 0.25 M NaOH

solution was added to the tube and centrifuged at 10 000 rpm for five seconds. The tube containing the DNA (pellet at the bottom of the tube) was left overnight in a waterbath at 25° C. The DNA was then centrifuged at 10 000 rpm for five seconds, incubated at 99 °C for three minutes and allowed to cool at room temperature. Subsequently the solution was then centrifuged again for five seconds whereafter 4 µl 1 M HCl, 5 µl 1 M Tris-HCl (pH 8.0) and 5 µl 2 % Triton X-100 were added. The solution was again centrifuged for five seconds, allowed to cool at room temperature, centrifuged for a further five seconds and then placed in a 20 °C freezer and left overnight. Two microlitres of root-knot nematode DNA was subsequently used for SCAR-amplification reactions.

3.2.3. SCAR amplification

Table 1. Sequence of the Primers (Zijlstra, 2000; Zijlstra *et al.*, 2000).

<i>Meloidogyne</i> species identification		
Species ID	<i>Farenaria:</i>	F:Forward
	<i>Rarenaria:</i>	R:Reverse
	<i>Fincognita:</i>	F:Forward
	<i>Rincognita</i>	R:Reverse
	<i>Fjavanica</i>	F:Forward
	<i>Rjavanica</i>	R:Reverse
	<i>Ffallax</i>	F:Forward
	<i>Rfallax</i>	R:Reverse
	<i>Fhapla</i>	F:Forward
	<i>Rhapla</i>	R:Reverse
	<i>Fchitwoodi</i>	F:Forward
	<i>Rchitwoodi</i>	R:Reverse

PCR was carried out in a total volume of 19 µl, containing 8.5 µl dH₂O, promega buffer 4.0 µl, 1.2 µl MgCl₂, 3.2 µl dNTP, 1.0 µl forward primer, 1.0 µl reverse primer and 0.15 µl Tag. DNA amplification for *M. incognita* race 2 was done in a ThermoHybaid Thermal Cycler programmed as follows: denaturation at 94 °C for two minutes (one cycle), followed by 40 cycles of

denaturation at 94 °C for 30 seconds, annealing at 54 °C for 30 seconds and extension at 72 °C for one minute, followed by a final extension step at 72 °C for five minutes. The same programme was used for *M. javanica* with the exception of the annealing temperature at 64 °C, while for *M. chitwoodi*, *M. fallax* and *M. hapla* the annealing temperature was set at 58°C and for *M. arenaria* at 61°C (Zijlstra, 2000; Zijlstra *et al.*, 2000).

DNA products resulting from the amplification process of J2 were analysed by electrophoresis in a 3-% agarose gel with 1 x TBE running buffer (89 mM Tris-borate, 2.5 mM EDTA, pH 8.3). Two microlitres of 15 % Ficol (0.75g Ficol, 0.012g Bromophenolblue) was used as loading buffer. Ethidium bromide (1 µg/ml) was added to the gel. One blank reaction (no DNA), one reference sample of the appropriate root-knot nematode species (if available) and Lambda DNA restricted with HindIII and EcoRI as molecular weight markers were loaded on the gel to establish the size of the DNA bands for each root-knot nematode species. The gel containing DNA products of the respective juveniles was electrophoresed for 2 hours at 80 V and banding patterns were visualised with ultraviolet (UV) illumination. A photograph was taken of each gel containing the root-knot nematode J2 DNA-banding patterns.

3.2.4. Results

PCR with the *M. hapla*-specific SCAR primers resulted in amplification of the *M. hapla* 610-bp SCAR fragment for root-knot nematode J2 from the reference population (Fig. 1) as well as from the following 14 sites in the eastern region, namely Gamaboya, Gamagongwa, Gamagongwa Zone 2, Makoko, Malekutu, Malepetleke, Mbonisweni, Numbi, Sakhisizwe, Sibiziwe, Sishwamukile, Sukumani, Obonjaneni and White River.

In terms of *M. javanica*, the 670-bp *M. javanica* SCAR fragment was amplified during PCR reactions with the *M. javanica*-specific SCAR markers (Fig. 2) for J2 from the reference population as well as J2 obtained from 20 sites in the eastern region, namely Centani, Clarkbury, Emoyeni, Fundani, Gam-Gam clinic, Gam-Gam field, Hlanganani, Mente, Mthokozi, Okhombe, Phola,

Phuthumani, Qakazana, Qunu, Reserve C, Sihlangene, Siyaqoba, Sizamangwane, Sondelani and Willowvale. In the western region *M. javanica* J2 were also positively identified from 10 sites namely, Bushbuck, Magojaneng, Mapoteng, Maruping, Matlhabanelong, Mothibistadt, Tsineng, Gaserutege, Goodhope and Dikhing.

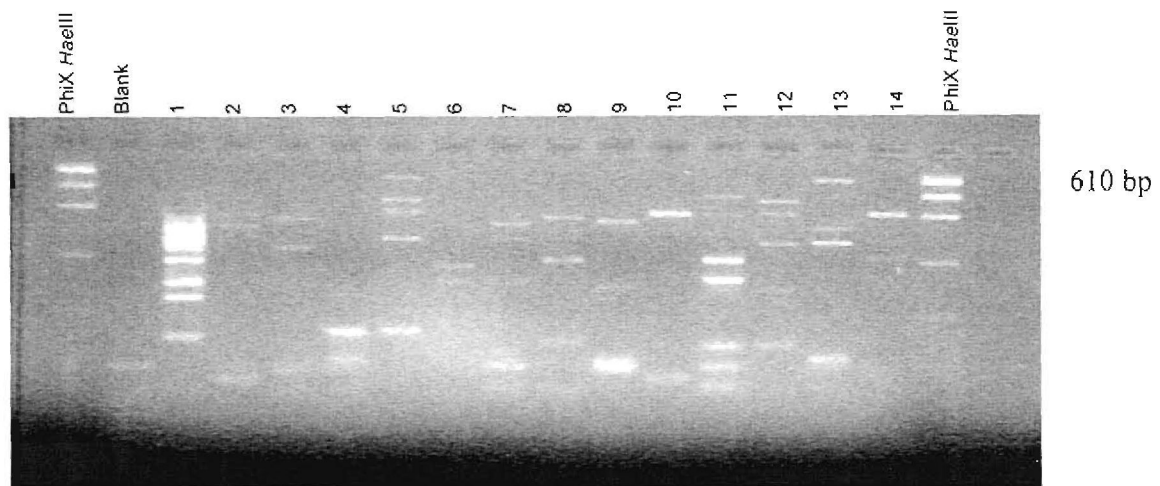


Figure 1. Amplification products of PCR reactions using primers Fh and Rh for *M. hapla* template DNA of J2 from Gamaboya (1), Gamagongwa Zone 2 (2), White River (3), Makoko (4), Numbi (5), Sishwamukile (6), Sibiziwe (7), Obonjaneni (8), Malepetleke (9), Malekutu (10), Mbonisweni (11), Gamagongwa (12), Sukumani (13) and Sakhisizwe (14).

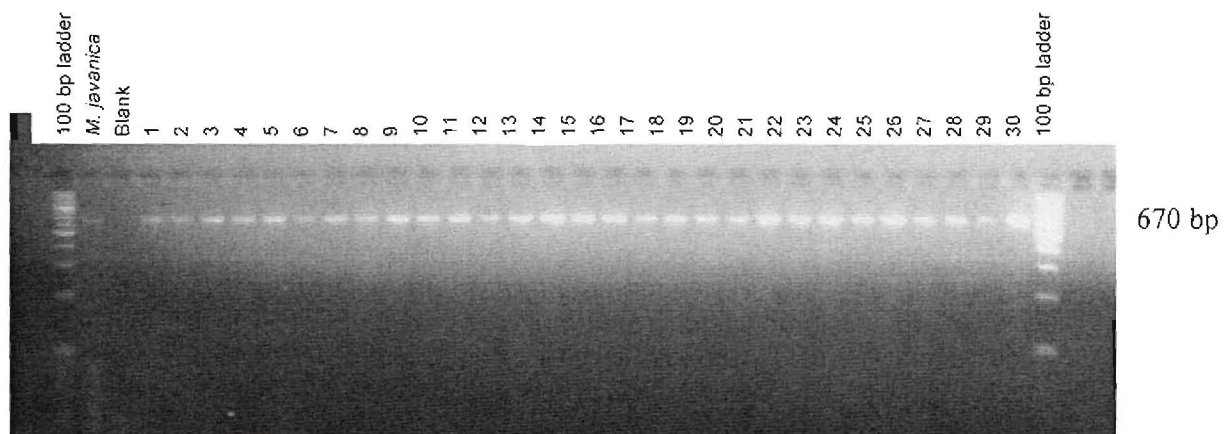


Figure 2. Amplification products of PCR reactions using primers Fjav and Rjav for *M. javanica* and DNA of *Meloidogyne* J2 from Okhombe (1), Mthokozi (2), Fundani (3), Emoyeni (4), Phuthumani (5), Phola (6), Reserve C (7), Siyaqoba (8), Sondelani (9), Sihlangene (10), Hlanganani (11), Clarkbury (12), Willowvale (13), Qakazana (14), Gam-Gam field (15), Mothibistadt (16), Gam-Gam clinic (17), Bushbuck (18), Mente (19), Tsineng (20), Dikhing (21), Maruping (22), Centani (23), Mapoteng (24), Matlhabanelong (25), Sakhisizwe (26), Goodhope (27), Magojaneng (28), Gaserutege (29) and Sizamangwane (30).

PCR with the *M. incognita*-specific SCAR primers resulted in amplification of the 1 200-bp SCAR fragment for root-knot nematode J2 from the reference population, but was not amplified for J2 from any of the 44 sites sampled during this survey (Fig. 3).

Although no reference DNA was available for *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla* PCR with specific primers for these respective species resulted in no amplification of the respective SCAR fragments (420 for *M. arenaria* (Fig. 4), 800 bp for *M. chitwoodi* (Fig. 5) and 520 for and *M. fallax* (Fig. 6) for J2 from all 44 sites sampled during the survey.

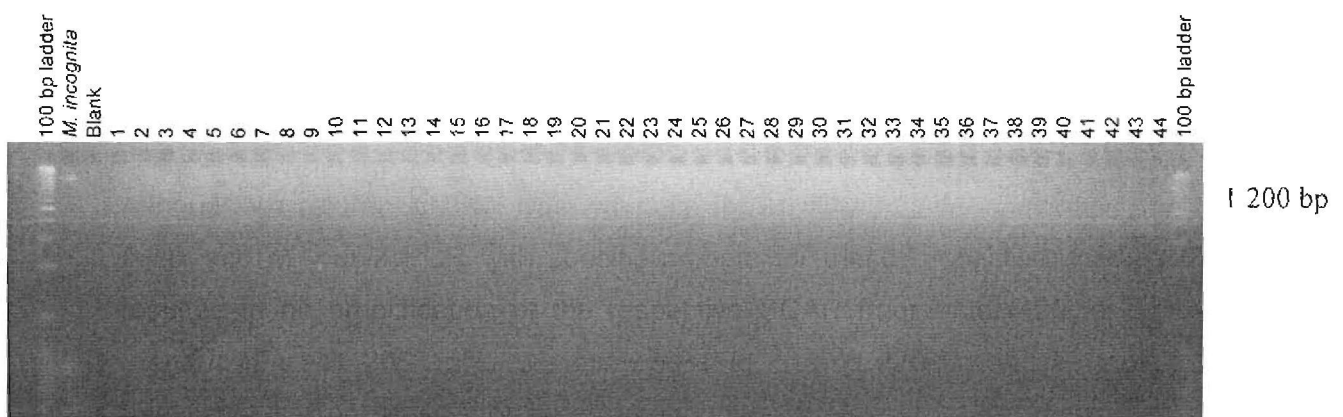


Figure 3. Amplification products of PCR reactions using primers Fincog and Rincog for *M. incognita* and DNA of *Meloidogyne* J2 from all 44 sites sampled.

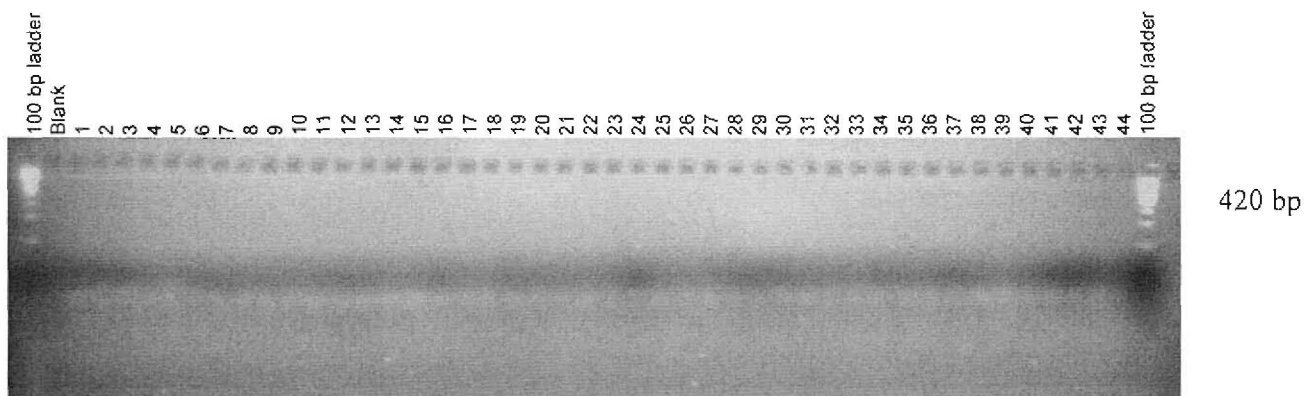


Figure 4. Amplification products of PCR reactions using primers Fa and Ra for *M. arenaria* template DNA of J2 from all 44 sites sampled.

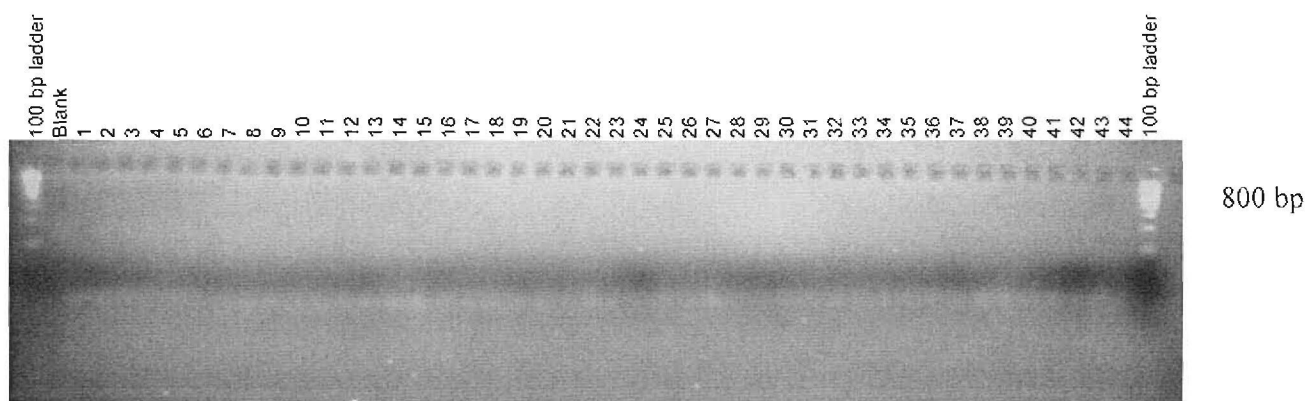


Figure 5. Amplification products of PCR reactions using primers Fc and Rc for *M. chitwoodi* template DNA of J2 from all 44 sites sampled.

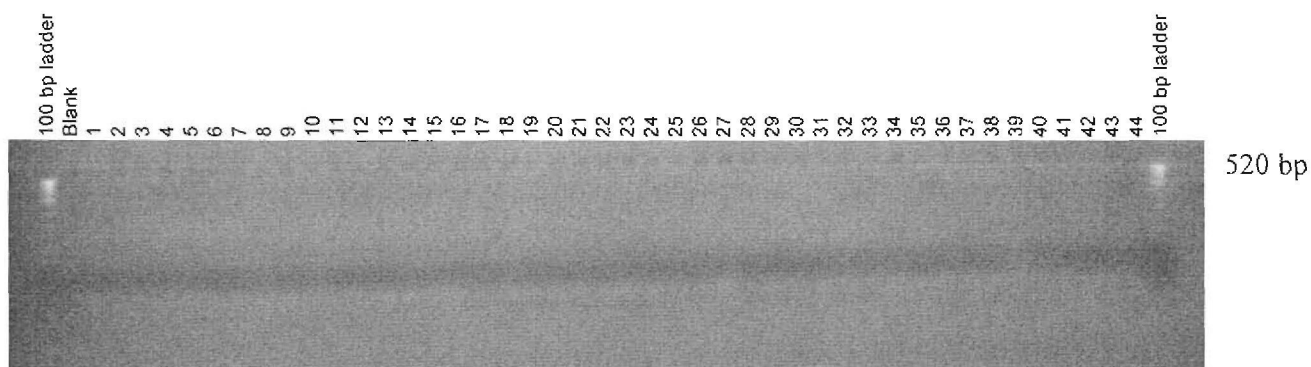


Figure 6. Amplification products of PCR reactions using primers Ff and Rf for *M. fallax* template DNA of J2 from all 44 sites sampled.

3.2.5. Discussion

DNA-based identification was successful in this study in terms of verifying and confirming the identity of two economically important root-knot nematode species occurring on a variety of weeds sampled at the 44 sites in small-scale farming systems.

The SCAR-PCR technique used in this study confirmed the presence of monospecific populations of *M. javanica* and *M. hapla* as the predominant root-knot nematode species occurring on weeds sampled. While *M. hapla* was only identified from sites in the eastern region of South Africa, *M. javanica* occurred in both regions. This is in agreement with reports by Kleynhans (1991) who identified *M. hapla* generally from sites in the eastern region of South Africa from agricultural soils as well as from native plants that grow in undisturbed areas. In terms of *M. javanica*, Kleynhans (1991) reported it as the most common root-knot nematode locally in both the western and eastern regions of South Africa in agricultural soils and natural vegetation. In addition to the latter two root-knot nematode species, Keetch and Buckley (1984) also listed *M. incognita* and *M. arenaria* as parasites of weeds in southern Africa. No positive identification of *M. arenaria* or *M. incognita* was, however, obtained during this study in association with weeds. The same is applicable to *M. chitwoodi* and *M. fallax*, which were, however, earlier identified from groundnut pods and tomato roots from two localities in South Africa by Fourie and Mc Donald (2001). These latter four root-knot nematode may, however, be identified as parasites of local weeds when more sites are sampled during continuation of this study. It is furthermore important to bear in mind that molecular identification of these four species has to be repeated using reference populations to substantiate our results in this regard.

Results from this study again confirmed that the SCAR-PCR method is a reliable tool for routine diagnostic identification of root-knot nematodes. The use of this molecular identification technique added value to our study because the identity of *Meloidogyne* spp. that was present at the sites sampled could be established. This information is critical to the establishment of cropping systems, because certain species of root-knot nematodes are

more damaging to certain cultivars of a specific crop or crops. Molecular identification of root-knot nematode species could thus play an important role in identifying the species in a certain cropping system and subsequently choose a follow-up cultivar that is not susceptible to it. Furthermore, molecular identification of particularly root-knot nematodes has become indispensable in nematode research, advisory and quarantine services and regulatory programmes worldwide.

Chapter 4: Conclusions

The need exists to maintain plant-parasitic nematode populations below damage-threshold levels and consequently increase crop yield without increasing production costs. This will ensure food security and stabilise household income. The capability of weeds that commonly occur in small-scale farming systems of South Africa to support proliferation of economically important plant-parasitic nematodes emphasises the need for proper weed management programmes. Although the use of herbicides and cultural techniques such as crop rotation and hand weeding do provide reasonable weed control, failure to manage weeds properly may result in a build-up of plant-parasitic nematode populations in roots/tubers of succeeding crops, with subsequent yield and quality losses.

Twenty plant-parasitic nematodes species and five genera were reported for the first time in association with weeds in South Africa as a result of this study. Root-knot nematodes were identified as the predominant genus in both root and soil samples from weeds, followed by *P. zaeae*, *H. dihystra* and *R. unisex*. Using molecular techniques, it was determined that *Meloidogyne javanica* is the predominant root-knot nematode species that infected weeds at the majority of the sites surveyed, followed by *M. hapla*.

Although this study focused mainly on plant-parasitic nematodes, free-living individuals of Rhabditidae as well as predatory Mononchidae were also recorded. Individuals from these families are common in South African soils and are usually abundant in the presence of decomposing organic material in soil (Kleynhans, 1991).

With regard to the occurrence of weed genera/species, *C. dactylon* occurred at most of the 44 sites sampled and was also predominant in eastern as well as western regions. All four predominant plant-parasitic nematode genera/species identified during this study also had the highest frequency of occurrence in both root and soil samples from *C. dactylon*. Therefore this weed species could be used as an indicator for the presence of these

parasites. Not only were there distinct differences with regard to the occurrence of weeds between the two regions, but they also differed with regard to their ability to host the four predominant plant-parasitic nematode genera/species. Farmers should be aware that *C. virgata*, *A. hybridus* and *N. physalodes* are more prone to root-knot nematode infestation in the eastern region. The same is true for *F. bidentis*, *Sparganium* spp. and *Bryophyllum* spp. in the western region. Results from this study also indicate that *T. minuta* generally maintained relatively high numbers of root-knot nematodes. Therefore, this weed should not be considered as a poor host for these parasites.

Results from this study emphasised the wide range of weeds that could be considered as good hosts and that may maintain populations of plant-parasitic nematodes. Since this may have a negative impact on succeeding crops, it is emphasised that weeds should not be neglected in nematode management strategies. It is important that this survey should be extended to more sites to ensure the identification of a wider range of plant-parasitic nematode genera/species associated with weeds. Additionally, field trials should be conducted under natural environmental conditions to evaluate weed species for host suitability to root-knot nematodes to ensure that farmers obtain optimal benefit from this research.

Chapter 5: References

- Abawi, G. S. and Chen, J. 1998. Concomitant pathogen and pest interaction. *In*: Barker, K. R., Pederson, G. A. Windham, G.L. (Eds), Plant and nematode interactions. Madison. Wisconsin, USA. pp. 135-157.
- Agrios, G. 1997. Plant pathology, 4th edition. Academic Press, London, UK, pp. 565-597.
- Baker, K. R. 1985. Nematode extraction and bioassay. *In*: Barker, K. R., Carter, C. C. and Sasser, J. N. (Eds), An advanced Treatise on *Meloidogyne*, Volume II. Methodology. Department of Plant Pathology. North Carolina State University and the U. S. Agency for International Development, North Carolina, U.S. A. pp. 20-25.
- Baker, H. G. 1974. The evolution of weeds. Annual Review of Ecology and Systematics 5: 1-24.
- Barker, K. R. and Nusbaum, C. J. 1971. *In*: Zukerman, B. M., Mai, W. F. and Rohde, R. A. (Eds), Diagnostic and advisory programs. Plant-parasitic nematodes. Volume 1. New York: Academic Press. pp. 281-301.
- Bélair, G. and Benoit, D. L. 1996. Host suitability of 32 common weeds to *Meloidogyne hapla* in organic soils of Southwestern Quebec. Supplement to Journal of Nematology 28: 643-647.
- Blok, V. C., Phillips, M. S., McNicol, J. W. and Fargette, M. 1997. Genetic variation in tropical *Meloidogyne* species as shown by RAPDs. Fundamental and Applied Nematology 20: 127-133.
- Boerma, H. G. and Hussey, R. S. 1992. Breeding plants for resistance to nematodes. Journal of Nematology 24: 242-252.

- Bogdan, A. V. 1977. Tropical pasture and fodder plants. Longman Inc., New York. pp. 77-86.
- Botha, C. 2001. Common Weeds of Crops and Gardens in Southern Africa. ARC-Grain Crops Institute, Potchefstroom, RSA. pp. 1-381.
- Bridge, J. 1996. Nematode management in sustainable and subsistence agriculture. Annual Review of Phytopathology 34: 201-225.
- Bridges, D. C. 1994. Impact of weeds in human endeavors. Weed Technology 8: 392-395.
- Brodie, B. B., Good, J. M and Jaworski, C. A. 1969. Population dynamics of plant nematodes in cultivated soil: Effect of summer crops in old agriculture land. Journal of Nematology 2: 147-151.
- Bromilow, C. 2001. Problem Plants of South Africa. Briza Publications, Pretoria. pp. 1-258.
- Castagnone-Sereno, P., Leroy, F., Bongiovanni, M., Zijlstra, C. and Abad, P. 1999. Specific diagnosis of two root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax*, with satellite DNA probes. Phytopathology 89: 380-384.
- Castagnone-Sereno, P., Piotte, P., Uijthof, J., Abad, P., Wajnberg, E., Vanlerberghe-Masuti, F., Bongiovanni, M. and Dalmasso, A. 1993. Phylogenetic relationships between amphimictic and parthenogenetic nematodes of the genus *Meloidogyne* as inferred from repetitive DNA analysis. Heredity 70: 195-204.
- Castagnone-Sereno, P., Piotte, P., Abad, C., Bongiovanni, P. and Dalmasso, A. 1991. Isolation of a repeated DNA probe showing polymorphism among *Meloidogyne incognita* populations. Journal of Nematology 23: 316-320.

Caveness, F. E., and Jensen, H. J. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs in soil. *Proceedings of the Helminthological Society of Washington* 22: 87-89.

Cenis, J. L., Opperman, C. H., Triantapylou, A. C. 1992. Cytogenic, enzymatic and restriction fragment length polymorphism variation of *Meloidogyne* spp. from Spain. *Phytopathology* 82: 527-531.

Chester, E. W., Wofford, B. E. and Kral, R. 1997. Atlas of Tennessee vascular plants. Vol.2. Miscellaneous Publication, No. 13. Center for Field Biology, Austin Peay State University, Clarksville. pp. 69-74.

Cobb, N. A. 1918. Estimating the mean population of the soil. *Agricultural Tech. Circ. Bur. Pl. Ind. Us. Dep. Agric.*, No 1. 48p.

Coolen, W. A., and D'Herde, C. J. 1972. A method for the quantitative extraction of nematodes from plant tissue. Ghent State Agricultural Research Station, Merelbeke, Belgium. 77p.

Cooper, J. I. and Harrison, B. D. 1973. The role of weed hosts and the distribution and activity of vector nematodes in the ecology of tobacco rattle virus. *Annals of Applied Biology* 73: 53-66.

Coyne, D. L., Plowright, R. A. and Fofana, B. 1999. Observations on the susceptibility of *Oryza sativa* and resistance of *Oryza glaberrima* to the cyst nematode (*Heterodera sacchari*) and the influence of weed management in upland rice in Ivory Coast. *International Journal of Pest Management* 45: 255-258.

Crow, W. T. and Welch, J. K. 2004. Root reductions of St. Augustinegrass (*Stenotaphrum secundatum*) and hybrid bermudagrass (*Cynodon dactylon* x

C. transvaalensis) induced by *Trichodorus obtusus* and *Paratrichodorus minor*. *Nematropica* 34: 31-37.

Curran, J., McClure, M. A. and Webster, J. M. 1986. Genotypic differentiation of *Meloidogyne* populations by detection of restriction fragment length difference in total DNA. *Journal of Nematology* 18: 83-86.

Curran, J., Baillie, D. L. and Webster, J. M. 1985. Use of genomic DNA restriction fragment length differences to identify nematode species. *Parasitology* 90: 137-144.

Davis, R. F. and Webster, T. M. 2005. Relative host status of selected weeds and crops for *Meloidogyne incognita* and *Rotylenchulus reniformis*. *Journal of Cotton Science* 9: 41-46.

De Grisse, A. T. 1965. Redescription ou modifications de quelques techniques utilisees dans l' etude des nematodes phytoparasitaires. *Medelingen Rijksfaculteit Landbouwetenschappen. Gent.* 34: 351-359.

De Waele, D. and Elsen, A. 2007. Challenges in Tropical Plant Nematology. *Annual Review of Phytopathology* 45: 457-485.

De Waele, D., Jordaan, E. M. and Basson, S. 1990. Host status of seven weed species and their effects on *Ditylenchus destructor* of peanut. *Journal of Nematology* 22: 103-107.

De Waele, D. and Jordaan, E. M. 1988. Plant-parasitic nematodes in field crops in South Africa. 2. Sorghum. *Revue Nématology* 11: 203-212.

De Waele, E., De Waele, D. and Wilken, R. 1987. Effect of root mass on the efficacy of methods for extracting root-lesion nematodes from maize roots. *Phytophylactica* 19: 473-474.

Dropkin, V. H. 1980. Introduction to Plant Nematology. A Wiley-Interscience Publication, USA. pp. 38-256.

Enama, M. 1994. Culture: The missing nexus in ecological economics perspective. *Ecological Economics* 10: 93-95.

Fargette, M., Phillips, M. S., Blok, V. C., Waugh, R., and Trudgill, D. L. 1996. An RFLP study of relationships between species, populations, and resistance-breaking lines of tropical species of *Meloidogyne*. *Fundamental and Applied Nematology* 19: 193-200.

Ferraz, L. C. C .B. and Brown, D. J. F. 2002. An Introduction to nematodes. Plant Nematology. A student's textbook. Pensoft Publishers, Sofia, Bulgaria. 221p.

Fourie, H., Zijlstra, C. and Mc Donald, A. H. 2001. Identification of root-knot nematode species occurring in South Africa using the SCAR-PCR technique. *Nematology* 3: 675-80.

Fourie, H. and Mc Donald, A. H. 2001. Annual report: Project M151/10 (Integrated nematode control in Maize). ARC-Grain Crops Institute, Potchefstroom. 11p.

Freed, V. H. 1980. Weed Science: The emergence of a vital technology. *Weed Science* 28: 621-625.

Garate, T., Robinson, M. P., Chacon, M. R. and Parkhouse, R. M. E. 1991. Characterisation of species and races of the genus *Meloidogyne* by restriction enzyme analysis. *Journal of Nematology* 23: 414-420.

Griffin, G. D. 1982. Differences in response of certain weed host populations to *Heterodera schachtii*. *Journal of Nematology* 14: 174–182.

Hance, R. J. and Holly, K. 1990. Weed Control Handbook: Principles. 8th Edition. Blackwell Scientific Publication, Oxford London Edinburgh, Boston Melbourne. 1p.

Heyns, J. 1971. A guide to the plant and soil nematodes of South Africa. Pretoria. South Africa.

Hillocks, R. J., Stokes, S. and Jones, M. 1995. Reproduction of *Meloidogyne javanica* on legume crops and some weed species associated with their cultivation in Malawi. *Nematologica* 41: 1-553.

Hogger, C. H. and Bird, G. W. 1976. Weed and indicator hosts of plant-parasitic nematodes in Georgia cotton and soybean fields. *Plant Disease Reporter* 60: 223-226.

Holm, L. G., Plocknett, D. L., Pancho, J. V. and Herberger, J. P. 1977. The World's worst weeds: Distribution and biology. Honolulu, HI: University Press of Hawaii. Pp. 609.

Hollis, J. P. 1977. Loss in yield of rice caused by the ring nematode. *Criconemoides onoensis* revealed by the elimination of yellow nutsedge, *Cyperus esculentus*. *Nematologica* 23: 71-78.

Hooper, D. J. and Stone, A. R. 1981. Role of wild plants and weeds in the ecology of plant-parasitic nematodes. *In*: Thresh, J. M. and Boston, M. A. (Eds), Pests, pathogens and vegetation: The role of weeds and wild plants in the ecology of crop pests diseases. USA, Pitman Advanced Publishing Program. pp. 192-215.

Huang, C. S. 1985. Formation, anatomy and physiology of giant cells induced by root-knot nematodes. *In*: Sasser, J. N. and Carter, C. C. (Eds), An advanced treatise on *Meloidogyne*, Vol. 1. Biology and Control. North Carolina State University Graphics, Raleigh, USA. pp. 143-153.

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

Ishaq, E. 1992. Plant parasitic nematodes associated with weeds in spring cereal field in the region of Wielkopolska. *Roczn. Nauk Roln., Ser. E*, 22: 7-30.

Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, UK. 256p.

Johnson, A. W. 1985. The role of nematicides in nematode management. *In*: Sasser, J. N. and Carter, C. C. (Eds), *An advanced treatise on Meloidogyne*. Volume 1. Biology and control. North Carolina State Graphics, Raleigh, North Carolina, USA, pp. 249-301.

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

Kaplan, D. T. and MacGowan, J. B. 1982. Ability of selected common weeds and ornamentals to host *Pratylenchus coffeae*. *Nematropica* 12: 165-171.

Keetch, D. P. 1989. A perspective of plant nematology in South Africa. *South African Journal of Science* 85: 506-508.

Keetch, D. P. and Buckley, N. H. 1984. A check-list of the plant-parasitic nematodes of Southern Africa. Technical communication. Department of Agriculture, Republic of South Africa, No. 195p.

King, L. J. 1966. *Weeds of the world: Biology and control*. Interscience Publishers, New York, NY. 526p.

Kinloch, R. A. 1998. Soybean. *In*: Barker, K. R., Pederson, G. A. and Windham, G. L. (Eds), *Plant and nematode interactions*. Madison, Wisconsin,

USA. pp. 317-333.

Kleynhans, K. P. N. 1991. The root-knot nematodes of South Africa. Department of Agricultural Development, Plant Protection Research Institute, Pretoria, SA. Technical Communication, No. 23: 37-40.

Lesufi, M. M., Swart, A. and Tiedt, L. R. 2006. The introduction and possible spread of *Aphelenchoides arachidis* in South Africa. African Plant Protection 12: 28-39.

Luc, M., Bridge, J. and Sikora, R. A. 2005. Reflections on Nematology in Subtropical and Tropical Agriculture. In: Luc, M., Sikora, R. A. and Bridge, J. (Eds), Plant-parasitic nematodes in subtropical and tropical agriculture. 2nd edition. CAB International. UK. pp. 1-10.

Luc, M., Sikora, R. A. and Bridge, J. 1993. Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Aberystwyth, UK. 629p.

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

Martin, G. C. 1959. Plants attacked by root-knot nematodes in the Federation of Rhodesia and Nyasaland. Rhodesia Agricultural Journal 56: 162-175.

McLain, D. K., Rai, K. S. and Fraser, J. M. 1987. Intraspecific and interspecific variation in the sequence and abundance of highly repeated DNA among populations of the *Aedes albopictus* subgroup. Heredity 58: 373-381.

McSorley, R. 1996. Impact of crop management practices on soil nematode populations. Procurement of Soil Crop Sciences Society 55: 63-66.

McSorley, R. and Campbell, C. W. 1980. Relationship between nematode and weed densities in avocado groves. Journal of Nematology 12: 231-232.

- Monaco, T. J., Weller, S. C. and Ashton, F. M. 2002. Weed Science: Principles and Practices. 4th edition. John Wiley & Sons, INC. New York. pp. 146-156.
- Motsinger, R. E., Moody, E. H., and Gay, C. M. 1977. Reaction of certain French marigold (*Tagetes patula*) cultivars to three *Meloidogyne* spp. Journal of Nematology 9: 278 (Abstract).
- Mullin, B. A., Abawi, G. S., Pastor-Corrales, M. A. and Kornegay, J. L. 1991. Reactions of selected bean pure lines and accessions to *Meloidogyne* species. Plant Disease 75: 1212-1216.
- Murray, J. J., Poole, T. E. and Ostazeki, S. A. 1986. Techniques for determining reproduction of *Meloidogyne graminis* on Zoysiagrass and Bermudagrass. Plant Disease 70: 559-560.
- Navas, M. L. 1991. Using plant population biology in weed research: A strategy to improve weed management. Weed Research 31: 171-179.
- Netscher, C. and Sikora, R. A. 1993. Nematode parasites of vegetables. In: Luc, M., Sikora, R. A. and Bridge, J. (Eds), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, UK. pp. 237-238.
- Piemiesel, R. L. 1954. Replacement control: Changes in vegetation in relation to control of pests and diseases. Botany Revolution 20: 1-32.
- Pimentel, D., Lach, L., Zuniga, R. and Morrison, D. 1999. Environmental and economic costs associated with non-indigenous species in the United States. Bioscience 50: 53-65.
- Piotte, C., Castagnone-Sereno, P., Uijthof, J., Abad, P., Bongiovanni, M. and Dalmasso, A. 1992. Molecular characterisation of species and populations of

Meloidogyne from various geographic origins with repeated-DNA homologous probes. *Fundamental and Applied Nematology* 15: 271-276.

Ploeg, A. T. 2002. Effect of selected marigold varieties on root-knot nematodes and tomato and melon yields. *Plant Disease* 86: 505-508.

Powers, T. O., Platzer, E. G. and Hyman, B. C. 1986. Species-specific restriction site polymorphism in root-knot nematode mitochondrial DNA. *Journal of Nematology* 18: 288-293.

Quénéhervé, P., Chabrier, C., Auwerkerken, A., Topart, P., Martiny, B. and Marie-Luce, S. 2006. Status of weeds as reservoirs of plant-parasitic nematodes in banana fields in Martinique. *Plant Disease* 86: 505-508.

Quénéhervé, P., Drob, F. and Topart, P. 1995. Host status of some weeds to *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp. and *Rotylenchus reniformis* associated with vegetables cultivated in polytunnels in Martinique. *Nematropica* 25: 149-157.

Quénéhervé, P. 2000. Enzyme phenotypes of *Meloidogyne* species associated with bananas in Martinique (Abstract). *Nematropica* 30: 145p.

Rao, V. S. 2000. *Principles of Weed Science*. 2nd edition. International Consultant, Weed Science Santa Clara, California, USA. pp. 1-555.

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

Robinson, A. F., Inserra, R. N., Caswell-Chen, E. P., Volvlas, N. and Troccoli, A. 1997. *Rotylenchus* species: Identification, distribution, host ranges and crop plant resistance. *Nematropica* 27: 127-180.

Salawu, E. O., Ambursa, A. S. and Manga, Y. B. 1991. Weed and Crop hosts for root-knot nematode, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. *In: North West Nigeria. Pak. Journal of Nematology* 9: 109-117.

Sasser, J. N. 1980. Root-knot nematodes: A global menace to crop production. *Plant Disease* 64: 36-41.

Sasser, J. N. and Freckman, D. W. 1987. A world perspective on nematology: the role of the society. *In: Veech, J. A. and Dickson, D. W. (Eds), Vistas on Nematology. Society of Nematologists, Hyatsville, Maryland, USA. pp. 7-14.*

Schulze, R. E. 1997. South African Atlas of Agrohydrology and Climatology. Department of Agricultural Engineering University of Natal, Pietermaritzburg, South Africa. pp. 1- 276.

Shepherd, J. A. and Barker, K. R. 1993. Nematode parasites of tobacco. *In: Luc, M., Sikora, R. A. and Bridge J. (Eds), Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Aberystwyth, UK. pp. 493-517.*

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

Sikora, E. J., Guertal, E. A. and Bowen, K. L. 2001. Plant-parasitic nematodes associated with hybrid bermudagrass and creeping bentgrass putting green in Alabama. *Nematropica* 31: 303-307.

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

Stanton, J. M., McNicol, C. D. and Steele, V. 1998. Non-manual lysis of second-stage *Meloidogyne* juveniles for identification of pure and mixed samples based on the polymerase chain reaction. *Australasian Plant Pathology* 27: 112-115.

Starr, J. L. and Page, J. L. 1990. Nematode parasites of cotton and other tropical fibre crops. *In*: Luc, M., Sikora, R. A. and Bridge, J. (Eds), *Plant-parasitic nematodes in Subtropical and Tropical Agriculture*. CABI, Wallingford, UK. pp. 733-735.

Swarup, G. and Sosa-Moss, C. 1993. Nematode parasites of cereals. *In*: Luc, M., Sikora, R. A. and Bridge, J. (Eds), *Plant parasitic nematodes in subtropical and tropical agriculture*. CAB International, Aberystwyth, UK. pp. 109-136.

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

Thill, D. C., Lish, J. M., Callihan, R. H. and Bechinski, E. J. 1991. Integrated weed management - A component of integrated pest management: A critical review. *Weed Technology* 5: 648-656.

Timper, P., Newton, G. L., Johnson, A. W. and Gascho, G. J. 2004. Nematode densities in year-round forage production system utilizing manure fertilization. *Nematropica* 34: 219-227.

Trudgill, D. L. 1997. Parthenogenetic root-knot nematodes (*Meloidogyne* spp.), how can these biotrophic endoparasites have such an enormous host range? *Plant Pathology* 46: 26-32.

Uva, R. H., Neal, J. C. and Ditomaso, J. M. 1997. *Weeds of the Northeast*, Ithaca, NY: Cornell University Press. pp. 214-217.

Webster, T. M. and MacDonald, G. E. 2001. A survey of weeds in various crops in Georgia. *Weed Tech.* 15: 771-790.

Whitehead, A. G. 1969. The distribution of root-knot nematodes (*Meloidogyne* spp.) in tropical Africa. *Nematologica* 15: 315-333.

Wong, A. T. S. and Tylka, G. L. 1994. Eight nonhost weed species of *Heterodera glycines* in Iowa. *Plant Disease* 78: 365-367.

Wyse, D. L. 1994. New technologies and approaches for weed management in sustainable agriculture systems. *Weed Technology* 8: 403-407.

Xue, B., Baillie, D. L., Beckenbach, K. and Webster, J. M. 1992. DNA hybridization probes for studying the affinities of three *Meloidogyne* populations. *Fundamental and Applied Nematology* 15: 35-41.

Xu, J., Narabu, T., Mazibuko, T. and Hibi, T. 2001. A molecular marker correlated with selected virulence against the tomato resistance gene *Mi* in *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. *Phytopathology* 91: 377-382.

Young, J. A. 1991. Tumbleweed. *Scientific American* 264: 82-87.

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

Zijlstra, C., Donkers-Venne, D. T. H. M. and Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR. *Nematology* 2: 847-853.

Zimdahl, R. L. 1999. Fundamentals of Weed Science. 2nd edition. Academic Press. San Diego, California. pp.1-556.