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External	None

Summary

An increase in awareness of root-knot nematode parasitism in maize over the last decade prompted identification and validation of resistance in order to control these parasites cost-effectively over the long term. Value was added to the project by conducting a nematode survey in fields of commercial producers and in Mother-and-Baby trials in resource-poor areas to review the importance and extent of plant-parasitic nematodes in local maize crops. Resistance in exotic maize lines was subsequently introgressed into local maize germplasm. Validation of resistance in F_2 and F_6 maize inbred-line populations that resulted from crosses made between exotic lines (root-knot nematode resistant donors) and germplasm that is adapted to local environmental conditions was done in a greenhouse and small field trial respectively. Finally, DNA fingerprinting of six S_8 maize populations, resulting from the abovementioned crosses has also been conducted with the respective parents of these populations being included. Three maize OPV's were furthermore planted in two separate microplot trials to evaluate the effect of initial inoculation densities of root-knot nematode resistance. Two field trials were also planted at sites that are naturally infested with root-knot nematodes at Potchefstroom (North-West Province) and Nelspruit (Mpumalanga Province) to validate the RKN resistance in local OPV QS-Obatanpa and USA line MP712w in these diverse regions under prevailing local environmental conditions. Finally, to confirm root-knot nematode resistance, the comparative penetration rate of *M. incognita* race 2 and *M. javanica* into roots of the susceptible maize hybrid AFG4410 as well as two resistant genotypes MP712w (exotic USA inbred line) and QS-Obatanpa (OPV) were subsequently determined. For the survey in maize fields, 16 plant-parasitic nematode species and 17 genera were identified with root-knot nematodes being the predominant genus. In terms of the host suitability of maize, root-knot nematode resistance has been identified in a number of commercial cultivars as well as open-pollinated varieties. Resistance was also identified in several wheat cultivars as well as in Vetiver grass. Introgression of root-knot nematode resistance was successfully done into locally adapted maize genotypes. Although fingerprinting of five F_8 maize populations were done, root-knot nematode data could not be merged with these results since screening trials of these populations were unsuccessful. Susceptible parental standards included in the latter trials did not yield accurate data and should be repeated. In terms of validation of root-knot nematode resistance

introgressed into locally adapted genotypes as well as those identified in commercial hybrids and open-pollinated varieties, data from both microplot and field trials showed that the resistant genotypes maintained significantly lower root-knot nematode numbers compared to the susceptible standards. This was substantiated by penetration rate trials, which indicated that the resistant genotypes exhibit superior resistance against these parasites.

1. INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop for human consumption in many parts of the world, with global production exceeding 600 million tonnes (mt) in recent years (FAO, 2007). Approximately 60% of the world's maize is produced in developing countries (McDonald & Nicol, 2005), with 14.8 mt having been produced in southern Africa during 2006 (FAO, 2007). Maize is the most important agricultural crop by far in South Africa. Annual production is highly variable due to periodic droughts but other abiotic and biotic constraints could also have localised or more extensive effects on the crop (McDonald & Nicol, 2005).

Several authors have indicated that plant-parasitic nematodes (PPN) are of great economic importance in maize production since they cause significant yield losses worldwide (Riggs & Niblack, 1993; De Waele & Jordaan, 1988; McDonald & Nicol, 2005). From a global perspective the predominant PPN genera that infect maize are root-knot nematodes *(*RKN: *Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus* spp.) and cyst nematodes (*Heterodera* spp.) (Riggs & Niblack, 1993; McDonald & Nicol, 2005). *M. incognita* race 2 and *M. javanica* are the most common and predominant species in the western maize production areas of South Africa (Riekert, 1996). These two species are, therefore, regarded to have the greatest damage potential on maize in this country and region due to the high population levels they could reach on the crop, often resulting in yield losses (Riekert, 1996).

Although a variety of chemical, cultural and biological strategies exist for the control of PPN in crops such as maize (Kerry, 1987; Stirling, 1991; McDonald & Nicol, 2005), host-plant resistance offers one of the few really cost-effective options to producers of this crop (De Brito & Antonio, 1989; Cook & Starr, 2006). Under conditions of severe nematode infection and/or inadequate levels of resistance the latter could still serve to complement other control strategies.

The objectives of this project were thus to i) assess the status of plant-parasitic nematodes (PPN) in local maize-producing areas, ii) identify maize and other crop cultivars with resistance to root-knot nematodes, iii) introgress root-knot nematode resistance into locally adapted maize genotypes, iv) verify resistance in maize genotypes and v) determine the penetration rate of root-knot nematodes in resistant genotypes.

2. MATERIAL AND METHODS

2.1. Surveys

A nematode survey was conducted to establish the occurrence and prominence of plant-parasitic nematodes in local, maize-based cropping systems. The survey was conducted during 2008 in 73 maize fields of both local commercial and developing farmers fields. Root and soil samples were taken for plant-parasitic nematode extraction, counting and identification during flowering of the maize crops. The extent of plant-parasitic nematode infestation was quantified by calculating their population densities, frequency of occurrence (%) and prominence values (PV). In addition, the effect on the prominence values (PV) of root-knot nematodes by other factors such as clay percentage (%) of soils, total rainfall, rainfall incidence, cultivation practices as well as cropping systems was investigated by means of principal component analysis (PCA: ADE-4).

2.2. Screening of crops for host suitability to root-knot nematodes

Maize, cowpea, dry bean wheat genotypes as well as Vetiver grass were screened for resistance to *M. incognita* race 2 and *M. javanica* in separate greenhouse trials since this project commenced. An appropriate susceptible as well as resistant standard were included for each trial. The trial layouts for the respective crops consisted of randomised complete block designs (RCBD) with six replicates. Seedlings of each crop were artificially inoculated with ±10 000 *M. incognita* race 2 or *M. javanica* eggs and juveniles 14 to 16 days after emergence. Root systems of plants were removed 56 days after inoculation (DAI) when assessments were made *viz*. i) the number of eggs/root system and ii) calculation of Rf values. Data were subjected to analyses of variance using Statgraphics 5 for Windows.

2.3. Introgression of *M. incognita* race-2 resistance into local maize genotypes.

Based on data from greenhouse screenings of maize genotypes, crosses were made using three exotic lines (MP710, MP711 & MP 712) with low Rf values (indicating resistance to root-knot nematodes) and four popular, local white maize genotypes (I137W, K2539W, R119W and FR827W). The latter genotypes exhibit resistance to northern corn leaf blight and grey leaf spot.

One-hundred-and-sixty-five F_1 maize plants, resulting from a cross between the root-knot nematode resistant line MP712w and the susceptible local line R119w were verified for resistance to a mixed population of *M. javanica* and *M. incognita* race 2 in a field experiment at Potchefstroom during 2002. Successive populations resulting from the latter crosses were selected for further screening and progeny advancement on account of their preferred agronomical traits as well as the low populations of root-knot nematode numbers/root system of individual plants. Subsequently, during the 2007/08 growing season at least 150 seeds of six S_6 populations of maize were planted in a field trial at Potchefstroom to validate the root-knot nematode resistance in these breeding lines. Each maize seed was inoculated with approximately 10 000 *M. incognita* race 2 eggs and second-stage juveniles (J2). Resulting from the latter trial, five hundred and seventy-six seeds of six F_7 populations of maize were selected on account of their poor host suitability to root-knot nematodes identified in a field trial. These seeds were planted along with their respective parents and were inoculated with *M. incognita* race 2 during January 2009 in a greenhouse trial to finally validate the root-knot nematodes resistance exhibited by them. Each maize seed was inoculated with approximately 10 000 *M. incognita* race 2 eggs and second-stage juveniles (J2) at planting.

Finally, DNA fingerprinting of six S_8 maize populations, resulting from crosses between foreign root-knot nematode-resistant and local susceptible maize lines has also been conducted. Seeds from the six maize populations, the respective parents as well as the three exotic lines were germinated and DNA extracted from lypholised leaf tissue. DNA was analysed with SSR using primers as published by CIMMYT (CIMMYT, 2005). Methods used in the molecular analyses of breeding lines and hybrids were adapted from recommendations by the Applied Molecular Genetics Laboratory, CIMMYT, Mexico. The samples were used to optimise the fingerprinting technique using fluorescently labelled microsatellites for automatic analysis with an AB13130xl genetic analyser. Electropheresis results were analysed with Genemapper 4.0 software (Applied Biosystems). Statistical analysis was carried out with Powermaker v3.25 (Lui & Muse, 2005) using Euclidian distances and UPGIMA clustering.

2.4. Verification of root-knot nematode resistance present in open-pollinated varieties under natural-occurring environmental conditions.

Three maize OPV's were planted in two separate microplot trials to evaluate the effect of initial inoculation densities of root-knot nematodes. For the *M. incognita* race 2-trial, PAN6549 (susceptible standard cultivar), QS-Ob (resistant standard cultivar) and MP712w (resistant standard line) were used. On the other hand, AFG4410 (susceptible standard cultivar), QS-Ob (resistant standard cultivar) and MP712w (resistant standard liner) were used for the *M. javanica* trial. Two field trials were also planted at sites that are naturally infested with RKN nematodes at Potchefstroom (North-West Province) and Nelspruit (Mpumalanga Province) to validate the RKN resistance in local OPV QS-Obatanpa and USA line MP712w in these diverse regions under prevailing local environmental conditions. RKN-susceptible standards in the latter field trials were included.

2.5. Penetration rate

To confirm RKN resistance, the comparative penetration rate of *M. incognita* race 2 and *M. javanica* into roots of the susceptible maize hybrid AFG4410 as well as two resistant genotypes MP712w (exotic USA inbred line) and QS-Obatanpa (OPV) were subsequently determined. Two separate trials were thus conducted for the respective root-knot nematode species in a greenhouse during February 2009. Both trials consisted of five treatments, with sampling of maize roots at 2, 4, 10, 16 and 20 days after inoculation (DAI). Seven replicates were included in each trial. Seven-day-old maize seedlings were grown in 500-ml Styrofoam cups and each was inoculated with ±2 000 second-stage juveniles (J2) of the respective RKN species seven days after plant germination. Maize roots of the three genotypes were removed, weighed and stained with acid fuchsin during each of the five sampling times and the respective juvenile stages present in roots counted.

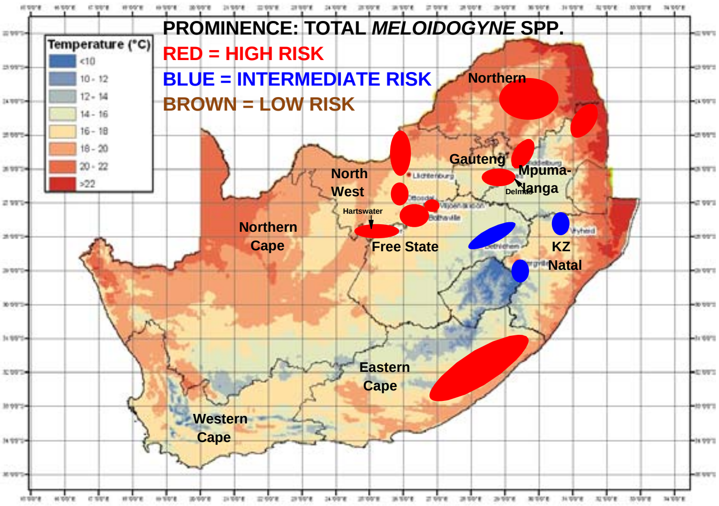
3. RESULTS

3.1. Survey

For the survey in maize fields, sixteen plant-parasitic nematode species and 17 genera were identified as a result of the nematode survey (Table 1). Root-knot nematodes were identified as the predominant genus, followed by the family Hoplolaimidae, *Tylenchorhynchus* and *Pratylenchus* spp. Predominant root-knot nematode species identified by means of molecular techniques were *M. javanica, M. incognita* and *M. arenaria*. The PV for root-knot nematodes was significantly lower at localities with soil that had clay contents higher than 30%, as opposed to those with clay contents of less than 10% and those with clay contents between 10% and 20%. Maize grown on irrigated land further showed significantly higher PV for root-knot nematodes compared to those of rain-fed fields. In terms of cultivation practices the combination of ploughing and seedbed preparation resulted in a significantly higher PV for root-knot nematodes when compared to a combination of ploughing and ripping, minimum tillage as well as no-till practices. For cropping sequences, wheat-potato rotations resulted in the highest PV for root-knot nematodes, followed by barley-groundnut, maize-sunflower, maize in monoculture and maize-cowpea rotations.

Table 1 Mean root-knot nematode (RKN) numbers, prominence values (PV), population densities (PD) and frequency of occurrence (%) for maize sampled at 73 localities during 2008 in maize-producing areas of South Africa.

Area	Mean RKN	PV	PD	Frequency of occurrence (%)
1. Lichtenburg	12 290	12 290	12 290	100
2. Vaalharts	3 464	3 464	3 464	100
3. Ottosdal	2 399	2 399	2 399	100
4. Bothaville	1 581	1 581	1 581	100
5. Eastern Cape	1 009	1 009	1 009	100
6. Bushbuckridge	817	817	817	100
7. Viljoenskroon	697	697	697	100
8. Delmas	387	387	387	100
9. Polokwane	197	197	197	100
10. Middelburg	146	146	146	100
11. Vryheid	96	99	103	94
12. Bethlehem	48	59	73	66
13. Bergville	30	51	124	24



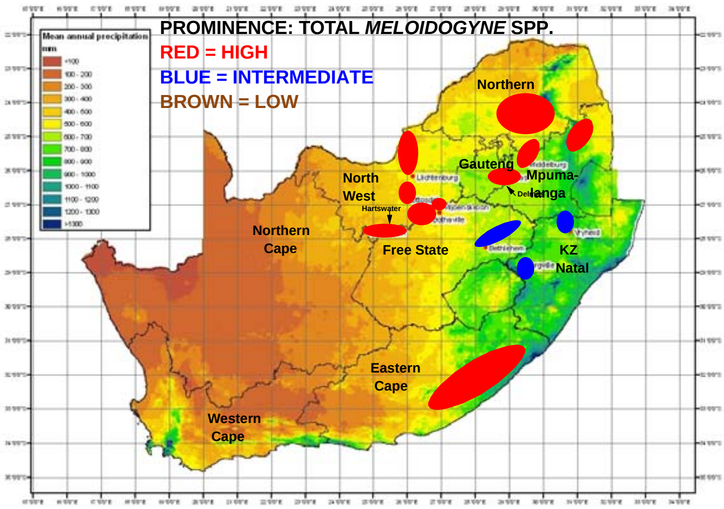


Fig. 1 Distribution of root-knot nematodes in maize producing areas of South Africa as indicated on a map that indicates the various temperature zones.

Fig. 2 Distribution of root-knot nematodes in maize producing areas of South Africa as indicated on a map that show the various rainfall zones.

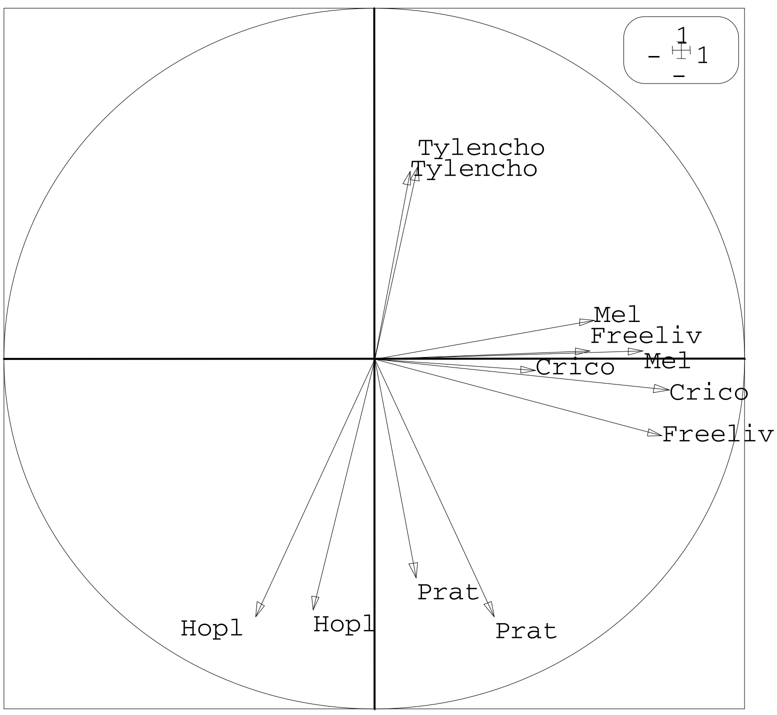


Fig. 3 Occurrence of various plant- as well as non-parasitic nematodes at 73 localities in maize-producing areas of South Africa.

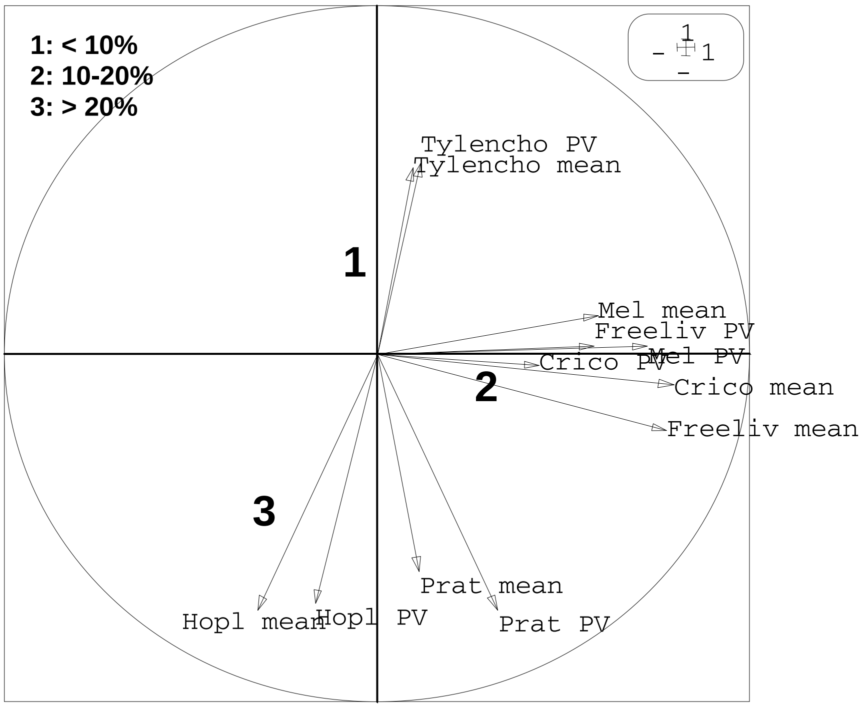


Fig. 4 Occurrence of various plant- as well as non-parasitic nematodes at 73 localities in soils with various clay regimes in maize-producing areas of South Africa.

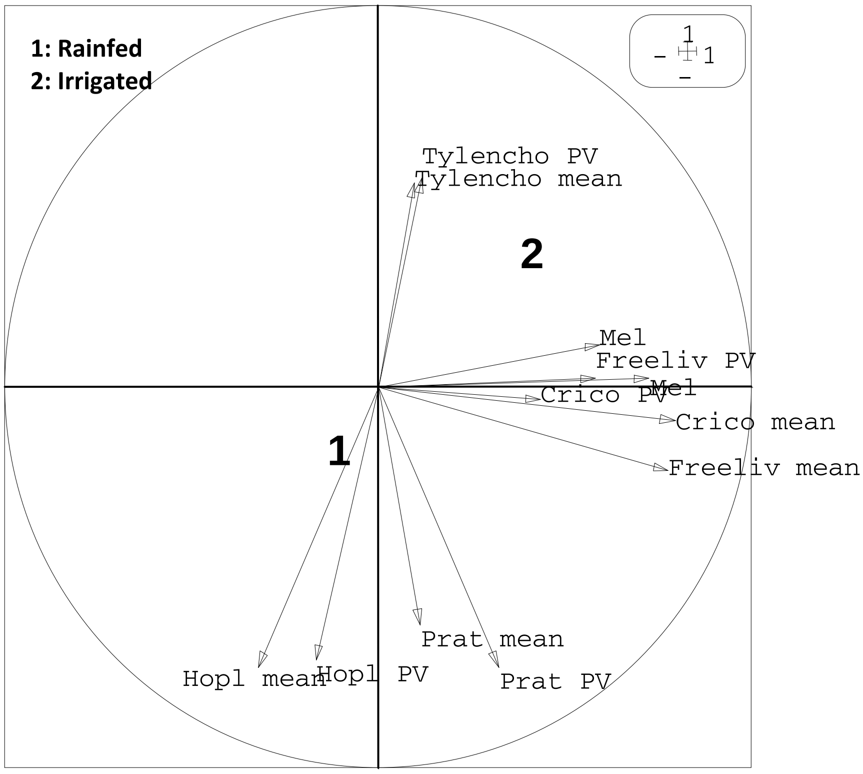


Fig. 5 Occurrence of various plant- as well as non-parasitic nematodes at 73 localities in irrigated and rain-fed fields in maize-producing areas of South Africa.

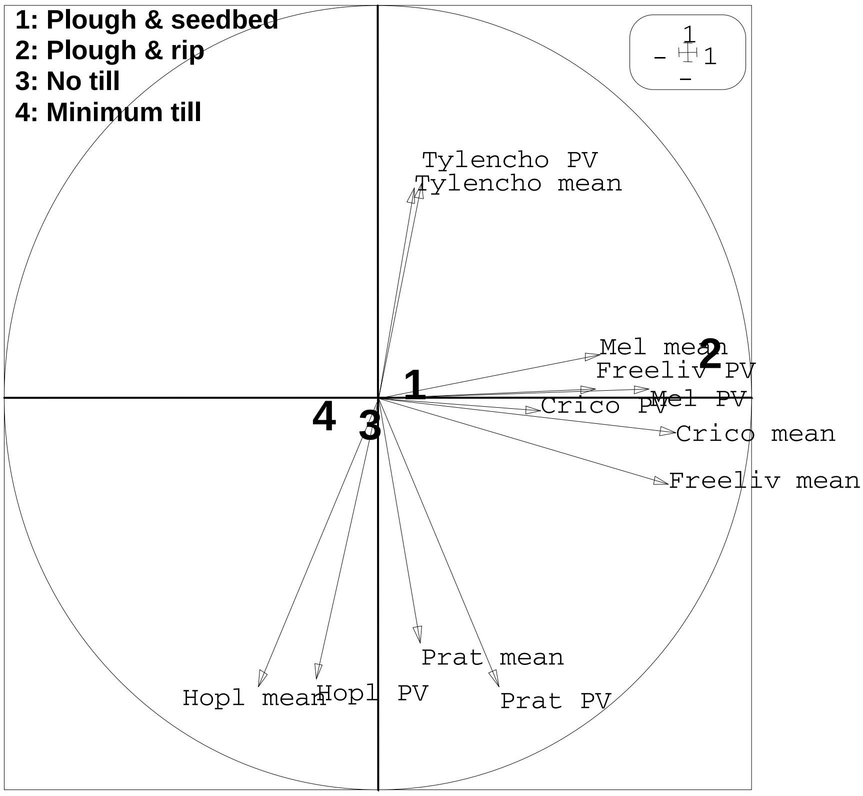


Fig. 6 Occurrence of various plant- as well as non-parasitic nematodes at 73 localities in fields where different practices in terms of soil preparation were done in maize-producing areas of South Africa.

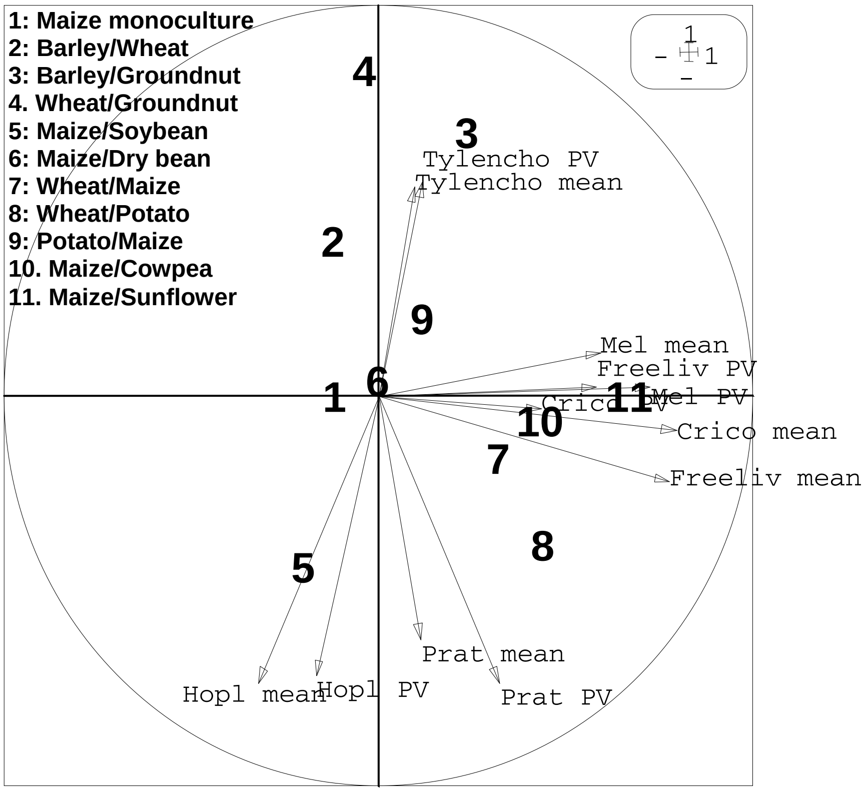


Fig. 7 Occurrence of various plant- as well as non-parasitic nematodes at 73 localities in fields where different cropping sequences were implemented in maize-producing areas of South Africa.

3.2. Screening of crops for host suitability to root-knot nematodes

Several maize genotypes were identified with resistance to *M. incognita* race 2 and *M. javanica*, based on RKN reproduction factor values (Rf values) lower than 1 (Table 2). Sixteen wheat genotypes have been identified with resistance to *M. incognita* race 2. Wheat genotypes, however, still need to be evaluated for resistance to *M. javanica*. Vetiver grass (*Vetiveria zizanoides*) also exhibited Rf values lower than 1 for both *M. incognita* race 2 and *M. javanica*. No dry bean genotypes evaluated had Rf values lower than 1, indicating that all genotypes were susceptible to the respective root-knot nematode species. Screening of cowpea, sorghum and sunflower genotypes have to be repeated since the inoculation of root-knot nematodes were not successful in these experiments.

Table 2 Crop genotypes as well as Vetiver grass identified with resistance to root-knot nematodes.

Crop	***Meloidogyne incognita* race 2**	***Meloidogyne javanica***	**Crop**	***Meloidogyne incognita* race 2**
	Genotype	**Genotype**	Wheat	Falcon
Maize	LS8507	LS8507		Tugela DN
	PAN6777	PAN6777		Limpopo
	QS7707	QS7707		Gariep
	PAN6549	PAN6549		Olifants
	PAN6114	PAN6966		Scheepers
	PANTHERA	PAN6146		Matlabas
		DKC61-25		Kowati
Vetiver	*Vetiveria zizanoides*	*Vetiveria zizanoides*		Elands
				Steenbras
				Witteberg
				SWK001
				Heros
				Overberg
				Maluti
				Drakensberg

3.3. Introgression of *M. incognita* race-2 resistance into local maize genotypes.

3.3.1. Nematode data

Root-knot nematode numbers were generally low in the majority of F_2 plants from a cross between MP712w and FR827w and ranged between zero and 113 17 /50g roots (Fig. 8A). Fifty-one percent of these F_2 plants maintained between zero and 100 root-knot nematode individuals/50g roots. Although root-knot nematode numbers from plants from the other four F_2 populations were also generally low, the majority of these plants maintained more than 100 root-knot nematode individuals/50 g roots (Figs. 8 B-E).

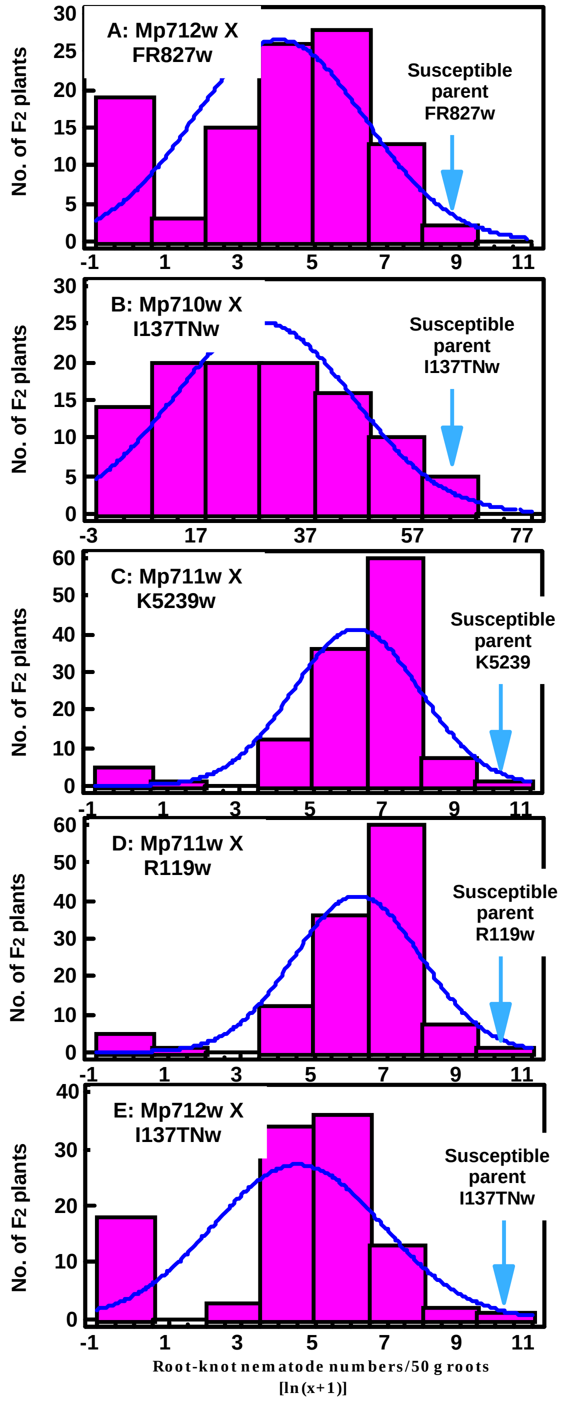


Fig. 8 Distribution graphs of F_2 progenies resulting from crosses between exotic maize lines with resistance to root-knot nematodes and local genotypes in terms of their host status when screened to root-knot nematodes.

Evaluation of F_3 to F_5 material in terms of its host suitability status to root-knot nematodes was not successful since heavy rains adversely affected extraction and counts of these nematodes from root systems of fully grown plants from field trials. Individuals from the latter generations were thus only evaluated for their agronomical traits, with host suitability assessments in terms of root-knot nematodes only commencing with individuals from the F_6 generation. For the latter generation the following nematode data were obtained for plants originated from the original Mp712w x FR827w crosses: RKN resistant parent Mp712w maintained a mean of 10 root-knot nematodes/root system, while susceptible parent FR827 maintained 2 192 root-knot nematodes/root system. Thirty-two percent of these plants showed root-knot nematode reproduction less than 5% of that of the susceptible parent. Therefore these plants are classified as resistant to these root-knot nematode populations. In terms of the Mp712w x I137TNw cross F_6 plants from the root-knot nematode resistant parent Mp712w maintained a mean of 500 root-knot nematodes/root system, while susceptible parent I137TNw maintained 74 908 root-knot nematodes/root system. Seventy-one percent of S_6 progeny plants showed less than 1% root-knot nematode reproduction compared to the susceptible standard and are thus classified as resistant to these root-knot nematode populations. For the Mp711w x K5239w cross, plants from the root-knot nematode-resistant parent Mp712w maintained a mean of 7 root-knot nematodes/root system compared to the susceptible parent K5239w with 6 683 RKN/root system. Twenty-four percent of these plants showed root-knot nematode reproduction less than 5% of that of the susceptible parent and are categorised as resistant to these root-knot nematode populations. In terms of the Mp711w x R119w cross, plants from the root-knot nematode-resistant parent Mp711w maintained a mean of 252 root-knot nematodes/root system, while susceptible parent R119w maintained 8 183 root-knot nematodes/root system. Twenty-two percent of these S_6-progeny plants showed less than 5% root-knot nematode reproduction compared to that of the susceptible parents and is classified as resistant to these root-knot nematode populations. For the MP710w x R119w cross, plants from the root-knot nematode-resistant parent Mp710w maintained 1 326 root-knot nematodes/root system, while susceptible parent R119w maintained 8 183 root-knot nematodes/root system. Forty-two percent of these S_6-progeny plants showed less than 5% root-knot nematode reproduction compared to the susceptible standard parent and are classified as resistant to these root-knot nematode populations. In terms of the MP710w x I137TNw cross, plants from the root-knot nematode-resistant Mp710w maintained 1 879 root-knot nematodes/root system, while I137TNW maintained 20 847 root-knot nematodes/root system. Fifteen percent of these S_6-plants maintained less than 5% root-knot nematode reproduction compared to that of the susceptible parent and are therefore resistant to these root-knot nematode populations.

3.3.2. Fingerprinting data

Maize exotic lines as well parental genotypes were fingerprinted with 71 SSR (simple sequence repeat) markers located throughout the maize genome using an ABI3130xl genetic analyser (Applied Biosystems). Fragment sizes were automatically calculated by Genemapper 4.0 software and analysed with Powermarker 3.0 (K. Liu and S.V. Muse, 2005) using the dissimilarity coefficient of Rogers (1972). Dendrograms were constructed using the unweighted pair-group method using arithmetic average (UPGMA). Fingerprinting data of parental genotypes and the three exotic maize lines used for introgression of root-knot nematode resistance is illustrated in Figs. 9 - 14. Root-knot nematode data could not be inserted in these data trees since the susceptible parental standards included in screening trials for each of the five populations did not yield sufficient root-knot nematode populations/root system. Screening trials thus have to be repeated.

The trees reflect a relatively close relationship between parental lines K2539, FR827 and MP711. Line MP712 was genetically more distant from the rest of the genotypes. The close relationship of F_8 progeny with both parental lines can clearly be deducted from the dendrograms as expected, indicating successful introgression of genetic material from both parents.

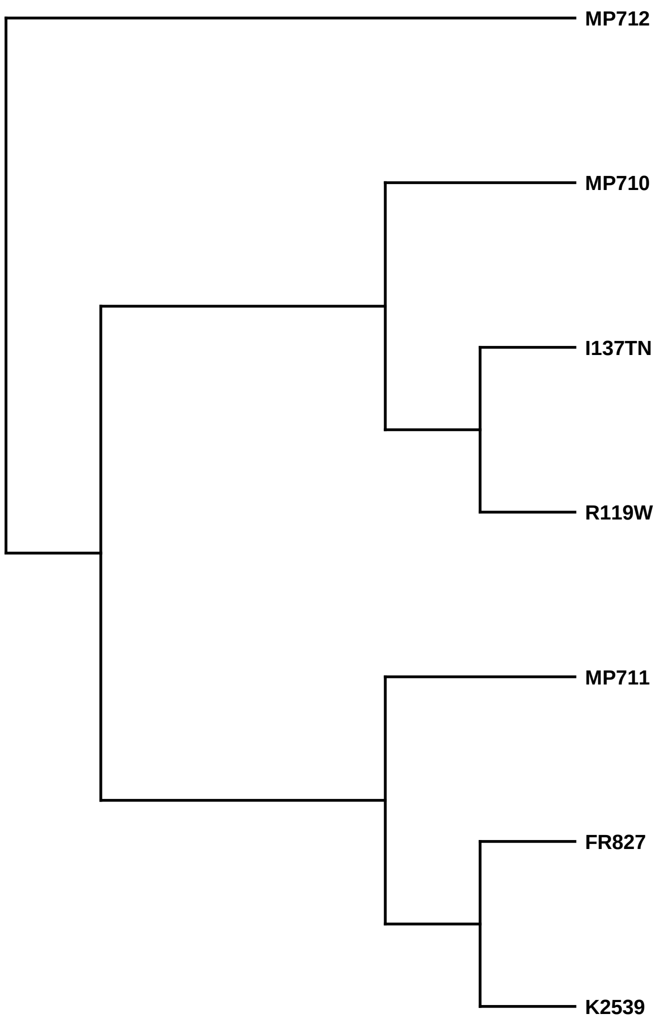


Fig. 9 Dendrogram of exotic maize lines that exhibit resistance to root-knot nematodes as well as those of local genotypes used to perform crosses based on genetic distance values using SSR markers.

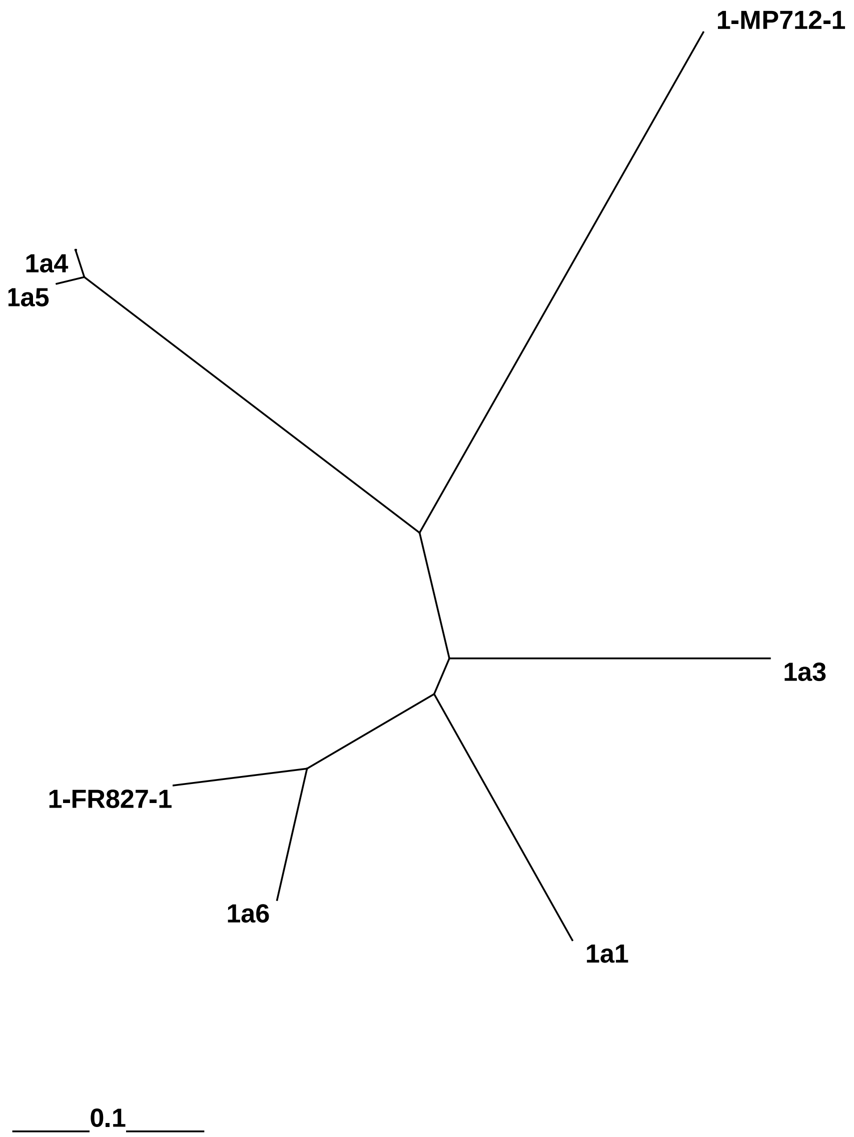


Fig. 10 Fingerprinting of selected plants from an F_8 progeny that resulted from original crosses between the local maize genotype FR827w and the root-knot nematode-resistant breeding line MP712w.

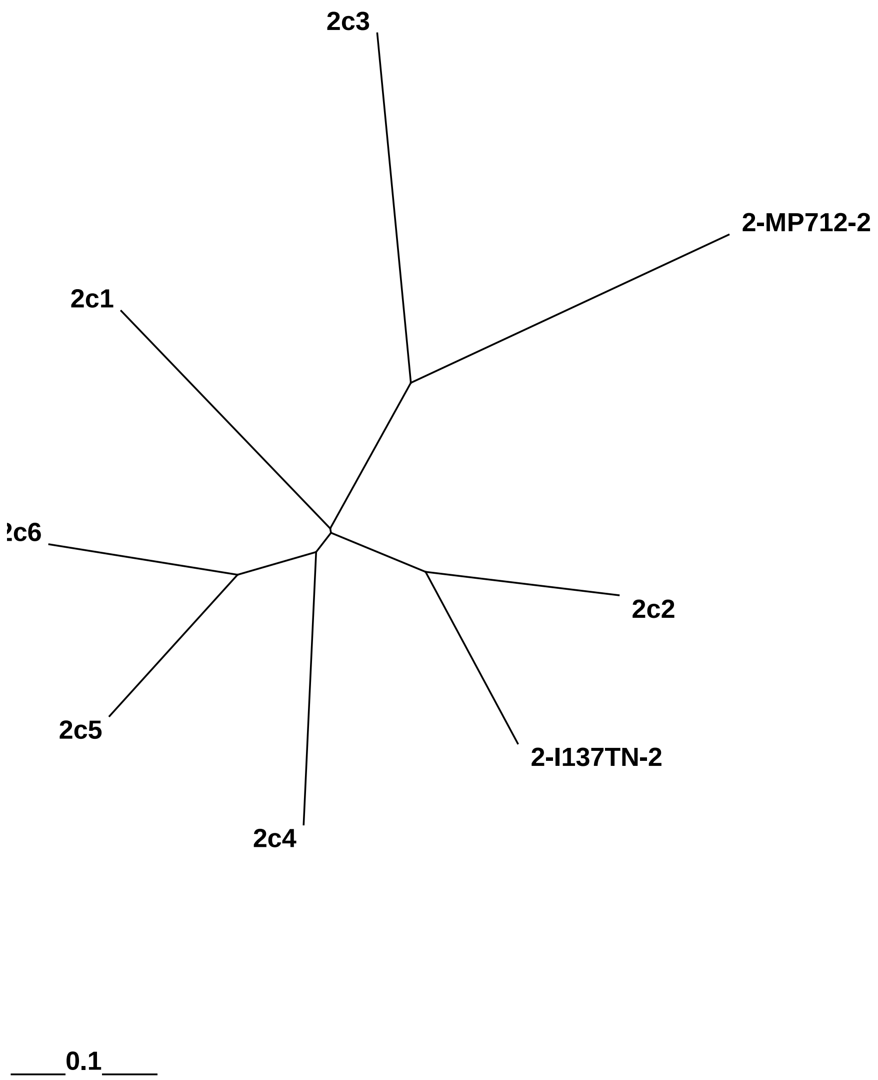


Fig. 11 Fingerprinting of selected plants from an F_8 progeny that resulted from original crosses between the local maize genotype FR827w and the root-knot nematode-resistant breeding line MP712w.

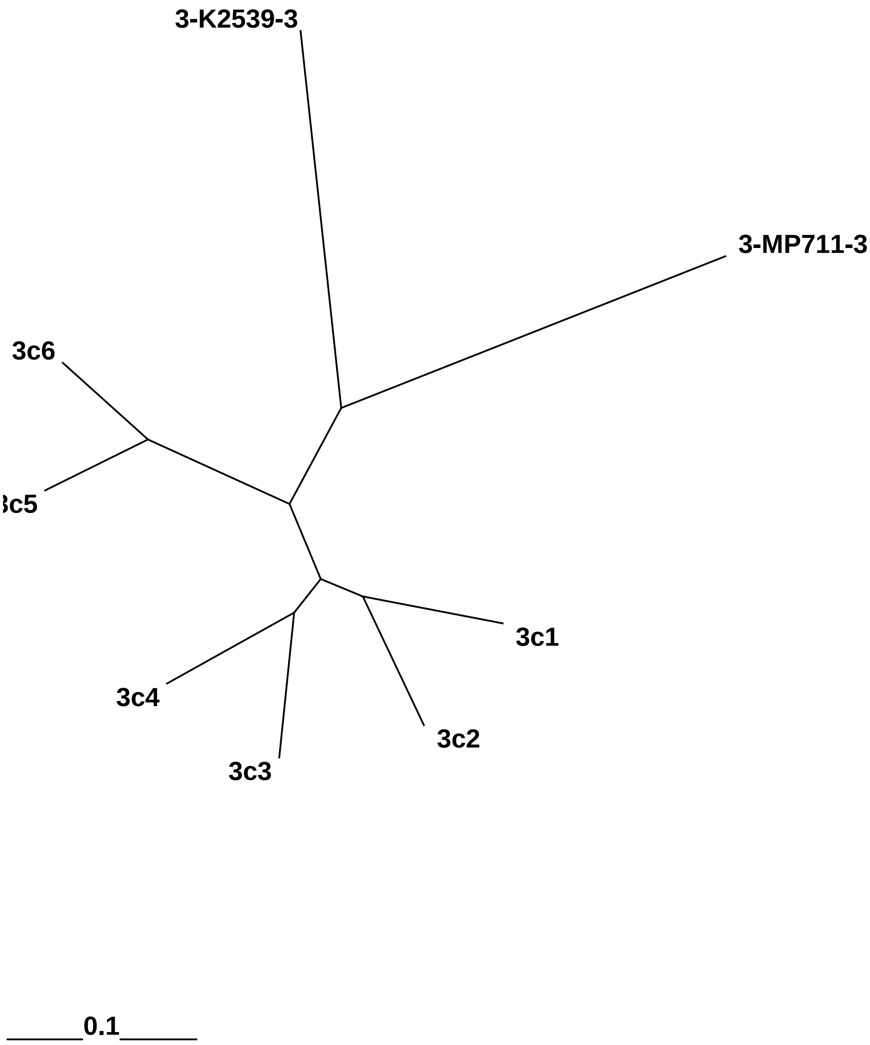


Fig. 12 Fingerprinting of selected plants from an F_8 progeny that resulted from original crosses between the local maize genotype FR827w and the root-knot nematode-resistant breeding line MP711w.

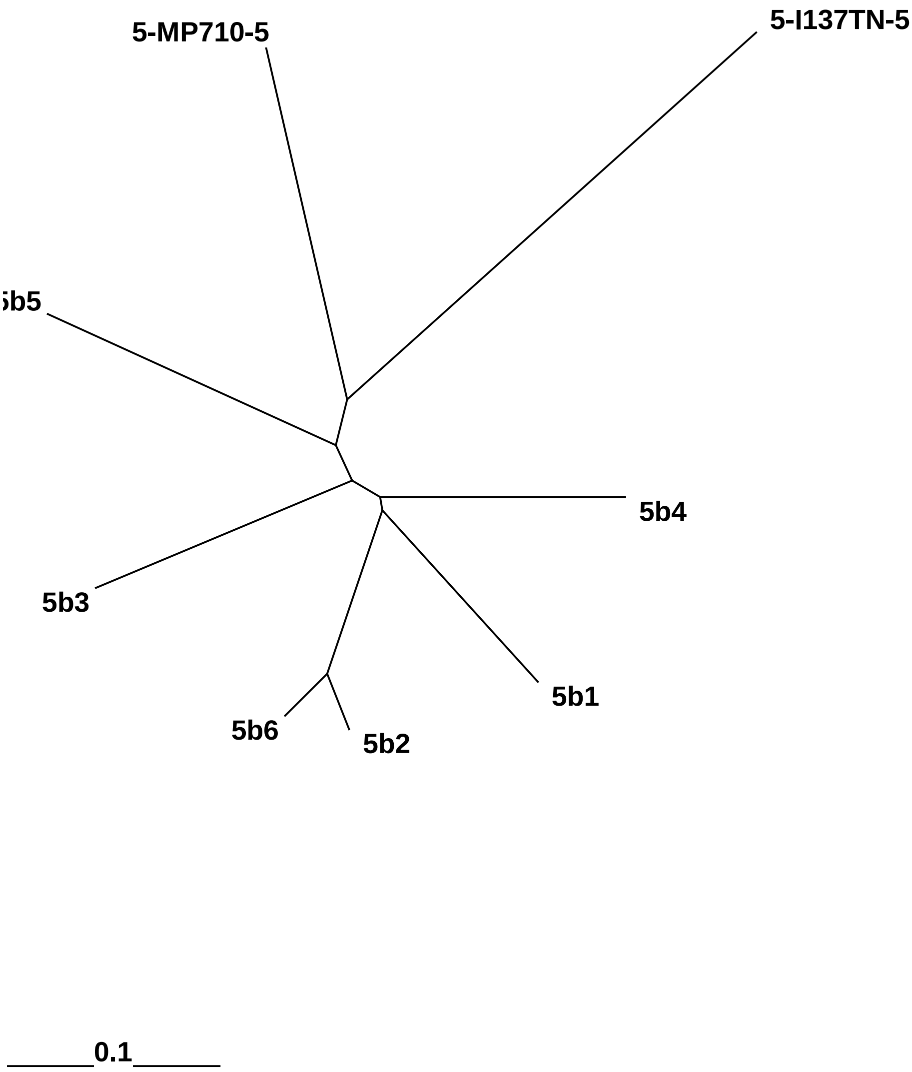


Fig. 13 Fingerprinting of selected plants from an F_8 progeny that resulted from original crosses between the local maize genotype FR827w and the root-knot nematode-resistant breeding line MP712w.

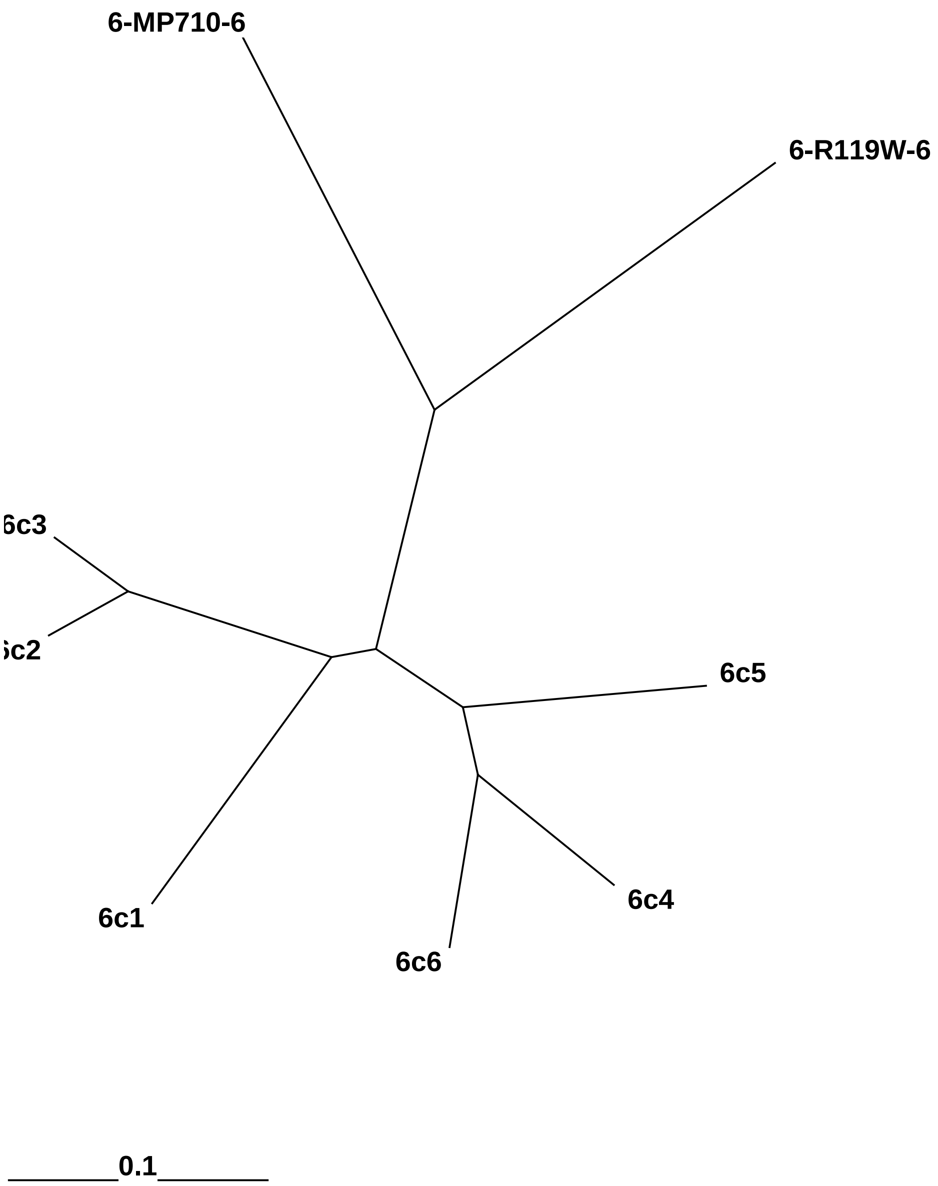


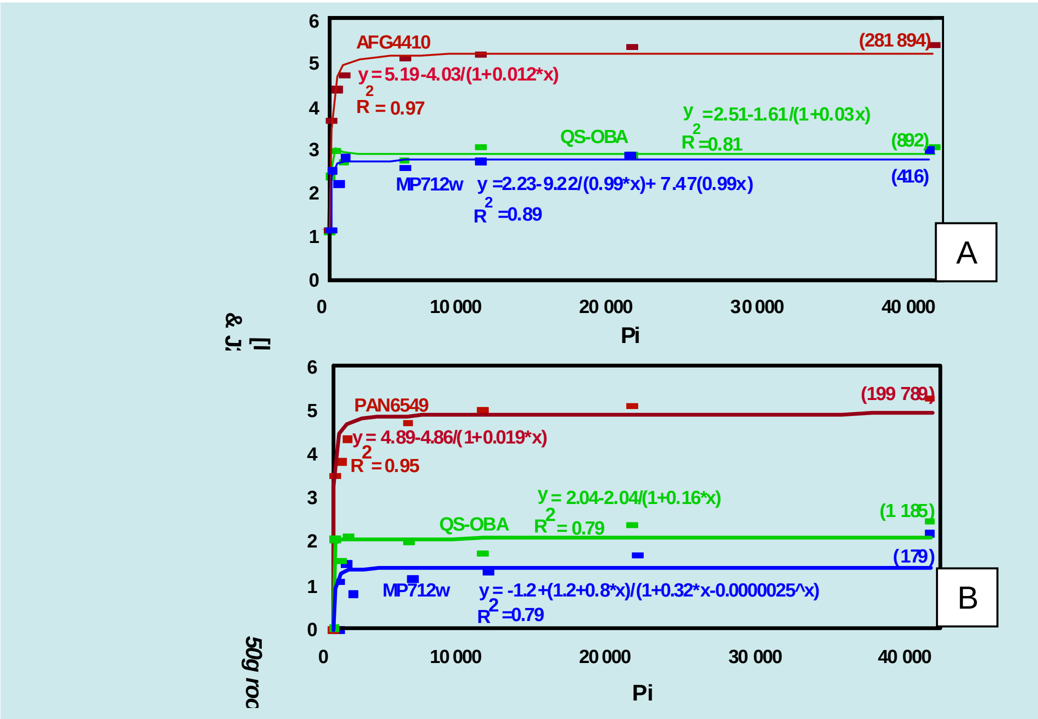
Fig. 14 Fingerprinting of selected plants from an F_8 progeny that resulted from original crosses between the local maize genotype FR827w and the root-knot nematode-resistant breeding line MP710w.

3.4. Verification of root-knot nematode resistance present in open-pollinated varieties under natural-occurring environmental conditions.

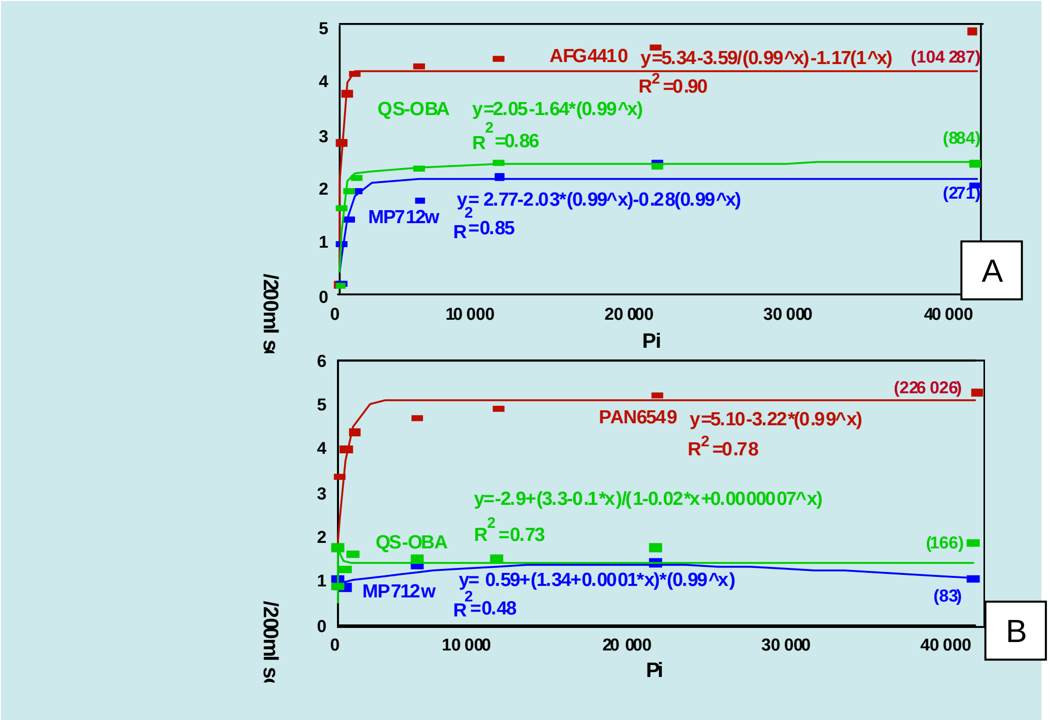
3.4.1. Microplot trials

3.4.1.1. Nematode data

QS-Ob and MP712 maintained significantly lower *M. javanica* and *M. incognita* race 2 numbers in both trials compared to the respective susceptible standard genotypes. Non-linear relationships were evident for both resistant and susceptible genotypes for each of the root-knot nematode species with regard to the number of eggs and J2/root system as well as J2/200ml soil (Figs. 15A & B; Figs. 16A & B). The genotypes with resistance to root-knot nematode species maintained significantly lower root-knot nematode numbers compared to the susceptible standards. The effect of Pi on the genotypes QS-Ob and MP712W showed Rf values lower than 1 (indicating resistance) at Pi's of 100 to 40 000 for *M. incognita* race 2. Rf values for the latter cultivar ranged between 7 and 47 at Pi = 40 000 and Pi = 100, respectively. The same trend was evident for the two resistant genotypes QS-Ob and MP712w when screened against *M. javanica.* While QS-Ob and MP712w had Rf values lower than 1 for Pi = 100 to Pi = 40 000, AFG4410 exhibited RF values ranging between 5 (Pi = 40 000) and 44 (Pi = 100).



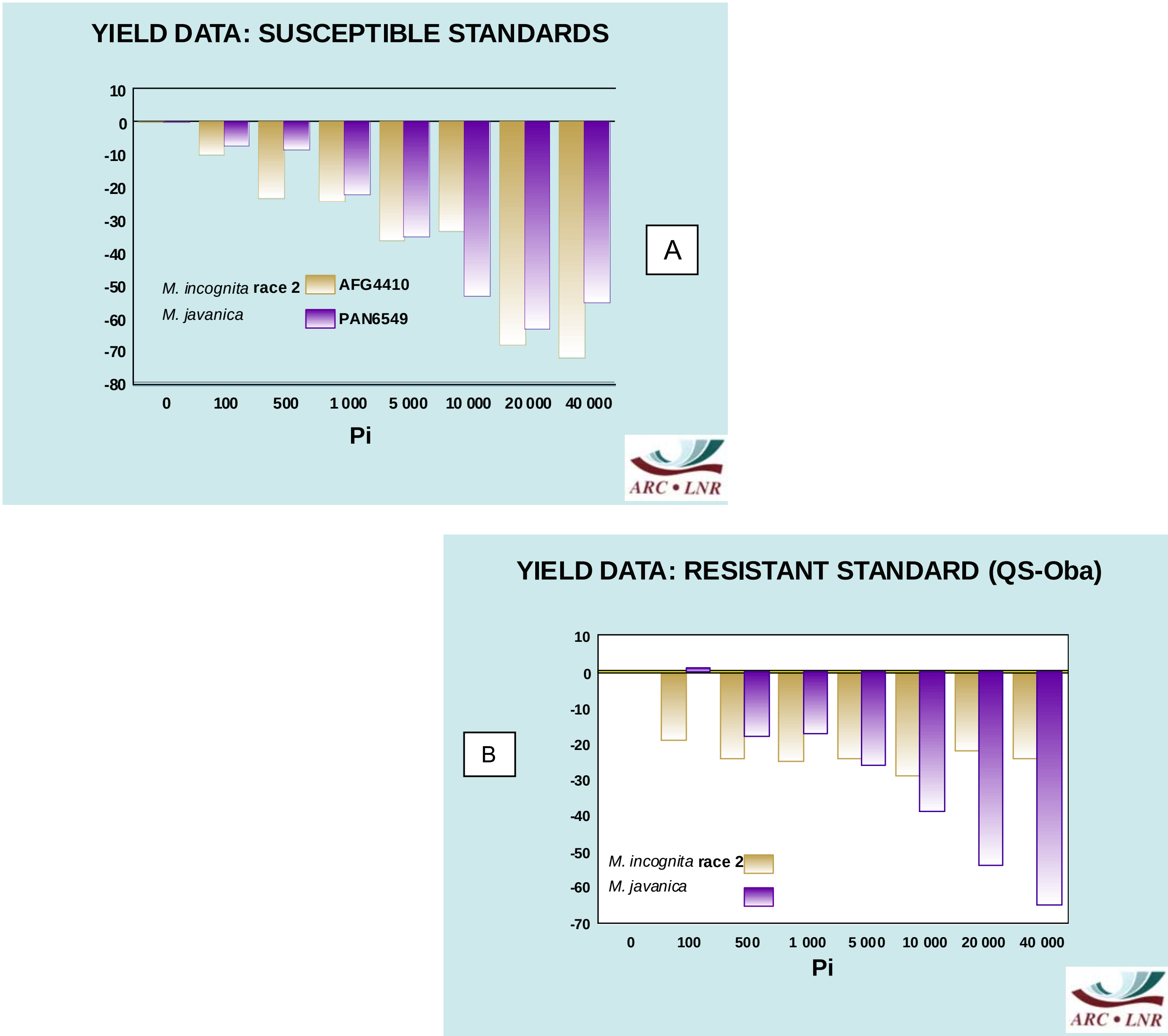
Figs. 15 A & B Regression data for susceptible and resistant maize genotypes (50g roots) inoculated with a range of initial population densities of *M. incognita* race 2 as well as *M. javanica* in a microplot trial at Potchefstroom.



Figs. 16 A & B Regression data for susceptible and resistant maize genotypes (200ml soil) inoculated with a range of initial population densities of *M. incognita* race 2 as well as *M. javanica* in a microplot trial at Potchefstroom.

3.4.1.2. Yield data

Yield losses for the susceptible standard were substantial over the Pi range, viz. from 0 at Pi = 0 to Pi = 72% for *M. incognita* and 55% for *M. javanica* (Figs. 17A & B). For local exotic cv. Mp712w yield increases were explained for *M. incognita*, while yield losses of up to 63% were recorded for this genotype for *M. javanica*. The local OPV (QS-OBA) showed yield losses for both spp. over the Pi range of up to 25% for *M. incognita* and 63% for *M. javanica*.



Figs. 17 A & B Regression data for yield of susceptible and resistant maize genotypes inoculated with a range of initial population densities of *M. incognita* race 2 as well as *M. javanica* in a microplot trial at Potchefstroom.

3.4.2. Field trials

Verification of RKN resistance in the USA inbred-line Mp712w and OPV QS-Obatanpa in field trials showed that significantly ($P \leq 0.05$) lower RKN eggs and J2/50g roots were recorded for both these genotypes in the Nelspruit and Potchefstroom trials compared to the respective susceptible standards. RKN eggs and J2/50g roots ranged from 6 for both Mp712w and QS-Obatanpa to 9 483 for AFG4410 and 9 785 for PAN6549 in the Nelspruit trial. For the Potchefstroom trial, RKN eggs and J2 were 12/50g roots for Mp712w and 15 for QS-Obatanpa, while it were 9 746 and11 528 for the respective susceptible parents AFG4410 and PAN6549. Statistical comparisons of yield are not possible since a line (Mp712w), an OPV (QS-Obatanpa) and two hybrids were tested in these trials. However, the resistant checks produced low mean seed masses/plot, namely 340 g for Mp712w and 994 g for QS-Obatanpa, while 3 441 g/plot were recorded for AFG4410 and 4 353 g for PAN6549 in the Nelspruit trial. The same trend was observed for the Potchefstroom trial, where Mp712w and QS-Obatanpa had yields of 271 g and 1 907 g/plot respectively, compared to those of AFG4410 and PAN6549 of 6 728 g and 6 107 g/plot, respectively.

3.4.3. Penetration rate

The penetration rate of root-knot nematodes J2 into roots of the three genotypes did not differ significantly at 2 and 10 DAI. However, both the resistant genotypes had significantly lower root-knot nematode individuals in their roots at 4, 16 and 20 DAI than the susceptible AFG4410. The number of swollen females as well as developing juveniles of both root-knot nematode species was significantly higher in the roots of AFG4410 from 10DAI onwards than those of both resistant genotypes. At 20 DAI roots of MP712 and QS-Obatanpa contained, respectively, 89% and 88% less developing *M. incognita* race-2 females than the susceptible AFG4410. The same trend was evident for *M. javanica*, with MP712W and QS-Obatanpa containing, respectively, 92% and 88% less developing juveniles than AFG4410. These results confirm that different levels of resistance to *M. incognita* race 2 and *M. javanica* exist in maize genotypes. Although genotypes with high levels of resistance to root-knot nematodes are immediately available to producers, the resistance should better be introgressed into commercial material that is well adapted to environmental conditions and with good combining ability. Use of host-plant resistance in combination with improved, integrated nematode-control strategies was the main objective of this research.

4. DISCUSSION

Results obtained from the surveys contributed substantially towards available knowledge about the occurrence, impact and management of PPN, in particular RKN in local maize-based cropping systems. For the survey in commercial maize-producing areas results showed that RKN was the predominant PPN in the Lichtenburg-, Vaalharts, Ottosdal- and Bothaville areas with the predominant spp. identified namely, *M. javanica*, *M. incognita* and *M. arenaria*. The survey in developing agricultural areas also showed that RKN were the predominant group parasitizing various crops that are planted in maize-based cropping systems in the North West, Limpopo, Gauteng, Mpumalanga, Eastern Cape and KwaZulu-Natal Provinces. The predominance of particularly RKN in the majority of fields sampled during these surveys, emphasizes the potential adverse impact this nematode group may have on local maize production.

Other crop cultivars identified with resistance to RKN include wheat and sorghum as well as Vetiver grass, which are used as a cover crop. Inclusion of these crops in maize-based systems could assist in alleviating the RKN problem in both commercial and small-scale systems. Screening of cowpea genotypes, which is adapted to drought should, however, be repeated since RKN numbers in the susceptible standard were low and thus indicate that the data are not accurate.

An increase in awareness of RKN parasitism in maize over the last decade prompted identification and validation of resistance in order to control these parasites cost-effectively over the long term. RKN resistance has thus been identified in a number of commercial maize cultivars as well as open-pollinated varieties (OPV's), while resistance in exotic maize lines has been successfully introgressed into local maize germplasm. Progenies resulting from crosses made between exotic RKN resistant and susceptible maize lines have been verified for RKN resistance in microplot and greenhouse trials during the 2007/08 and 2008/09 growing seasons. Seed from such RKN populations was multiplied during the 2009/10 growing season in a commercial greenhouse at the GCI and should finally be evaluated in either a greenhouse/microplot/field trial to confirm their RKN resistance levels. After this final validation RKN data should be merged with fingerprinting data that has already been done. The breeding lines should then be made available to SANSOR for use by interested maize breeders.

In terms of RKN resistance in OPVs, resistance in OPV QS-Ob was confirmed during microplot trials when it was compared to resistance exhibited by the exotic line MP712W. QS-Obatanpa could thus be used by developing farmers who prefer it to reduce RKN numbers effectively. High levels of resistance to RKN in the latter OPV were further demonstrated by the rate of penetration of RKN into its roots and supporting data from both microplot and field trials. Results from this study demonstrate the benefits that poor-host status of local OPV's would have in controlling root-knot nematode numbers in fields, particularly for small-scale farmers. These local as well as the exotic germplasm with root-knot nematode resistance could also be used in breeding programmes to transfer resistance to OPV's or cultivars with preferred agronomic characteristics. Although genotypes with high levels of resistance to RKN are immediately available to producers, the resistance should better be introgressed into commercial material that is well adapted to local environmental conditions and have both good combining ability and high yields.

Since the use of host-plant resistance in combination with improved, integrated nematode-control strategies was the main objective of this research, Project M151/10 focussed on a wide variety of objectives that addressed the needs of both the commercial and small-scale farmers. For this reason an overview of the predominant research activities were done in this report. However, a list of all contributions in terms of research articles, symposia/congress presentations as well as other activities that contributed towards technology transfer since this project commenced are attached as Appendix 1.

5. LITERATURE CITED

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Appendix 1

i) Scientific articles published/accepted in peer-reviewed journals

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

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Riekert, H.F., 1996. Economic feasibility of nematode control in dryland maize in South Africa. *African Crop Science Journal*. 4(4), 477 - 481.

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Fourie, H., Venter, G.A. & Mc Donald, A.H. 2003. Optimum temperature and inoculation requirements for *in vitro* production of *Pratylenchus brachyurus* on carrot discs. South African Journal of Plant and Soil, 20:44 - 45.

Fourie, H., Leswifi, C., Mc Donald, A.H. & De Waele, D. 2007. Host suitability of vetiver grass to *Meloidogyne incognita* and *M. javanica*. Nematology, 9: 49 - 52.

Coyne, D., Fourie, H. & Moens, M. 2009. Current and Future Management. Strategies in Subsistence Agriculture in Resource-poor Regions. In: Perry, R., Moens, M. & Starr, J. (Eds), Root-knot nematodes. CABI, Wallingford, UK. Pp. 444 - 473.

Ngobeni, G. L., Fourie, H., Mc Donald, A.H. & Mashela, W., 2010. Host suitability of selected South African maize genotypes to the root-knot nematode species Meloidogyne incognita race 2 and Meloidogyne javanica: A preliminary study. *SA Journal of Plant and Soil* (accepted for publication).

ii) Invited contributions: International

Fourie, H., Mc Donald, A.H. & Riekert, H.F. 2000. Nematology research at the ARC-GCI. Plant Research International, Wageningen, The Netherlands, February 2000. (Guest Speaker)

Fourie, H. & Mc Donald, A.H. 2008. Evaluation of abamectin seed treatment for reducing root-knot nematode populations in various crops, particularly maize. Syngenta Seed Care Corn Production Conference, 18 – 20 February 2008, Tucson, Arizona, USA. (Guest Speaker)

Fourie, H., Mc Donald, A.H., De Waele, D. & Van Biljon, E.R. 2009. Control strategies to manage root-knot nematodes in cropping systems in South Africa. African Crop Science Society Conference, Cape Town. (Keynote)

iii) Invited contributions: National

Fourie, H., Mc Donald, A.H., De Waele, D. & Van Biljon, E.R. 2009. Control strategies to manage root-knot nematodes in cropping systems in South Africa. 19TH NSSA Symposium, Casa Do Sol Hotel and Resort, Hazyview. (Guest Speaker - Workshop)

Fourie, H. 2009. Nematodes and soil health. Soil Health Workshop, Free State University. (Guest Speaker - Workshop).

iv) Local scientific congresses/symposia contributions

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Riekert, H.F. 1996. Importance of root-knot nematodes in local maize breeding and production. Maize breeding symposium

Riekert, H.F. 1996. Economic important nematodes in grain crops. Plant Protection Forum

Riekert, H.F. 1996. Economic aspects of chemical nematode control. Plant Protection Forum,

Riekert, H.F. 1996. Pretoria. Prelimary results on the effect of rotation crops of maize on root-knot nematode infestation levels. Nematology workshop

Riekert, H.F. 1997. Effect of crop rotation on root-knot nematode infestation of maize. 13th NSSA.

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Riekert, H.F. 1997. Knopwortelaalwurm geassosieer met sand gronde. Grondbelangegroep, NWLOI.

Riekert, H.F. 1998. The effect of cowpea groundnut and soybean rotations on root-knot nematode infestation of maize. Congress of the South African Crop Production Society.

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v) International symposia contributions

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vii) Expert advice

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Fourie, H. 2008. Importance and fundamental value of Temik for the agricultural and horticultural industries, particularly in South Africa. Supplied informative document to Mr Pieter Fourie from BayerCropScience for submission to international committee which will meet during October 2008 to decide whether Temik should be withdrawn or degraded to the Pic 2 list.

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viii) Publications: popular

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ix) Radio talks

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Riekert, H.F. 1994. Metodes van aalwurmbeheer op mielies.

Riekert, H.F. 1995. Die ekonomiese regverdigbaarheid van chemiese aalwurmbeheer op mielies.

Riekert, H.F. 1996. Navorsingstendense in Nematologie soos waargeneem tydens die Derde Internationale Nematologie Kongres te Guadelope.

Riekert, H.F. 1997. Die effek van wisselbou met peulgewasse op knopwortelaalwurmbesmetting van mielies in 'n mieliegebasseerde wisselboustelsel.

Riekert, H.F. 2000. Swamkweker termietskade in mielies. (Landbou- radio)

Riekert, H.F. 2000. Fungus growing termite damage in maize (15 Rural radio stations)

Riekert, H.F. 2000. Bestuur en beheer van knopwortelaalwurm in akkerboustelsels. Opname Radio Pretoria.

Riekert, H.F. 2001. Knopwortelaalwurm monitering en monsterneming in graan en ander gewasse. Opname Radio Pretoria.

x) Leaflets

Riekert, H.F. 1998. Root-knot nematodes in maize. Crop Protection Series No. 10b.

Riekert, H.F. 1998. Knopwortelaalwurm by mielies Gewasbeskermingreeks No. 10a.

Riekert, H.F. & Mc Donald, A.H. 2001. Management of root-knot nematodes in field crop systems. Crop Protection Series No. 23b.

Riekert, H.F. & Mc Donald, A.H. 2001. Bestuur van knopwortelaalwurm in akkerboustelsels. Gewasbeskermingreeks No. 23a.

xi) Television broadcasts

Riekert, H.F. 1998. Knopwortelaalwurm by mielies. Nampo TV Channel.

Riekert, H.F. 1998. Die effek van gewasrotasie in ‘n mieliegebaseerde rotasiestelsel in knopwortelaalwurm besmette grond. Nampo TV Channel.

xii) Awards

Best poster:

Riekert, H.F. 1997. Screening for *Meloidogyne javanica* resistance in maize lines important in breeding for insect resistance. 13th NSSA Symposium, San Lameer, Southbroom.

E. De Beer, H. Fourie, A. H. Mc Donald & E.R. van Biljon. 2007. Evaluation of Abamectin seed treatment as a potential nematicide for plant-parasitic nematodes in cotton. 18th NSSA Symposium, Board Walk Centre, Port Elizabeth.

Other awards

Rhône-Poulenc Trophy:

Fourie, H. 2005. For the advancement of Nematology in Southern Africa. Received during 17th NSSA Symposium, Hans Merensky Estate, Phalaborwa, 2005.

Fourie, H. 2006. ARC-Grain Crop Institute's "Best Performing Scientist", ARC-GCI, Potchefstroom, South Africa.

Fourie, H. 2007. Agriculturalist of the Year for North West Province 2007. Awarded by the Agricultural Writers SA,Birchwood, Johannesburg, 7 November 2007.

Bayer CropScience Trophy:

Fourie, H. 2009. For the advancement of Nematology in Southern Africa. Received during 19th NSSA Symposium, Casa Do Sol, Hazyview.