View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Stellenbosch University SUNScholar Repository

Differentiation of South African potato cyst nematodes (PCN) by analysis of the rDNA internal transcribed spacer region

R Knoetze[1*], A P Malan[2] & C Mouton[1]

[1]Directorate Plant Health, Department of Agriculture, Private Bag X5015, Stellenbosch, 7599 South Africa
[2]Department of Entomology and Centre for Agricultural Biodiversity, Faculty of Agricultural and Forestry Sciences, University of Stellenbosch, Private Bag X1, Matieland, 7602 South Africa

Knoetze R, Malan A P & Mouton C 2006. Differentiation of South African potato cyst nematodes (PCN) by analysis of the rDNA internal transcribed spacer region. *African Plant Protection* **12**: 103–110.

Cysts from nematode-infested plots on eight potato farms in the Sandveld and Ceres regions of South Africa were analysed by means of rDNA-RFLP. The size of the PCR amplification products for all populations was typical for the genus *Globodera*. Restriction digestion of the amplified products with *Msp*I and *Hinf*I confirmed *Globodera rostochiensis* to be present in both the Sandveld and Ceres regions and *Globodera pallida* to be absent from South African potato cyst nematode populations. However, several populations from the Sandveld region showed no recognition of the *Hinf*I restriction site and no digestion took place, indicating them to be a distinct *Globodera* species. Sequencing of the ITS1 region confirmed the presence of *G. rostochiensis* and an unknown *Globodera* species, and the absence of *G. pallida* in South Africa. Species-specific primers for the identification of *G. rostochiensis* and *G. pallida* were optimised.

Key words: diagnostic, *Globodera rostochiensis,* PCN, PCR, potato cyst nematode, South Africa.

Potato (*Solanum tuberosum* L.) is a major world crop and the potato cyst nematodes (PCN) *Globodera rostochiensis* (Wollenweber, 1923) and *Globodera pallida* (Stone, 1973) are parasites of worldwide significance attacking this crop. These two species coevolved with the potato in South America several hundred thousand years ago (Stone 1979). They are sibling species within the genus *Globodera,* which contains some of the most specialised and successful plant-parasitic nematodes of agricultural crops.

G. rostochiensis was reported for the first time in South Africa in 1971 from an irrigated farm north of Pretoria and subsequently from smallholdings around Johannesburg and Bon Accord. Very strict quarantine measures were imposed to prevent the spread of this nematode to other potato-producing areas. In April 1999, almost 28 years later, it was reported for the first time in the Western Cape from the Ceres area (Knoetze et al. 2004). In both the Plant Improvement Act (Act No. 53 of 1976) and Agricultural Pest Act (Act No. 36 of 1983), *G. rostochiensis* is listed as a prohibited pest. Distribution of PCN by means of seed potatoes is prevented by the South African Seed Potato Certification Scheme of 15 May 1998, where no tolerance for infection is permissible.

Szalanski et al. (1997) compared the first internally transcribed spacer region (ITS1) from cyst nematode species by nucleotide sequencing and PCR-RFLP. The RFLP patterns from this region have been shown to discriminate between different *Globodera* species (Szalanski et al. 1997). The value of rDNA in cyst nematode diagnostics was also indicated in comparative studies of ribosomal sequence variation from PCN and other *Globodera* species (Ferris et al. 1995; Thiery & Mugniery 1996; Subbotin et al. 2000). Bulman & Marshall (1997) described a multiplex PCR-based method, which targeted the ITS1 and enabled the identification of *G. pallida, G. rostochiensis* and mixtures of the two species.

The differentiation of South African populations of PCN on a molecular level could provide further information on the composition of local populations. The presence of *G. pallida* in South Africa, the identity of an unknown *Globodera* from the Sandveld and the design of a reliable diagnostic test are the main areas of concern addressed in this study.

Materials and methods

Surveys for PCN

Through close collaboration between the National Department of Agriculture, the Independent Certification Council for Seed Potatoes and the Agricultural Research Council, the potato-producing areas of South Africa are systematically being sampled for the presence of PCN. Following the resurfacing of *G. rostochiensis* in 1999, all units planted for registered seed potato production from

*Author for correspondence. E-mail: rinusk@nda.agric.za

Table 1. Potato cyst nematode samples used in this study. Farm names have been substituted for reasons of confidentiality.

Farm	Region	Initial morphological identification	Number of cysts
C1	Ceres	*Globodera rostochiensis*	70
C2	Ceres	*G. rostochiensis*	28
S4	Sandveld	*G. rostochiensis* Unknown *Globodera*	12
S5	Sandveld	*G. rostochiensis* Unknown *Globodera*	2
S6	Sandveld	*G. rostochiensis* Unknown *Globodera*	8
S7	Sandveld	*G. rostochiensis* Unknown *Globodera*	4
S11	Sandveld	*G. rostochiensis* Unknown *Globodera*	21
S12	Sandveld	*G. rostochiensis* Unknown *Globodera*	7

1 January 2000 to 31 December 2000 were tested before planting for the presence of PCN. Since 1 January 2001, registered seed potato plantings in the Sandveld and Ceres regions, as well as all plantings intended for export throughout South Africa, are sampled on a compulsory basis. During 2002, potato producers in the Ceres area were also surveyed. In addition to this, all units registered for export were also sampled during harvesting. Up to 2005, a total of 12 000 ha of potato fields was tested.

Origin of cysts

Cysts were obtained from infested plots in the Ceres and Sandveld regions. The cysts with their origins and the number analysed are listed in Table 1. Since *G. pallida* is not known to occur in South Africa, DNA was obtained from L Waeyenberge of the Agricultural Research Centre, Department of Crop Protection, Merelbeke, Belgium.

Preparation of DNA templates

Cysts were cut open and the juveniles removed. Individual juveniles were selected and transferred to a 5 µl drop of 1 × PCR reaction buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.1 Tween-20) containing 60 µg ml^{-1} proteinase K in a sterile PCR tube. The nematode was then cut into small pieces with a sterile scalpel blade. The tube was kept at –80 °C for a minimum of 10 minutes, and then incubated at 60 °C for 15 minutes and a further five minutes at 95 °C.

Polymerase chain reaction

Two PCR amplification primers that amplify the ITS1 region as well as short sections of the 18 S and 5.8 S ribosomal genes were used. The rDNA2 primer (5′-TTGATTACGTCCCTGCCCTTT-3′) has been described by Vrain et al. (1992), and the rDNA1.58S primer (5′-ACGAGCCGAGTGATCC ACCG-3′) was designed by comparative sequence alignments of various nematode species by Szalanski et al. (1997). Primers were synthesised by Genosys Biotechnologies Ltd.

PCR amplifications were carried out in the same tube containing 5 µl of nematode lysate together with 0.5 µM of each primer, dATP, dCTP, dGTP and dTTP, each at 200 µM final concentration, 1 × Taq reaction buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.1 Tween-20), 1.5 mM $MgCl_2$ and 1 U Taq polymerase. The final reaction volume was 25 µl. Amplifications were performed on a Hybaid PCR Sprint thermal cycler. The cycling conditions were as follows: denaturation at 94 °C for 20 seconds, annealing at 57 °C for 30 seconds and extension at 72 °C for 45 seconds, repeated for 25 cycles. A two-minute incubation period at 72 °C followed the last cycle in order to complete any partially synthesised strands.

Restriction fragment length polymorphisms

Ten microlitres of each of the PCR products was digested with 10 U of a restriction enzyme in the appropriate buffer according to the manufacturer's instructions (Promega, Madison, WI) in a total volume of 20 µl. The amplified fragments of

Globodera were digested with *Msp*I and *Hinf*I. The digested DNA was loaded on a 2 % agarose gel, separated by electrophoresis, and visualised by ethidium bromide staining. A 100 bp DNA ladder (Promega, Madison, WI) was used as a size marker.

Morphometric measurements

Permanent mounts of individual cysts collected above were made as described by Turner (1998). Juveniles were fixed in hot (85 °C) FAA and processed to pure glycerine by using the short Seinhorst (1985) method. The following structures were investigated and measured using the drawing tube attached to a research microscope at ×1000 magnification: stylet length, stylet knob shape, cuticular ridges, vulval diameter, distance from anus to vulva, presence/absence of bullae. Granek's ratio (distance from anus to the nearest edge of the vulval basin, divided by the diameter of the vulval basin) was calculated.

DNA sequencing

PCR products of the ITS1 region of the different populations were cleaned up and sequenced by Inqaba Biotechnical Industries (Pty) Ltd, using a Spectrumedix SCE2410 genetic analysis system with 24 capillaries from SpectruMedix LLC, Pennsylvania, USA. BigDye version 3.1 dye terminator cycle sequencing kit from Applied Biosystems was used for the reactions.

Sequence alignment and primer optimisation

Sequences were edited using the Chromas (version 2.3) program (Copyright© 1998–2004 Technelysium Pty Ltd), aligned using the CLUSTALX program with default options (Thompson et al. 1997) and edited using Genedoc (version 2.6.002) (Nicholas & Nicholas 1997). Primers sagU1 (5′-GATTACGTCCCTGCCCTTTG-3′), sagR1 (5′-CAAGCGCAGACATGCCGCAA-3′) and sagP1 (5′-CGACAACAGCAATCGTCGAG-3′) were optimised by extension of the primers designed by Vrain et al. (1992) and Bulman & Marshall (1997) to facilitate melting temperatures that were compatible in a multiplex PCR.

Results

Nematode surveys

G. rostochiensis was found in 35 plots on 19 farms, in total comprising 500 hectares. These plots are situated in the Ceres and Sandveld regions (Knoetze et al. 2004). No *G. pallida* has yet been identified by conventional morphological means in any of these populations.

Amplification

The PCR amplification products of juveniles from all the cysts of the different populations were approximately 750 bp in size, but amplification products of some of the cysts from the Sandveld appeared slightly larger. Fig. 1 shows typical amplification products obtained from the *Globodera* populations.

Restriction fragment length polymorphisms

Restriction digestion with *Hinf*I and *Msp*I of the amplification products of several juveniles from

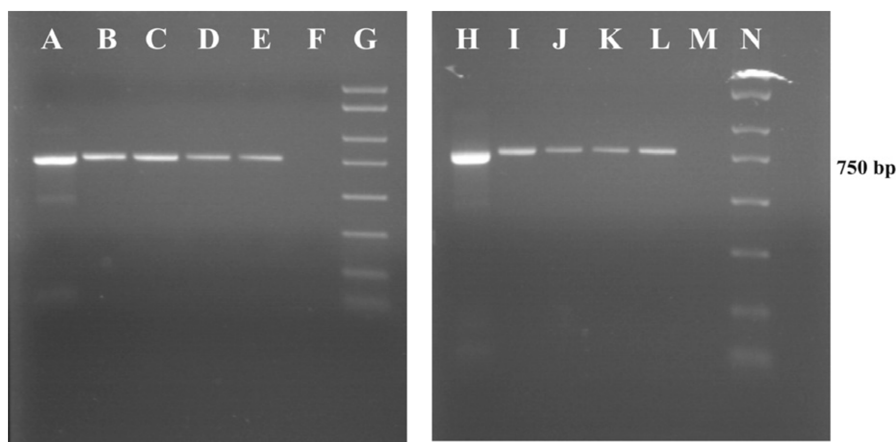

Fig. 1. Amplification products of selected cysts from *Globodera* populations visualised on a 1 % agarose gel. **A**: C1; **B**: S4; **C**: S5; **D**: s12; **E**: S12; **H:** C2; **I**: S6; **J**: S7; **K**: S7; **L**: S11. **F, M** = negative control; **G, N** = PCR marker (Sigma).

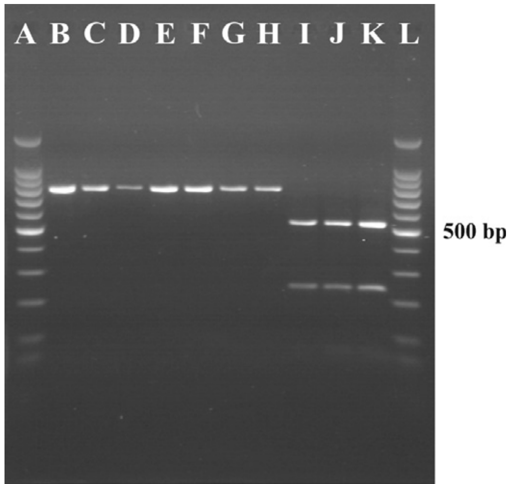

Fig. 2. *Hinf*I digestion products of the amplified ITS1 region of selected cysts from *Globodera* populations separated on a 2 % agarose gel. **B**: uncut PCR product; **C**: S4; **D**: S5; **E**: S6; **F**: S7; **G**: S11; **H**: S12; **I**: C1; **J**: C1; **K**: C2. **A, L** = 100 bp marker (Promega).

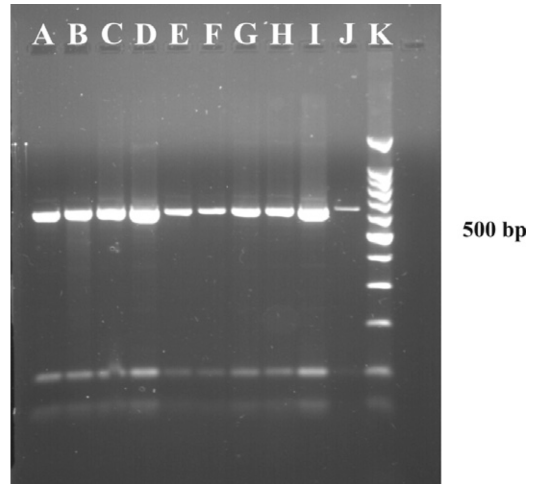

Fig. 3. *Msp*I digestion products of the amplified ITS1 region of selected cysts from *Globodera* populations separated on a 2 % agarose gel. **A**: C1 **B**: C1; **C**: C2; **D**: C2; **E**: S4; **F**: S5; **G**: S6; **H**: S7; **I**: S11; **J**: S12. **K** = 100 bp marker (Promega).

cysts identified morphologically as *G. rostochiensis* produced patterns with fragments of c. 520 bp and 230 bp for *Hinf*I, and c. 620 bp, 100 bp and 40 bp for *Msp*I. For several other juveniles from an unknown cyst, however, there was no *Hinf*I recognition site and no digestion took place, while the *Msp*I RFLP patterns for these juveniles were the same as for *G. rostochiensis.* The different RFLP patterns for the juveniles are shown in Figs 2 and 3.

Table 2 gives the approximate MspI and HinfI restriction fragment sizes present in individuals of the above populations. Results from the different populations were reproducible and consistent for that particular population.

Morphometric measurements

Tables 3 and 4 show the results of the measurements taken from the different populations. Stylet length of larvae from the Sandveld cysts were markedly longer than those from the Ceres area, whereas knob shape was inconclusive. Cuticular ridges, distance from anus to vulva, presence/absence of bullae and Granek's ratio differed markedly between the Ceres and Sandveld populations.

Sequence alignment

Products of 755 bp and 766 bp spanning the ITS1 region as well as short sections of the 18 S and 5.8 S ribosomal genes were amplified from South African populations of *Globodera*. After alignment of sequences from ten individuals from each population, a consensus sequence was derived. These sequences were submitted to GenBank (accession numbers DQ 887561 and DQ 887562). The aligned sequences from two populations are compared to sequences of *G. rostochiensis* (AF0 16878) and *G. pallida* (AF0 16871) obtained from Genbank in Fig. 4. The sequence from population 2 (Ceres) was almost identical to that of *G. rostochiensis,* the only differences being one point-mutation and one deletion in the ITS1 region. The sequence from population

Table 2. Number of cysts with DNA fragment sizes (bp) obtained after endonuclease digestion of ITS1 regions of *Globodera* spp.

Farm	*Hinf*I		*Msp*I
	Fragments 520 + 230	No digestion	Fragments 620 + 100 + 40
C1	45	0	45
C2	28	0	28
S4	1	10	11
S5	1	1	2
S6	7	1	8
S7	0	4	4
S11	6	15	21
S12	0	7	7

Table 3. Measurements of the L2 stylet from different populations of *Globodera* from the Ceres and Sandveld regions.

	Ceres		Sandveld		
Population	C1	C2	S7	S11	S12
Hinf I cut	Yes	Yes	No	No	No
n	49	41	9	32	19
Stylet length (µm)	21.6±1.2 (18.4–24.1)	22.1 ± 1.4 (18.9–25.5)	23.4 ± 1.5 (20.8–25.5)	24.1 ± 0.7 (22.6–25.0)	24.2 ± 1.0 (21.7–25.5)
			Stylet knobs		
n	41	29	7	31	18
Rounded (%)	78	55	71	13	28
Flattened (%)	0	0	29	77	72
Indented (%)	41	45	0	10	0

7 (Sandveld) was not similar to either *G. rostochiensis* or *G. pallida.* When compared to *G. rostochiensis,* the sequence of population 7 contained 38 point-mutations and 12 insertions. When compared to *G. pallida,* it contained 48 point-mutations and 12 insertions. Only one point-mutation was not situated in the ITS1, but in the 18 S gene.

Primer design

Primers sagU1 (5′-GATTACGTCCCTGCCCT TTG-3′), sagR1 (5′-CAAGCGCAGACATGCCG CAA-3′) and sagP1 (5′-CGACAACAGCAATC GTCGAG-3′) were used in a multiplex PCR to test their ability to distinguish between *G. rostochiensis, G. pallida* and the unknown *Globodera* sp. from the Sandveld. An amplification product of 575 bp was obtained when the PCR was performed with DNA from *G. rostochiensis* populations and a 403 bp product was amplified from *G. pallida* DNA (Fig. 5). A 575 bp product was, however, also obtained from populations of the unknown Sandveld cysts on some occasions,

Table 4. Measurements (in µm) of selective characteristics of cysts of different populations of *Globodera* from the Ceres and Sandveld regions.

	Ceres		Sandveld				
Population	C1	C2	S4	S6	S7	S11	S12
Hinf I cut	Yes	Yes	No	No	No	No	No
n	18	9		1		8	4
Cuticular ridges	16 ± 3 (25–12)	16 ± 3 (12–20)		13		12 ± 3 (11–13)	12 ± 1 (11–13)
n	18	18	1	1	2	13	4
Diameter of vulva	19.3 ± 2.8 (15.1–25.5)	20.0 ± 4 (12.7–27.4)	23.1	18.9	19.8 ± 2.7 (19.9–21.7)	19.9 ± 2.1 (17.0–23.6)	17.1 ± 2.2 (14.2–19.3)
n	20	17	1	1		11	4
Anus to vulva	59.1 ± 16.3 (35.9–93.4)	60.2 ± 16.4 (39.6–100)	35.4	33.0		34.7 ± 9.0 (17.9–53.8)	35.1 ± 3.4 (30.2–37.7)
n	20	17	1	1		10	4
Granek's ratio	3.12 ± 1.07 (1.89–5.50)	3.08 ± 0.78 (2.07–5.00)	1.53	1.75		1.74 ± 0.47 (1.06–2.71)	2.08 ± 0.32 (1.78–2.53)
n	29	19	1	1	3	15	4
Bullae present (%)	0	0	100	0	67	73	75
Bullae absent (%)	100	100	0	100	33	27	25

```
Gr:  TTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGGACTGAGCCATTTCGAGAAACTCG  70
G2:  ......................................................................  70
Gp:  ......................................................................  70
G7:  ......................................................................  70
Gr:  GGGACGATTATGCGTGTCGGCTTCGGTCGTCGCGTTGATTGGAACCGATTTAATCGCAGTGGCTTGAACC  140
G2:  ......................................................................  140
Gp:  ......................................................................  140
G7:  .........................................A............................  140
Gr:  GGGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCTGCTGGATCATTACCCAAGTGATACCAATTC  210
G2:  ......................................................................  210
Gp:  ......................................................................  210
G7:  .............................................................-........  209
Gr:  ACCACCTACCTGCTGTCCAGTTGAGTCAGTGTGGGCAACACCACATGCCTCCGTTTGTTGTT-GACGGAC  279
G2:  ..............................................................-.......  279
Gp:  ..............................................................-.......  279
G7:  ....GT................TG..............T...........T...........T.......  279
Gr:  -ACATGCCCGCTGTGTAT---TGGCTGGCACATTGACCAACAAT---GTACGGACAGCGCCCTGTGGGCA  342
G2:  -.................---.......................---.......................  342
Gp:  -...........A...T.---G..............T.......---...T...................  342
G7:  C.T.......T.GG....TTTG....................G.TGT...C...................  349
Gr:  CATGAGTGTTGGGGTGTAACCGATGTTGGTGGCCCTATGGGTGAGCCGACGATTGCTGCTGTCGTCGGGT  412
G2:  ......................................................................  412
Gp:  ...................................A........CT............T...........  412
G7:  ..A.....T........T.T.A..........T..G........GC.........T...CA.........  419
Gr:  CGCTGCGCCAACGGAGGAAGCACGCCCACAGGGCACCCGAACGGCTGTGCTGGCGTCTGTGCGTCGTTGA  482
G2:  ......................................T...............................  482
Gp:  ......A..........TG...................................................  482
G7:  .....TG...........A.T.................................................  489
Gr:  GCGGTTGTTGCGCCTTGCGCAGATATGCTAACATGGAGTGTAGGCTG--CTACTCCATGTTGTACGTGCC  549
G2:  ............................................-..--.....................  548
Gp:  ....................G........G.................--...T.......C.........  549
G7:  ....................T..C.......................TG...C.......T....A.A..  559
Gr:  GTACCTTGCGGCATGTCTGCGCTTGTGTGCTACGTCCGTGGCCGTGATGAGACGACGTGTTAGGACCCGT  619
G2:  ......................................................................  618
Gp:  .....CA...............................................................  619
G7:  .....T...........G....................................................  629
Gr:  GCCTGGCATTGGCACGTGGTTTAAGACTTGATGAGTGCCCGCAGGCACCGCCAGC-TTTTTCCCATTTTT  688
G2:  .......................................................-..............  687
Gp:  ................C......................T...............-......T.......  688
G7:  ..............T........................C...............G..............  699
Gr:  ATTTATTTTTT-AATGCAATTCGATTGCTAAAATATTCTAGTCTTATCGGTGGATCACTCGGCTCGT  754
G2:  ...........-.......................................................  753
Gp:  ..AA.......-..GT.....T...GT........................................  754
G7:  ..T.T......CC.TG.....C...TG........................................  766
```

Fig. 4. Alignment of the ribosomal internal transcribed spacer (ITS1) from *Globodera rostochiensis* (Gr) and *Globodera pallida* (Gp) with the sequences from two South African species (G2, G7). Sequence differences are shown, (.) represents identical base and (-) represents deletions. Estimated positions of the 18 S and 5.8 S genes are indicated by shading. Primer sequences are underlined.

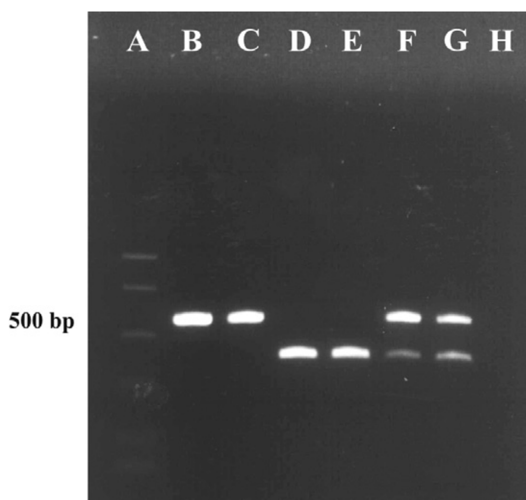

Fig. 5. Amplification products of *Globodera rostochiensis* (**B, C**), *Globodera pallida* (**D, E**) and a mixture of *G. rostochiensis* and *G. pallida* (**F, G**) with primers sagU1, sagR1 and sagP1 in a multiplex PCR. **H** = negative control; **A** = PCR marker (Sigma)

indicating a possible false-positive reaction of the *G. rostochiensis*-specific primer with these cysts.

Discussion

The size of amplification products obtained from all juveniles (c. 750 bp) from the different populations was the same as those reported in literature (Szalanski et al. 1997). The comparison of restriction patterns derived from the amplified ITS1 region, as well as morphological identification, showed that two different species of *Globodera* exist in some of the samples used in this study. Only *G. rostochiensis* was found in samples from the Ceres area. This was confirmed by morphological as well as molecular identification. The area from which the population originates is in a warmer temperate zone that experiences light frost. It is a winter-rainfall region, receiving 400–500 mm of rain per year. *G. rostochiensis* was also found in some samples from the Sandveld area. The area is also in a warmer temperate zone, with very light frost. It is also a winter-rainfall region, receiving about 250 mm of rain per year. The soil is almost 100 % sand. The number of cysts found in the Sandveld region varied between 1 and 15 per 100 cm^3 soil. No damage was reported from this area. This could be because the populations from the Sandveld were very low compared to those of Ceres. The low populations could be attributed to the high soil temperatures in this region. The most notable morphological differences between the *G. rostochiensis* from the Ceres and the Sandveld regions were the presence of bullae in the vulval region. No molecular differences could, however, be found between the populations by PCR-RFLP alone, which suggests that the morphological differences might be caused by environmental factors. A *Globodera* sp. morphologically remarkably close to *Globodera achilleae* (Golden & Klindic, 1973) and *Globodera millefoli* (Kirjanova & Krall, 1965) was also found in potato fields in the Sandveld area. PCR-RFLP confirmed the presence of a different *Globodera* species in the Sandveld with no restriction site for *Hinf* I in the amplification product, which distinguishes it from *G. rostochiensis, G. pallida* and *Globodera tabacum* (Lownsbery & Lownsberry, 1954). Morphological identification of the cysts that exhibited these restriction patterns showed that they were indeed those of a species close to *G. achilleae.* Sequence analysis and comparison to published sequences of the ITS1 region provided further evidence that the Sandveld species is different from *G. rostochiensis* (GenBank accession number AF0 16878), *G. pallida* (GenBank accession number AF0 16871) and *G. tabacum* (GenBank accession number AF 339502). The sequence also differed from a partial sequence of *G. achilleae* obtained from Genbank (accession number AY 599498), although it was morphologically similar to it. The comparison of the ITS1 from the different populations by PCR-RFLP and sequencing confirmed the presence of *G. rostochiensis* in both the Sandveld and Ceres regions in South Africa and a different species of *Globodera* in the Sandveld region. It also confirmed *G. pallida* not yet to be present in South Africa. Further morphological studies and phylogenetic analysis of the sequence data of the Sandveld cysts need to be undertaken in order to characterise the species properly. A comparison of sequence data from populations of *G. rostochiensis* from the Ceres and Sandveld regions also needs to be done to investigate the morphological differences between the populations. The development of primers in the ITS1 region that are specific to *G. rostochiensis* and *G. pallida* was successful, even though the primer that distinguishes *G. rostochiensis* from *G. pallida* seemed to give a false-positive result with the unknown

Sandveld cyst. This means that positive results for *G. rostochiensis* must be verified by restriction enzyme digest. Sequence analysis of the amplified fragments for *G. rostochiensis* and the unknown Sandveld cyst indicated that digesting the fragment with *Hinf* I would result in two fragments of 341 bp and 234 bp, while no digestion will occur in the fragment obtained from the unknown Sandveld cyst. This final step enables the specific primers to be used in a diagnostic test.

References

Bulman S R & Marshall J W 1997. Differentiation of Australasian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR). *New Zealand Journal of Crop and Horticultural Science* **25**: 123–129.

Ferris V R, Miller L I, Faghihi J & Ferris J M 1995. Ribosomal DNA comparisons of *Globodera* from two continents. *Journal of Nematology* **27**: 273–283.

Knoetze R, Malan A P, Swart A & Pieterse W 2004. Present status of potato cyst nematode in South Africa (Abst.). *African Plant Protection* **10**: 129.

Nicholas K B & Nicholas H B Jr. 1997. GeneDoc: analysis and visualization of genetic variation. Online: http://www.cris.com/~ketchup/genedoc.shtml

Seinhorst J W 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* **4**: 67–69.

Stone A R 1979. Co-evolution of nematodes and plants. *Symbolae Botanicae Uppsala* **22**: 46–61

Subbotin S A, Halford P D, Warry A & Perry R N 2000. Variations in ribosomal DNA sequences and phylogeny of *Globodera* parasitising solanaceous plants. *Nematology* **2**: 591–604.

Szalanski A L, Sui D D, Harris T S & Powers T O 1997. Identification of cyst nematodes of agronomic and regulatory concern with PCR-RFLP of ITS1. *Journal of Nematology* **29**: 255–267.

Thiery M & Mugniery D 1996. Interspecific rDNA restriction fragment length polymorphism in *Globodera* species, parasites of solanaceous plants. *Fundamental and Applied Nematology* **19**: 471–479.

Thompson J D, Gibson T J, Plewniak F, Jeanmougin F & Higgins D G 1997. The CLUSTALX windows interface; flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876–4882.

Turner S J 1998. Sample preparation, soil extraction and laboratory facilities for the detection of potato cyst nematodes. In: *Potato cyst nematodes. Biology, distribution and control,* 75–90 (Eds R J Marks & B B Brodie). CAB International, Wallingford.

Vrain T C, Wakarchuck D A, Lèvesque A C & Hamilton R I 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15**: 563–573.

Accepted 10 October 2006
Associated Editor was E van den Berg