

# RESEARCH/INVESTIGACIÓN

## OCCURRENCE AND PATHOGENICITY OF PLANT-PARASITIC NEMATODES ON COMMONLY GROWN BANANA CULTIVARS IN SOUTH AFRICA

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### ABSTRACT

Daneel, M., K. De Jager, I. Van den Bergh, M. De Smet, and D. De Waele. 2015. Occurrence and pathogenicity of plant-parasitic nematodes on commonly grown banana cultivars in South Africa. *Nematropica* 45:118-127.

A preliminary and limited nematode survey was conducted in the three main banana-producing areas of South Africa, namely Onderberg, Hazyview (both in Mpumalanga Province), and the South Coast of Kwazulu/Natal Province. Root and soil samples were taken from ‘Chinese Cavendish’ (AAA), ‘Dwarf Cavendish’ (AAA), ‘Grand Nain’ (AAA), ‘Williams’ (AAA) and ‘Goldfinger’ (syn. ‘FHIA-01’, AAAB). The burrowing nematode, *Radopholus similis*, the coffee root-lesion nematode, *Pratylenchus coffeae*, mixed populations of root-knot nematodes, *Meloidogyne incognita* and *Meloidogyne javanica*, and the spiral nematodes, *Helicotylenchus dihystera*, *H. multicinctus*, *H. pseudorobustus*, and *Scutellonema brachyurus* occurred in all three areas with the root-knot and spiral nematodes being most abundant. *Radopholus similis*, a well known damaging pest on banana, was detected at low population levels. Other species found were *Rotylenchulus reniformis*, *Paratylenchus minutus*, and *Paratrichodorus minor*.

‘Chinese Cavendish’, ‘Williams’, ‘Grand Nain’ and ‘High Noon’ (AAAB) were also evaluated in the glasshouse for their response to *R. similis* and mixed populations of *M. incognita* and *M. javanica*. ‘Grand Nain’ was more susceptible to *R. similis* than ‘High Noon’, which allowed lower final nematode densities. It was also found that large numbers of mixed populations of *M. incognita* and *M. javanica* did not have a negative effect on the growth of banana plants in the glasshouse. ‘Chinese Cavendish’ and ‘High Noon’ had high root gall ratings and nematode numbers in the roots, but the root systems still appeared fairly healthy.

*Key words:* *Helicotylenchus multicinctus*, host response, *Meloidogyne incognita*, *Meloidogyne javanica*, *Musa*, *Pratylenchus coffeae*, *Radopholus similis*, screening, survey.

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### RESUMEN

Daneel, M., K. De Jager, I. Van den Bergh, M. De Smet, and D. De Waele. 2015. Presencia y patogenicidad de nemátodos parásitos de plantas en cultivares de banana frecuentemente cultivados en Sudáfrica. *Nematropica* 45:118-127.

Se realizó un muestreo nematológico preliminar y limitado en las tres principales áreas productoras de banana en Sudáfrica, Onderberg, Hazyview (ambas en la provincia de Mpumalanga), y la costa sur de Kwazulu en la provincia de Natal. Se tomaron muestras de suelo y raíces de los cultivares ‘Chinese Cavendish’ (AAA), ‘Dwarf Cavendish’ (AAA), ‘Grand Nain’ (AAA), ‘Williams’ (AAA) and ‘Goldfinger’ (syn. ‘FHIA-01’, AAAB). El nematodo barrenador, *Radopholus similis*, el nematodo lesionado de raíces del café, *Pratylenchus coffeae*, poblaciones mezcladas de nematodos de las agallas de las raíces *Meloidogyne incognita* y *Meloidogyne javanica*, y nematodos espirales, *Helicotylenchus dihystera*, *H. multicinctus*, *H. pseudorobustus*, y *Scutellonema brachyurus* se encontraron en las tres áreas, siendo los nematodos de las agallas de las raíces y los nematodos espirales los más abundantes. *Radopholus similis*, conocida plaga dañina de la bananera, fue detectado en bajos niveles de población. Otras especies encontradas fueron *Rotylenchulus reniformis*, *Paratylenchus minutus*, y *Paratrichodorus minor*.

La respuesta de los cultivares ‘Chinese Cavendish’, ‘Williams’, ‘Grand Nain’ and ‘High Noon’ (AAAB) a

*R. similis* y a poblaciones mixtas de *M. incognita* y *M. javanica* fue evaluada en invernadero. ‘Grand Nain’ fue más susceptible a *R. similis* que ‘High Noon’, en el cual se obtuvieron densidades finales de nematodos menores. También, se observó poblaciones mixtas de *M. incognita* and *M. javanica* en altas densidades no tuvieron un efecto negativo sobre el crecimiento de las plantas de banana en invernadero. ‘Chinese Cavendish’ y ‘High Noon’ mostraron altos índices de agallamiento en las raíces y altas densidades de nematodos en las raíces, aunque el sistema radical se observó aparentemente bastante sano.

*Palabras clave:* *Helicotylenchus multicinctus*, respuesta hospedante, *Meloidogyne incognita*, *Meloidogyne javanica*, *Musa*, *Pratylenchus coffeae*, *Radopholus similis*, selección, muestreo.

## INTRODUCTION

Dessert bananas (*Musa acuminata* (AAA) Cavendish subgroup) are commercially cultivated in five regions of South Africa on an overall area of 11,360 ha (DAFF, 2011). These regions are Levubu (Limpopo Province), Hazyview, Onderberg (both in Mpumalanga Province) and the North and South Coast of Kwazulu/Natal Province. Cultivars most frequently grown are ‘Grand Nain’ (AAA), ‘Chinese Cavendish’ (AAA), and ‘Dwarf Cavendish’ (AAA) (Robinson, 1993).

Nematodes previously found on banana in South Africa were *Radopholus similis* (Cobb, 1893) Thorne, 1949, *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941, *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949 (Milne, 1973; Jones, 1979; Jones and Milne, 1982; De Jager, 1993; Daneel, 2007) and can cause as much as 60% yield loss without proper control measures (Keetch, 1989; Robinson, 1993). *Radopholus similis*, which is the most damaging species on banana, was imported into South Africa with planting material (Jones and Milne, 1982) and has since spread to all banana-growing areas except Levubu in the Limpopo Province (De Villiers *et al.*, 1970). *Radopholus similis* was observed on one farm in the Tzaneen area in the Limpopo Province (Jones and Milne, 1982), but due to proper control measures, it has never been found again.

*Pratylenchus coffeae*, *H. multicinctus*, *M. incognita*, and *M. javanica* are endemic to South Africa and are found on a wide range of hosts (Kleynhans *et al.*, 1996). In order to obtain information on the species composition of these plant-parasitic nematodes in bananas in South Africa, a study was undertaken to determine the occurrence and damage of these species on the commonly grown cultivars in the three main banana production areas. The response of these cultivars to *R. similis* and mixed populations of *M. incognita* and *M. javanica* was also investigated.

## MATERIALS AND METHODS

### Survey

A preliminary and limited survey was conducted in the three main banana-producing areas of South Africa, i.e., Onderberg, Hazyview, and the South Coast of Kwazulu/Natal (Fig. 1) during the dry winter months (July until September). The three production areas surveyed differ in temperature range, annual rainfall, and soil type. Kwazulu/Natal is situated at sea level with a sandy soil and average temperatures range from 16.5 to 24.3°C with a mean temperature of 20.4°C and an annual rainfall of 1,217 mm. Due to the summer rain, water logging can become a problem after heavy rain fall. Hazyview and Onderberg areas have a well-drained clay loam soil mostly with a clay content of >30%. Hazyview area is classified as cool subtropical climate with temperature ranges between 14.9 to 25.7°C, mean temperature of 20.2°C and total annual rainfall of 970 mm compared to Onderberg with temperature ranges of 16.3 to 28.5°C, a mean temperature of 22.5°C and total annual rainfall of 700 mm.

A limited sampling was conducted in each region and consisted of 5 samples from 15 banana plants per 100 ha (Robinson, 1993) resulting in a total of 404 samples taken from 134 plantations. Nematode samples were taken from ‘Chinese Cavendish’, ‘Dwarf Cavendish’, ‘Grand Nain’, ‘Williams’ (AAA) and ‘Goldfinger’ (syn. ‘FHIA-01’, AAAB). ‘Goldfinger’ was included because it was planted commercially (although very limited) in two of the three areas and alleged to be tolerant to *R. similis* (Rowe and Rosales, 1993). Individual samples, consisting of soil and roots, were taken from three plants per plantation. A sample of 30 cm<sup>3</sup> of soil and roots was collected from each plant using a spade. Plants used for sampling were in the flowering stage. Root samples, consisting of five 10-cm pieces of root, were evaluated for root damage by the nematode species present in the surveyed areas using the Root Necrosis Index (RNI) (Spejier and De Waele, 1997), Root Knot Index (RKI) and

Root Infection Index (RII). The RNI expresses the percentage of roots parasitized with nematode life stages of both the endoparasitic migratory (*P. coffeae* and *R. similis*) and sedentary (*M. incognita* and *M. javanica*) species as well as ectoparasitic species including *H. multicinctus* after cutting open the roots. The RNI reflects the abundance of galled roots while RII reflects the amount of root tissue infected by *M. incognita* and *M. javanica* egg masses and swollen females, respectively. Both these indexes were expressed according to a scale from 0 to 4, where 0 = no root galling/infection and 4 = 100% root galling/infection.

Nematodes were extracted from soil using the sugar flotation technique (Jenkins, 1964) and from roots using a combination of maceration and sugar flotation techniques (Coolen and D'Herde, 1972; Coolen 1979). They were recorded as number of nematodes in 250 cm<sup>3</sup> soil and 30 g roots, respectively.

#### Host response evaluation

'Chinese Cavendish', 'Williams', 'Grand Nain' and 'High Noon' (syn. 'SH 3640', AAAB) were evaluated for their response to *R. similis*, *M. incognita* and *M. javanica*. The Cavendish cultivars were selected because of their importance in the South African banana industry, while 'High Noon' was a newly imported FHIA hybrid that was evaluated for Fusarium wilt (Eckstein *et al.*, 1998).

*Radopholus similis* was collected from the field and reared on carrot discs at 28°C (Spejier and De Waele, 1997). Eggs and second-stage juveniles (J2) of a mixed population of *M. incognita* and *M. javanica* (20:80) were collected in the field (Merensky Experimental Farm, Tzaneen, Limpopo Province) and used for inoculation.

Five-centimeter-high banana tissue-culture plantlets were planted in 20 cm diam. plastic bags containing 1 dm<sup>3</sup> of nematode free potting soil [1/3 Braaks medium mixed with 2/3 sand] and kept in a glasshouse for 3 wk. Thereafter, a group of plants was inoculated with the initial densities (Pi) of 0, 500, and 2,000 *R. similis* per plant and another group with the same initial densities (Pi) of a mixed population of *M. incognita* and *M. javanica* J2's in the proportion of 20:80. Plants were kept in a glasshouse at 25°C. Each treatment consisted of 10 replicates. Plant height and pseudostem diameter were measured fortnightly. Eight weeks after inoculation, plants were removed, and fresh shoot and root mass, as well as leaf area of the first and the third leaves, were determined. The experiments were repeated twice. The screening experiments were a factorial experiment with 2 factors (cultivar and inoculation level).

To evaluate the effect of *R. similis*, a rating was

given for the damage observed on the roots, with 0 = no damage and 5 = severe necrosis. For *Meloidogyne* spp., ratings were given for the general health of the roots as well as root gall ratings (Zech, 1971). Final soil and root densities (Pf) for the two nematodes were determined with the techniques used for the survey. The reproductive factor (Pf/Pi) for the two nematodes was calculated for each inoculum level.

Data were analyzed statistically using the program STATISTICA. Two factor variance analysis was conducted to analyze the interactions between cultivars and nematode populations.

## RESULTS

### Survey

The burrowing (*R. similis*) and coffee root lesion (*P. coffeae*) nematodes, and mixed populations of root-knot (*M. incognita* and *M. javanica*) and spiral (*H. dihystra* (Cobb, 1893) Sher, 1961, *H. multicinctus*, *H. pseudorobustus* (Steiner, 1914) Golden, 1956 and *Scutellonema brachyurus* (Steiner, 1938) Andrassy, 1958) nematodes were all found in the three areas surveyed. Root-knot and spiral nematodes were the most abundant taxa and made up more than 89% of the total root nematode population in Onderberg, 79% in Hazyview, and more than 60% in the South Coast of Kwazulu/Natal (Tables 1 and 2). These nematodes were also found in 94.5 and 93.2% of the samples respectively (Table 1). Besides *P. coffeae*, *P. neglectus* was also found in the Hazyview area. The coffee root lesion nematode was found in 25.9% of the samples and was distributed in the three banana-producing areas but mean population numbers were low.

*Radopholus similis* was present in a few farms in the three banana-producing areas surveyed, but at low population levels. The nematode was in 4 farms (12 plantations) in Onderberg, in 4 farms (9 plantations) in Hazyview, and in 3 farms (8 plantations) in the South Coast of Kwazulu/Natal. In total, *R. similis* was only found in 18.5% of all samples.

Besides the above-mentioned species, other species occurring in considerable numbers in the different areas were *Paratylenchus minutus* Linford, Oliveira & Ishi, 1963, *Paratrichodorus minor* (Colbran, 1965) Siddiqi, 1973 and *Rotylenchulus reniformis* Linford & Oliveira, 1940 at population levels reported in Table 1. The occurrence of the four main nematode species per cultivar in the three areas surveyed is shown in Table 2. Numbers of root-knot nematode species listed above were high in the Onderberg area for all of the cultivars evaluated. Soil type in the area ranged from sandy clay loam to clay. In the South Coast of Kwazulu/

Table 1. Mean number of nematodes found on banana in the three main banana-growing areas in South Africa (250 ml soil and 30 g root samples) and frequency of occurrence (%).

Areas	Soil			Roots			% frequency of occurrence
	O <sup>x</sup>	H <sup>y</sup>	KZN <sup>z</sup>	O <sup>x</sup>	H <sup>y</sup>	KZN <sup>z</sup>	
Number of samples	163	124	117	166	119	117	
<i>Radopholus similis</i>	3	14	11	79	45	41	18.5
<i>Pratylenchus coffeae</i>	17	55	15	37	76	26	25.9
<i>Helicotylenchus</i> spp.	474	1142	1953	1059	1292	1532	94.5
<i>Meloidogyne</i> spp.	730	749	835	1571	571	750	93.2
<i>Paratylenchus</i>	0	0	185	0	0	266	22.2
<i>Rotylenchulus</i>	279	1	0	7	0	0	2.4
<i>Paratrichodorus/Nanidorus</i>	3	31	30	0	0	0	4.3

<sup>x</sup> O = Onderberg<sup>y</sup> H = Hazyview<sup>z</sup> KZN = South Coast of Kwazulu/Natal

Table 2. Mean number of the four most important nematode species on five major banana cultivars in the three main banana-growing areas in South Africa (30 g root samples).

	Williams (AAA)	Grand Nain (AAA)	Chinese Cavendish (AAA)	Dwarf Cavendish (AAA)	Goldfinger (AAAB)
Onderberg (n) <sup>z</sup>	51	42	40	27	0
<i>Radopholus</i>	79 (0-2350)	65 (0-1400)	36 (0-700)	161 (0-2350)	
<i>Pratylenchus</i>	18 (0-600)	30 (0-1050)	-	147 (0-500)	
<i>Helicotylenchus</i>	1370 (0-13750)	888 (0-13950)	613 (0-4100)	1669 (0-5650)	
<i>Meloidogyne</i>	589 (0-4950)	2160 (0-11600)	1880 (0-20450)	1003 (0-13150)	
Hazyview (n)	46	15	36	20	9
<i>Radopholus</i>	53 (0-800)	-	16 (0-200)	163 (0-1900)	-
<i>Pratylenchus</i>	94 (0-1050)	43 (0-350)	93 (0-600)	-	211 (0-1000)
<i>Helicotylenchus</i>	1468 (0-5550)	2129 (0-6150)	1238 (0-4050)	818 (0-2450)	886 (0-4950)
<i>Meloidogyne</i>	474 (0-6450)	623 (0-3000)	517 (0-7300)	700 (0-4900)	247 (0-825)
Kwazulu/Natal (n)	75	6	24	12	3
<i>Radopholus</i>	62 (0-1250)	-	-	8 (0-100)	-
<i>Pratylenchus</i>	39 (0-1400)	-	21 (0-400)	-	33 (0-100)
<i>Helicotylenchus</i>	1483 (0-7150)	320 (0-650)	1384 (0-5850)	2532 (0-5650)	4583 (2100-8200)
<i>Meloidogyne</i>	731 (0-12500)	295 (0-650)	1024 (0-5450)	446 (0-1200)	517 (350-1150)

<sup>z</sup> (n) indicates number of samples taken.

Natal, the soil type was always sandy loam and the densities of the spiral nematode species listed above were higher than in the other areas for all cultivars except Grand Nain. 'Chinese Cavendish' had the highest mean numbers of *Meloidogyne* spp. in the three areas. The highest numbers of *R. similis* and *P. coffeae* occurred on 'Dwarf Cavendish' in the Onderberg and Hazyview areas, with *R. similis* most abundant in the Onderberg area. *Radopholus similis*

was not found on 'Goldfinger'. Although only a few 'Goldfinger' plants were sampled because of the limited distribution of the cultivar, this observation agrees well with those of previous studies (Rowe and Rosales, 1993; Robinson, 1996) where 'Goldfinger' is stated to be tolerant to *R. similis*. In both areas, *P. coffeae* was observed on 'Goldfinger'.

The root damage (RNI, RII, and RKI) per cultivar in the three areas is shown in Table 3. Root necrosis

Table 3. Mean values of root necrosis index (RNI), root infection index (RII) and root knot index (RKI) of five major banana cultivars in the three main banana-growing areas in South Africa

	Williams (AAA)	Grand Nain (AAA)	Chinese Cavendish (AAA)	Dwarf Cavendish (AAA)	Goldfinger (AAAB)
Onderberg (n) <sup>x</sup>	51	42	40	27	0
RNI	9.75 ab/x <sup>yz</sup>	7.29 ab/x	5.95 a/x	8.11 a/x	
RII	0.46 a/x	0.77 a/x	0.48 ab/x	0.37 a/x	
RKI	0.26 a/x	0.49 a/x	0.43 a/x	0.26 a/x	
Hazyview (n)	46	15	36	20	9
RNI	7.62 b/x	13.27 a/y	11.61 a/xy	10.40 a/xy	5.94 a/x
RII	0.49 a/x	0.73 ab/x	0.28 b/x	0.23 a/x	0.33 a/x
RKI	0.22 a/x	0.10 a/x	0.10 a/x	0.10 a/x	0.11 a/x
Kwazulu/Natal (n)	75	6	24	12	3
RNI	12.08 a/x	1.00 b/x	8.15 a/x	14.00 a/x	9.17 a/x
RII	0.43 a/xy	0.00 b/y	0.67 a/x	0.46 a/xy	1.00 a/x
RKI	0.19 a/x	0.00 a/x	0.25 a/x	0.08 a/x	0.00 a/x

<sup>x</sup> n = Number of samples

<sup>y</sup> Different letters (a,b,x,y) show a significant difference between mean values of root damage factors per area (a,b) and cultivar (x,y)

<sup>z</sup> Means within a column/row followed by the same letter do not differ significantly at  $P = 0.05$

Table 4. Mean values of root necrosis index (RNI), root infection index (RII) and root knot index (RKI) in the three main banana-growing areas in South Africa

	Onderberg n = 160	Hazyview n = 126	Kwazulu/Natal n = 120
RNI	7.90 a <sup>z</sup>	9.90 a	10.86 a
RII	0.52 a	0.40 a	0.47 a
RKI	0.36 a	0.14 b	0.18 b
Numbers of <i>Meloidogyne</i>	0 - 20450	0 - 7300	0 - 12200
<i>Meloidogyne</i> frequency	70.5%	71.2%	80.2%

<sup>z</sup> Means within a row followed by the same letter do not differ significantly at  $P = 0.05$ .

caused by burrowing, lesion, spiral, and root-knot nematodes was limited since RNI values did not exceed 14%. Within the areas, between the different cultivars little difference was observed except for Hazyview, where 'Grand Nain' had a higher RNI than 'Williams' and 'Goldfinger' ( $P < 0.05$ ). If cultivars were compared between the different areas, 'Williams' had a higher RNI in South Coast of Kwazulu/Natal while 'Grand Nain' had a lower RNI in the same area ( $P < 0.05$ ). Hazyview had the lowest RNI for 'Williams' and highest RNI for 'Grand Nain' ( $P < 0.05$ ). The other cultivars showed very little difference between the different areas. The values of RII and RKI, which reflect the damage caused by root-knot nematodes, were low in the three surveyed areas. The response of the five banana cultivars to

the nematodes as reflected by these indices varied not significantly except for RII in the South Coast of Kwazulu/Natal with 'Chinese Cavendish' and 'Goldfinger' having a higher value than 'Grand Nain' ( $P < 0.05$ ). Between the three different areas, RII showed differences for 'Grand Nain' and 'Chinese Cavendish' ( $P < 0.05$ ) (Table 3).

Root-knot Index (RKI) differed between Onderberg and the two other areas (Table 4). Root-knot Index (RKI) is directly linked to *Meloidogyne* damage, thus indicating that *Meloidogyne* damage was higher in the Onderberg area. This was also observed in Table 2. Root Necrosis Index (RNI) was the highest in South Coast of Kwazulu/Natal, although not significantly different from the other areas.

Table 5. Root mass, plant mass, root damage, and nematodes per root system of different banana cultivars exposed for eight weeks to increasing initial densities of *Radopholus similis* in a glass house experiment.

Cultivars	Root mass (g)			Plant mass (g)			Damage (1-5)			Nematodes / root system		
	0	500	2000	0	500	2000	0	500	2000	0	500	2000
CC <sup>y</sup>	15.3 bc <sup>z</sup>	13.5 bcd	10.7 d	18.4 ab	16.5 abc	13.3 c	0.1 a	1.9 b	3.6 e	0 a	152 ab	659 cd
GN <sup>w</sup>	15.3 bc	17.2 ab	14.1 bcd	18.8 a	20.1 a	19.3 a	0.1 a	2.4 bcd	3.1 de	6 a	435 bc	509 c
HN <sup>x</sup>	17.5 ab	19.9 a	14.4 bcd	17.6 abc	18.6 a	13.8 bc	0 a	2 b	2.3 bc	7 a	76 ab	281 ab
W <sup>y</sup>	13.4 bcd	12.0 cd	11.9 cd	17.5 abc	13.4 c	13.7 c	0.2 a	1.9 b	3 cde	20 a	250 ab	505 c

<sup>y</sup> CC = Chinese Cavendish

<sup>w</sup> GN = Grand Nain

<sup>x</sup> HN = High Noon

<sup>y</sup> W = Williams

<sup>z</sup> Means for each parameter (rows and columns) followed by the same letter do not differ significantly at  $P=0.05$

Table 6. Root mass, plant mass, gall index, and nematodes per root system of different banana cultivars exposed for eight weeks to increasing initial densities of a mixed population of *Meloidogyne incognita* and *M. javanica* in a glasshouse experiment.

Cultivars	Root mass (g)			Plant mass (g)			Gall index (1-10)			Nematodes / root system		
	0	500	2000	0	500	2000	0	500	2000	0	500	2000
CC <sup>y</sup>	48.6 de <sup>z</sup>	67.5 bc	72.5 b	126.7 bc	146.1 ab	169.3 a	0.0 a	4.5 d	5.5 e	100 a	32270 cde	139128 e
GN <sup>w</sup>	18.3 f	45.4 de	49.3 d	47.8 f	85.6 de	90.4 de	0.0 a	3.3 b	5.0 de	143 a	3591 bc	16861 bcd
HN <sup>x</sup>	53.8 cd	79.4 ab	92.0 a	105.7 cd	153.7 a	162.2 a	0.0 a	5.1 de	4.8 de	99 a	45464 cde	48361 cde
W <sup>y</sup>	33.4 ef	50.7 d	48.7 de	72.1 ef	101.6 cd	90.5 de	0.0 a	3.6 bc	4.3 c	818 ab	6907 bc	20881 bcd

<sup>y</sup> CC = Chinese Cavendish

<sup>w</sup> GN = Grand Nain

<sup>x</sup> HN = High Noon

<sup>y</sup> W = Williams

<sup>z</sup> Means for each parameter (rows and columns) followed by the same letter do not differ significantly at  $P=0.05$

### *Response of different banana cultivars against R. similis*

The main effect of both cultivar and inoculation level caused differences in root mass (Table 5). No matter what the inoculation level, the cultivar caused a difference in root mass, with 'High Noon' having a significantly higher root mass than 'Williams' and 'Chinese Cavendish'. As inoculation level increased, root mass decreased, although significant differences were only observed in 'Chinese Cavendish' between the control and highest inoculum level (2,000). In general, 'High Noon' displayed vigorous root growth and abundant hair roots when compared to the other plants.

For plant mass, the main effect was found in the cultivar, independent of inoculation level. Plant mass of 'Grand Nain' was significantly higher than 'Williams'. Plants exposed to the highest inoculum level (2,000) were slightly smaller than the control plants with significant differences only observed in 'Chinese Cavendish' between untreated and the highest inoculum level (2,000).

An interaction was observed between cultivar and inoculation level for damage index. The control plants differed significantly from the inoculated plants for all cultivars with higher indices at the higher inoculation levels (Table 5). 'High Noon' had a lower index than the other cultivars at 2,000 inoculation level and, except for 'Williams', the difference was significant (Table 5). This seems to show that High Noon is more tolerant to *R. similis*.

An interaction was also observed between cultivar and inoculation level for nematode numbers per root system. The higher the inoculum, the higher the number of nematodes found in the root systems. 'High Noon' had a lower number of nematodes per root system than the other cultivars at both inoculation levels although it was only significantly different at the highest inoculum level (2,000).

*Radopholus similis* numbers and thus reproduction potential were highest in 'Grand Nain' followed by 'Williams' and 'Chinese Cavendish' at the inoculum level of 500. However at an inoculum level of 2,000 both 'Grand Nain' and 'Williams' showed reduced potential of reproduction indicating that such high numbers might already be detrimental to these small plants. In contrast 'Chinese Cavendish' showed similar reproduction levels at both inoculum levels, which may indicate a slightly higher potential to withstand *R. similis* infections. In contrast to root-knot nematodes where high reproduction rates were found after 8 wk (Table 6), in our experiments with *R. similis*, reproduction was low even at 8 wk after inoculation. Numbers very seldom reached the numbers observed in other field and glasshouse

studies (Fogain and Gowen, 1997; Barekeya *et al.*, 2000). This might be due to a lower pathogenicity of *R. similis* found in South Africa although this was never proven in a study. But other studies confirmed that differences in reproductive capabilities are present between different populations (Marin *et al.*, 1999; Stoffelen *et al.*, 1999). However, *R. similis* had a negative effect on plant growth, and the effect was more prominent with the higher number of nematodes.

### *Resistance of different banana cultivars against Meloidogyne spp.*

The main effect of both cultivar and inoculation level caused differences in root mass (Table 6). No matter what the inoculation level, the cultivar caused a difference in root mass, with 'High Noon' having the highest root mass followed by 'Chinese Cavendish'. Both are different from 'Grand Nain' and 'Williams' ( $P < 0.05$ ). Similar results can be seen for shoot mass. The inoculation level caused a difference in root mass and shoot mass independent of the cultivar with the both inoculation levels having a higher root mass and shoot mass than the control plants.

An interaction was observed between cultivar and inoculation level for nematode numbers per root system. The higher the inoculum, the higher the number of nematodes found in the roots. 'Chinese Cavendish' had high nematode numbers in the roots, but their root systems still appeared healthy. 'High Noon' showed little difference between inoculation numbers of 500 and 2,000 *Meloidogyne*. Gall rating was also similar between the two levels of inoculation. Despite high nematode numbers, the root systems appeared healthy and vigorous growth was observed. 'Williams' and 'Grand Nain' had lower *Meloidogyne* numbers than the other cultivars. It seemed as if reproduction was therefore much higher on 'Chinese Cavendish'. This compared well with the results obtained from the survey (Table 2).

However, with inoculation levels of 500 and 2,000 *Meloidogyne* per plant, no negative effect was observed on the growth of banana plants in the glasshouse. At the numbers evaluated, it even appeared as if *Meloidogyne* spp. stimulated growth. Nematodes found in the control treatment were possibly due to contamination of the water during watering.

## DISCUSSION

Various nematode genera were found in the survey in the banana production areas in South Africa with *Meloidogyne*, *Helicotylenchus*, *Radopholus*, and *Pratylenchus* being the most widespread. This

agrees with Gowen and Quénehervé (2005) who reported that in bananas, the root system is attacked by several nematode species causing simultaneous infections. In this present survey, the root-knot and spiral nematodes listed above were the most frequent and widely distributed. These nematodes were found in all areas and on all cultivars. In a survey carried out in household gardens in South Africa and Swaziland, the population densities of these species were higher than those of *P. coffeae* and *R. similis* and occurred in more than 90% of the root samples while *R. similis* was found in 9% and *P. coffeae* in 3% of the samples (Daneel *et al.*, 2002). Results of that study were similar to what was observed in the present survey.

Although many surveys confirm the presence of these four genera on bananas, abundance and frequency differ between genera. In a recent survey conducted in the Democratic Republic of Congo (Kamira *et al.*, 2013), *H. multincinctus* was present in 89% of the samples whereas *Meloidogyne* was found in 54% of the samples. On the other hand, *R. similis*, was present in 30% of the samples which is considerably higher than in the Southern African region. Distribution seemed to be influenced by altitude, which is not a factor in South Africa as banana production areas are all below 1300 m altitude. In a survey carried out in the Rusitu Valley in Zimbabwe (Chitamba *et al.*, 2013), the four species found in order of absolute frequency (95-75%) and abundance were *Meloidogyne* species, *R. similis*, *P. coffeae*, and *Helicotylenchus* species. Again *R. similis* and *Pratylenchus* seemed to be more widely distributed than in the present survey. Although Chitamba *et al.* (2013) states the long-term monoculture practices as an important reason for the high population densities and frequencies, it is believed that the subtropical conditions might limit *R. similis* and *Pratylenchus* distribution. Van den Bergh *et al.* (2006) have found similar results in Vietnam with *R. similis* being absent and *Meloidogyne* being the most prevalent taxon. Climatic conditions in Vietnam are also subtropical.

From the survey, it can be concluded that *R. similis* is present in the sampled banana growing areas of South Africa, but at low population densities. *Radopholus similis* was originally imported and distributed by means of infested plant material as was often the case in banana production areas (Gowen and Quénehervé, 2005). Due to a very effective quarantine system where movement of infected material was prohibited between areas and even farms (Anonymous, 1975, 1979), distribution of *R. similis* is now limited to single farms, with the highest presence in the South Coast of Kwazulu/Natal. The use of tissue-culture banana plants has also limited the spread of this nematode. It is only when infested

fields are found in close proximity to new plantations that chances are high of spreading *R. similis*.

However, when *R. similis* was present, serious damage was observed in the plantation. De Jager *et al.* (1999) investigated the pathogenicity of *R. similis* isolated from different areas and found that the population isolated from Kwazulu/Natal had the highest pathogenicity, which might explain why *R. similis* had a higher presence in Kwazulu/Natal. However, no studies from different *R. similis* pathotypes have been conducted to determine the damaging effect on banana. A phylogenetic study by Kaplan *et al.* (2000) was conducted to determine which pathotypes were able to infect citrus among a large number of *R. similis* populations from distant geographical areas. This study included two isolates from Mpumalanga, South Africa, which were found to belong to the *R. similis* pathotype that does not parasitize citrus.

However, as in the case of Van den Bergh *et al.* (2005), *R. similis* damage in South Africa might be similar to what they found of *P. coffeae*, which never increased to dramatic levels due to cool winter and periods of abundant rainfall. South Africa also has a cool, dry winter period, which might be in part responsible for preventing dramatic *R. similis* levels in banana plantations.

Although pathogenicity tests of *Meloidogyne* showed no significant damage to the plants at the inoculation levels used, this does not mean that *Meloidogyne* cannot cause problems on bananas. When root-knot nematodes and other stress factors such as drought and cold occur, it can result in the decrease of plant growth or the development of False Panama disease (Deacon *et al.*, 1985; Rabie, 1991; De Beer *et al.*, 2001).

The survey showed that *R. reniformis* was mostly limited to the Onderberg area. De Villiers *et al.* (1970) did not record *R. reniformis* on bananas. *Paratylenchus minutus* was limited to the South Coast of Kwazulu/Natal, in contrast to a previous study (De Villiers *et al.*, 1970) where *P. minutus* was found in Mpumalanga as well. *Pratylenchus coffeae* and *P. minor* were present in all areas, but in limited numbers.

Differences observed during the survey between cultivars and areas were limited, but 'Chinese Cavendish' seemed to have a tendency to accommodate higher numbers of *Meloidogyne*.

The glasshouse experiments indicated some differences in the response between cultivars to *R. similis* and *M. incognita* and *M. javanica*. 'Grand Nain' and 'Williams' were most sensitive to *R. similis*, whereas 'High Noon' allowed less reproduction of this nematode. *Meloidogyne incognita* and *M.*



*javanica* reproduced less in ‘Grand Nain’ and ‘Williams’ and showed the highest final population in densities ‘Chinese Cavendish’ and ‘High Noon’.

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