Pest Manag Sci 57:1068–1074 (online: 2001)
DOI: 10.1002/ps.397

Evaluation of PCR, IEF and ELISA techniques for the detection and identification of potato cyst nematodes from field soil samples in England and Wales

Said K Ibrahim,[1]* Stephen T Minnis,[1] Anthony DP Barker,[2] Mike D Russell,[2] Patrick PJ Haydock,[1] Kenneth Evans,[2] Ivan G Grove,[1] Simon R Woods[1] and Andrew Wilcox[1]

[1]*Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire TF10 8NB, UK*
[2]*IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK*

Abstract: Effective management of potato cyst nematodes (PCNs) requires simple, rapid and accurate identification and quantification of field populations. Soil samples from a survey of 484 fields in potato rotations in England and Wales were used to compare the identification and quantification of PCNs using IEF, PCR, ELISA and bait plant tests. The cyst counts and bait plant test revealed that 64.3% of field samples contained PCNs. Bait plant tests increased the detection rate of PCNs in field samples by 4–6.4%. This means that some infestations are cryptic and would not normally be detected by standard counts. IEF, PCR and ELISA methods distinguished between *Globodera rostochiensis* and *G pallida* and were able to register mixed populations; however they were not in full agreement. All methods suggested that *G pallida* is the dominant species in the field samples tested. The PCR results indicated that 66% of field samples contained pure *G pallida*, 8% contained pure *G rostochiensis* and 26% contained mixtures of the two species. Estimates of the relative process times taken per sample in the PCR, IEF and ELISA techniques are given.

Keywords: bait test; cyst nematodes; DNA; ELISA; *Globodera pallida*; *Globodera rostochiensis*; IEF; PCN; PCR; rITS

1 INTRODUCTION

The potato cyst nematodes (PCNs) *Globodera pallida* (Stone) Behrens and *G rostochiensis* (Wollenw) Behrens are serious pests in potato-growing areas of the UK.[1] *G pallida* is difficult to control because there are no fully resistant potato cultivars, and non-fumigant nematicides are less effective against this species than against *G rostochiensis*. This is probably due to their relatively short persistence in the soil and the prolonged period of emergence of *G pallida* juveniles.[2] PCNs are quarantine pests within the European Union and, in seed production areas, legislation imposes a minimum 10-year ban on the production of seed potatoes if a single cyst of a PCN is found in a field.[3] As the genetic resistance in host plants available to combat PCNs can be species-specific, correct identification is a crucial part of a successful integrated management programme.

Several procedures, using widely differing technologies, are available for routine use in the identification of PCN populations. These include the traditional approach of using morphological features,[4,5] two-dimensional electrophoretic analysis of protein profiles,[6] analysis of protein patterns by isoelectric focusing,[7,8] ELISA,[9–11] polymerase chain reaction (PCR)-based techniques,[12–15] randomly amplified polymorphic DNA (RAPD)-PCR markers,[16,17] restriction fragment length polymorphisms (RFLPs) and hybridisation techniques.[16,18] Each of these methods has problems for use in routine identification[13] and none of them has been fully tested and validated against the range of field populations found in the UK, as most of the work has been done only on known species from glasshouse cultures. The PCR technique offers the prospect of a simple, rapid and reliable diagnostic tool for plant parasitic nematodes that will

* Correspondence to: Said K Ibrahim, Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire TF10 8NB, UK
E-mail: sibrahim@harper-adams.ac.uk
Contract/grant sponsor: Dow AgroSciences
Contract/grant sponsor: Ministry of Agriculture, Fisheries and Food
Contract/grant sponsor: British Potato Council
Contract/grant sponsor: Zeneca
Contract/grant sponsor: Biotechnology and Biological Science Research Council of the UK
(*Received 14 March 2001; revised version received 1 June 2001; accepted 13 July 2001*)

enable advisers to identify and quantify populations of PCN from field samples,[16,19,20] as do ELISA-based procedures.[21]

The first unbiased survey of *G pallida* and *G rostochiensis* populations in England and Wales was carried out at Harper Adams University College in 1998/1999 by Minnis *et al.* [22] The survey provided a total of 484 soil samples taken from fields that had grown potatoes in the previous year. The objectives of the present study were to compare results for the detection, quantification and identification of PCN populations using standard counting, PCR, IEF, ELISA and bait plant techniques on a sub-set of the survey samples.

2 MATERIALS AND METHODS

2.1 Sample collection

Soil samples were collected from 484 sites throughout England and Wales that had been identified with the assistance of the British Potato Council. At sites 1–220, 50 cores (2.5 cm × 20 cm) were taken from an area of 4 ha in a grid pattern and bulked together to give approximately 2 kg of soil. These were kept at Harper Adams University College for PCN extraction. Two soil samples (2 kg each) were taken from sites 221–484. The first was put in a cotton bag and sent to Harper Adams University College for cyst extraction and processing in the IEF and PCR studies. The second was put in a plastic bag and sent to IACR-Rothamsted for bait plant testing and ELISA studies. A further 83 sub-samples, taken from those samples from batches 1–220 in which no cysts were found, were sent to Rothamsted for a bait plant test.

2.2 Standard PCN quantification

Soil samples were air-dried and sieved through a 4-mm sieve, thoroughly mixed, and a 200-g sub-sample taken from each for cyst extraction using a Fenwick can.[23] The numbers of PCN cysts present in each extract were counted under a stereomicroscope and, where possible, 50 cysts (all cysts if there were less than 50) were removed for egg counts. The cysts were soaked in water overnight before crushing to release the eggs. From the resulting suspensions the eggs in 1-ml aliquots were counted. A population density in eggs per gram of dried field soil was calculated for each sample in which PCNs were found.

2.3 Bait plant tests

Soils were mixed with 25% by weight of peat-based compost to prevent compaction in the pots, and 400 g of each modified soil (347 samples in all) were added to a 10-cm diameter plastic pot in which a single sprouted tuber plug of potato *Solanum tuberosum* L cv Désirée was planted. The plants were grown for 12 weeks, after which time any new cysts that had formed were extracted by Fenwick can.

2.4 DNA Extraction

DNA was extracted as described by Ibrahim *et al.* [20] The suspensions of crushed cysts remaining from the egg counts were used as a source of DNA template for the PCR reactions. The remaining suspension was centrifuged at 500 rev min^{-1} for 5 min, after which the pellet was re-suspended in water in a 1.5-ml micro-centrifuge tube before centrifuging at 12 000 rev min^{-1} for 5 min. The excess water was removed and lysis buffer [Tris (10 mM, pH 8.0), EDTA (1 mM), Nonidet P-40 (10 ml $litre^{-1}$), proteinase K (100 μg ml^{-1}); 20 μl] was added. The pellet was homogenised with a micro-homogeniser (Biomedix, UK) and the extract incubated at 95 °C for 5 min. The crude DNA extracts prepared in this way were suitable for PCR amplification without further treatment.

2.5 PCR Amplification

Two different sets of PCR primers were used in this study. PCR amplification and the first set of primers used were as described by Bulman and Marshall.[14] The G *rostochiensis* primer (5′-AGC GCA GAC ATG CCG CAA-3′) (PITSr3) and the *G pallida* primer (5′-ACA ACA GCA ATC GTC GAG-3′) (PITSP4) binds to the ITS1 region. They were used in combination with a universal primer (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) that binds to the 18S region and is called the ITS5 primer by White *et al.* [24] The second set of primers[13] is designed to bind to the ITS1 region and the 5.8S rRNA gene. The *G rostochiensis*-specific primer was 5′-TGT TGT ACG TGC CGT ACC TT-3′, the *G pallida*-specific primer 5′-GGT GAC TCG ACG ATT GCT GT-3′ which bind to the ITS1 region, and the universal primer was 5′-GCA GTT GGC TAG CGA TCT TC-3′ located in the 5.8S gene. Each sample was run at least three times with each set of primers. The methodology for the second set of primers was as described by Mulholland *et al*,[13] except that Taq polymerase (Thermostable DNA; Advanced Biotechnologies) was used.

2.6 Agarose gel electrophoresis

PCR products were separated on 1.2% agarose gel buffered in 1X TEB, containing 0.02 μg ml^{-1} ethidium bromide. DNA was visualised under UV light and records made with a digital camera attached to a Gel Doc-1000 box (Bio-Rad Ltd).

2.7 Protein extraction and IEF gel electrophoresis

Where sufficient cysts were available in a sample, 50 were taken and soaked in distilled water overnight. Where fewer than 50 cysts were available, as many as possible were taken (between 15 and 50). The cysts of each sample were transferred to aqueous glycerol (10 ml $litre^{-1}$; 100 μl) kept on ice and homogenised using a Biomedix homogeniser, and then centrifuged at 14 000 rev min^{-1} for 10 min at 4 °C. Proteins in the samples were separated using an LKB Multiphor isoelectric focusing (IEF) gel system with ampholine

pH range of 4 to 6 using the methods reported by Ibrahim and Rowe.[8]

Cathode and anode electrode strips were soaked in sodium hydroxide (1.0 M) and phosphoric acid (1.0 M), respectively. The gel was pre-focused for 30 min with 800 V at 4 °C, followed by electrophoresis for 2 h at 2000 V. Protein bands on the gels were stained with Coomassie Brilliant Blue R250 (5 g litre^{-1}) in ethanol + acetic acid + water (25 + 10 + 65 by volume) at 60 °C for 30 min. Coomassie-stained gels were subsequently destained with several changes of ethanol + acetic acid + water (50 + 7 + 43 by volume). Since silver staining techniques are up to 100-fold more sensitive than those using Coomassie Blue, gels that stained only faintly with Coomassie Blue were re-stained with silver, using the procedure described by Oakley *et al.*[25] Samples of standard, known populations of both *G pallida* and *G rostochiensis* cysts were included on the gel to help in identification of the species specific bands that occur with isoelectric points at pH 5.7 and 5.9 respectively. Each sample was run at least twice to confirm the reproducibility of the banding patterns.

2.8 Enzyme-linked immunosorbent assay (ELISA)

Twelve cysts were taken from each population. Where insufficient cysts were available from the first counts of cysts in samples, further cysts were extracted from any remaining soil. Where this was still insufficient, cysts were taken from the bait plant test. If fewer than 12 cysts were available, the ELISA identifications were not performed. Each cyst from each sample was homogenised individually in phosphate buffered saline (PBS; 10 µl) in 96-well culture plates using a micropipette tip as a homogeniser. The wells and pipette tips were washed with PBS (200 µl), and the resulting solution was divided between two wells of an ELISA microtitre plate, each of which was then serially diluted (×2) three times. Plates were left overnight at room temperature and then washed three times with PBST [Tween (0.5 ml litre^{-1}) in PBS] and probed with each of the two PCN species-specific monoclonal antibodies in PBSTM [Tween (0.5 ml litre^{-1}), + dried skimmed milk (5 g litre^{-1}) in PBS]. The secondary antibody used was rabbit anti-rat IgG conjugated with horseradish peroxidase. Following detection with substrate, the optical densities (OD) of the samples were read on a Titertek ELISA plate reader. Any cyst producing an OD of less than 50 was deemed unclassifiable; when ODs were between 50 and 100, a difference of greater than 20 OD units was required between antibodies before classification as one species or the other, when ODs were over 100, a difference of greater than 30 OD units was required between antibodies before classification as one species or the other. The OD readings of the individual cysts varied largely as a consequence of their differing egg contents, and the average OD per unequivocally classified cyst was calculated for each species in each sample. The ODs of these unequivocally classified cysts were also summed for each species in each sample.

2.9 Statistical analysis

To determine statistical differences between nematode sampling techniques for the generated binary response data (eg nematodes (cysts) are either present or absent), data were analysed by 'Statistica'[26] using a generalised linear model, assuming Poisson errors and a log-link function. This type of analysis is appropriate when there are no continuous explanatory variables associated with binary responses and the explanatory variables are factors.[27] Statistical calculations are based on maximum likelihood estimation and produce the Wald statistic, which is then tested against the chi-squared (χ^2) distribution for significance ($P < 0.05$).

3 RESULTS

The results of standard counts and bait plant tests are presented in Table 1. The counts revealed that 269 out of 484 (55.6%) of the field samples tested contained PCN cysts, but that cysts extracted from only 237 out of 484 (49%) of the samples contained live eggs. Similar results were obtained from the sub-set of samples (347) counted at both Harper Adams and Rothamsted, in which 38% and 36.3%, respectively, of samples contained cysts. However, this difference was not significant. Significant differences in the proportion of cysts containing eggs were observed at the two laboratories; 25.9% of cysts were found to

Table 1. Detection of potato cyst nematodes, *Globodera* spp, by direct extraction (using Fenwick can) of field soil or field soil in which bait plants had been grown. Numbers in parenthesis are 95% confidence limits

Method	*Number of samples tested*	*Number of samples containing cysts*	*% of samples containing cysts*	*Number of samples containing eggs*	*% of samples containing eggs*
Fenwick HAUC[a]	484	269	55.6	237	49.0
Fenwick HAUC[b]	347	132	38.0 (±5.1)	119	34.3 (±5.0)
Fenwick IACR[b]	347	126	36.3 (±5.1)	90	25.9 (±4.6)
Bait test	347	148	42.7 (±5.2)	128	36.9 (±5.1)
Wald χ^2 (2*df*)			1.90		6.96*
All methods	484	311	64.3	263	54.3

[a] Total samples tested at Harper Adams University College only.

[b] Sub-set of the total samples tested at both HAUC and IACR-Rothamsted.

* $P < 0.05$.

Table 2. Identification of potato cyst nematodes using different PCR primers, IEF and ELISA. Numbers in parenthesis are 95% confidence limits[a,b]

Method	*Number of samples tested*	*Samples containing solely Pa (%)*	*Samples containing solely Ro (%)*	*Samples containing Pa+Ro (%)*	*Samples with no species detected (%)*
PCR					
Bulman and Marshall[14]	269	53 (±6.0)	5 (±2.7)	17 (±4.5)	25 (±5.2)
Mulholland *et al*[13]	269	43 (±6.0)	10 (±4.0)	9 (±3.4)	38 (±5.9)
Both PCR methods	269	64 (±6.0)	7 (±3.1)	25 (±5.2)	4 (±2.3)
IEF	83	83 (±8.2)	12 (±7.2)	5 (±3.7)	0
ELISA	93	19 (±8.2)	19 (±8.2)	61 (±10.0)	0
Wald χ^2		42.33*** (4*df*)	16.42** (4*df*)	91.31*** (4*df*)	50.99*** (2*df*)

[a] Pa: *Globodera pallida*, Ro: *G rostochiensis*, Pa+Ro: *G pallida*+*G rostochiensis*.
[b] $^{**}P<0.01$, $^{***}P<0.001$.

contain eggs at Rothamsted, whilst eggs were found in 34.3% of cysts at Harper Adams. The bait plant tests revealed cysts in 148 samples, compared with the 126 that were positive from the first field soil count, but this difference was not significant. The bait test significantly improved detection of eggs when compared to the Rothamsted sub-sample set, but was almost directly comparable to the Harper Adams sub-sample. A combination of bait plant tests with the counts made directly on field soils increased the overall detection of PCNs in the field samples to 64.3% from the 55.6% found in the direct counts (Table 1).

The results of PCR, IEF and ELISA identification are summarised in Table 2. Simultaneous comparison of the identification tests showed that there were significant differences in detection levels for all the parameters measured. Both sets of PCR primers distinguished the two species of PCN and both identified some populations as mixtures. The combined results from the two sets of primers suggested that 64% of the samples tested contained pure *G pallida*, 7% pure *G rostochiensis*, 25% a mixture of the two species and 4% were not identified by both sets of primers. The Bulman and Marshall[14] primers alone revealed that 53% of the samples tested contained pure *G pallida*, 5% pure *G rostochiensis* and 17% a mixture of the two, whereas the Mulholland *et al*[13] primers registered 43% as pure *G pallida*, 10% as pure *G rostochiensis* and 9% as mixtures of the two species. Both sets of primers failed to amplify some of the field samples containing PCNs (Fig 1). The Bulman and Marshall[14] primers generated products in 75% of the 269 samples tested, and the Mulholland *et al*[13] primers in 62%. The two sets of primers were not always in full agreement for species identification, with, occasionally, one species identified by one set of primers but by not the other set (Fig 1). The lengths of the amplification products were 434 bp for the *G rostochiensis* fragment and 265 bp for the *G pallida* fragment using Bulman and Marshall[14] primers (Fig 1(A)), and 238 bp and 391 bp, respectively, using Mulholland *et al*[13] primers (Fig 1(B)). Samples of other cyst nematodes (*Heterodera* spp) and fungal fruiting bodies (both of which may be confused in appearance with PCN cysts) found in the field samples were also evaluated with the two sets of primers and no amplification products were observed (results not shown). IEF and ELISA tests could only be used on 83 and 93 samples respectively, because insufficient cysts were available. Protein banding patterns of all the populations examined were highly reproducible (results not shown). IEF suggested that 83% of the samples tested contained solely *G pallida*, 12% contained solely *G rostochiensis*, and 5% contained a

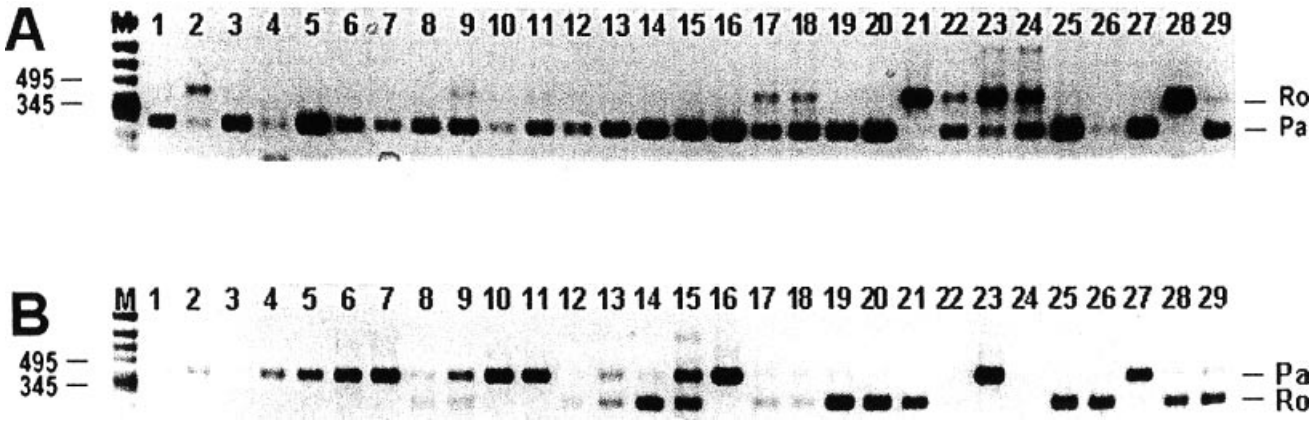

Figure 1. PCR differentiation of the potato cyst nematodes (PCNs) *Globodera rostochiensis* (Ro) and *G pallida* (Pa), using selected soil samples and two sets of primers: (A) PCR primers of Bulman and Marshall,[14] (B) PCR primers of Mulholland *et al.*[13] Lanes 1–29 from soil samples that contained PCNs, M: molecular marker (ΦX174 DNA Hinc II).

Table 3. Identification of potato cyst nematodes in 30 samples using different PCR primers, IEF and ELISA. Numbers in parenthesis are 95% confidence limits[a,b]

Method	*Number of samples tested*	*Samples containing solely Pa (%)*	*Samples containing solely Ro (%)*	*Samples containing Pa+Ro (%)*	*Samples with no species detected (%)*
PCR					
Bulman and Marshall[14]	30	47 (±18.9)	3 (±7.0)	10 (±11.0)	40 (±18.7)
Mulholland *et al*[13]	30	43 (±18.8)	17 (±14.1)	7 (±10.0)	33 (±17.9)
Both PCR	30	73 (±16.8)	7 (±9.4)	20 (±15)	0
IEF	30	80 (±15.2)	20 (±15.1)	0	0
ELISA	30	17 (±14.1)	17 (±14.3)	67 (±17.9)	0
Wald χ^2		13.23* (4*df*)	4.18 (4*df*)	20.20** (3*df*)	0.18 (1*df*)

[a] Pa: *Globodera pallida*, Ro: *G rostochiensis*, Pa+Ro: *G pallida*+*G rostochiensis*.
[b] $*P<0.05$, $**P<0.01$.

mixture of the two species. ELISA was performed on 93 samples but it was impossible to decide whether a cyst was one species or the other in many cases. From the cysts that were unequivocally identified, 61% of the samples appeared to contain mixtures of the two species, 19% to contain solely *G pallida*, and 19% to contain solely *G rostochiensis*. However, there were many cysts that could not be unequivocally ascribed to either species and only about one in three populations appeared to be either pure *G pallida*, or pure *G rostochiensis*.

Thirty samples from the survey were tested by all methods (Table 3) and again there were significant differences between methods of identification. The results from the two sets of PCR primers taken together were in moderate agreement with the results from IEF for the identification of *G pallida* but disagreed for *G rostochiensis* identification, whereas results from PCR using the Mulholland *et al*[13] primers, IEF and ELISA were in almost full agreement for the identification of *G rostochiensis*. IEF did not identify any population as a mixture of the two species. However, both PCR and ELISA registered populations as mixtures of the two species, but disagreed greatly on the number of mixed populations (20% and 67% respectively).

Table 4 shows the estimated time and costs per sample in PCR, IEF and ELISA methods for the identification of potato cyst nematodes. PCR requires more preparation time per sample than ELISA (17 vs 8 min, respectively), but the PCR process required less waiting time. The cost of reagents per sample for each method ranged from £0.20 (not including the cost of antibodies) for ELISA to £2.00 for IEF.

4 DISCUSSION

The main purpose of this study was to compare IEF, ELISA and PCR-based techniques when used for determination of species of PCNs in field samples, rather than PCNs grown under controlled conditions with new cysts full of new eggs. This means that some of the cysts were of very poor quality and difficult to identity. All the methods were laborious, with no significant advantage of any one noted over any of the others. All of them appeared to distinguish the two species of PCN and all were also able to register population mixtures, but they frequently were not in full agreement. A greater number of positive results was obtained with PCR than with any other method, indicating the greater sensitivity of this method. All 269 samples that were found to contain PCNs were able to be processed by PCR, while sufficient cysts were available in only 83 and 93 samples for processing by IEF and ELISA respectively. The IEF and PCR results suggested that *G pallida* (incidences of 66% and 80% respectively) is the dominant species, with only 12% and 8%, respectively, revealed to be *G rostochiensis*. This may be due to the frequent use of resistant cultivars against *G rostochiensis* for PCN management. Only partially resistant cultivars are available against *G pallida*. When the same samples were tested by all methods, some agreement was noted on the identification of pure samples of *G rostochiensis*, but ELISA failed to agree with other methods on the identification of pure and mixed samples of *G pallida*. This was due to partial cross-reactivity of the two antibodies used. With more specific antibodies, particularly against *G pallida*, the ELISA method will be able to discriminate and quantify the species in a sample in a single processing step. Furthermore, it has the potential to discriminate live from dead eggs.

The disagreement between the results obtained in this study may be explained by differences in the principles of operation and the sensitivity of each of the methods. The sensitivity of PCR is much greater than that of isoelectric focusing, with the DNA from single eggs or juveniles sufficient for identification. Protein isoelectric focusing is very useful for PCN identification, but requires large numbers of viable eggs. Cysts having eggs of low viability often contain insufficient protein for diagnosis.[28] The recent development of multiplex PCR tests may offer the way forward for nematode identification. Multiplex PCR tests have also been used to identify other nematodes such as *Heterodera*[28] and *Meloidogyne* species.[29]

Both sets of PCR primers failed to yield amplification products from some field samples known to contain eggs. The same set of primers[13] also failed previously to detect PCN DNA from field samples known to contain live eggs.[30] Several factors may affect the amplification of DNA in PCR. These include the

Methods/process	Preparation time (mins)	Total running time (mins)	Reagent cost per sample (£)
PCR			
Reagents preparation	1	10	0.35
DNA extraction	7	70	
PCR tube loading	3	30	
DNA amplification	—	120	
Gel—all stages	6	30	
Total	17	260	
IEF			
Reagents preparation	3	30	2.00
Protein extraction	5	10	
Centrifugation	—	10	
Gel set-up	5	30	
Sample loading	1	120	
Gel staining	1	30	
Gel destaining	1	120	
Total	16	350	
ELISA			
Reagents preparation	1	10	0.20 (+ Mabs)
Protein extraction	3	30	
Sample dilution	0.5	5	
(Incubation)	—	(overnight or 8h)	
Wash × 3	0.5	5	
Load AB 1	0.5	5	
(Incubation)	—	(120)	
Wash × 3	0.5	5	
Load AB 2	0.5	5	
(Incubation)	—	(60)	
Wash × 3	0.5	5	
Add developer	0.5	5	
(Incubation)	—	(10)	
Read	0.5	5	
Total	8	90 (plus 11h incubation)	

Table 4. Estimated cost and time involved per sample in PCR, IEF and ELISA techniques for batches of 10 samples

inhibition of DNA amplification at high concentrations of template DNA (Ibrahim SK, unpublished data), heterogeneity in the ITS sites,[14] genetic differences within the species G *pallida*,[31] genomic variation,[32,33] or the presence of different pathotypes in the same sample.[34,35] The sensitivity and specificity of PCR and ELISA make them obvious choices for determination and quantification of PCN species in field populations.

The standard cyst counts at Harper Adams revealed that 55.6% of the field samples tested contained PCNs, but only 49.8% contained viable eggs. The second standard cyst count and bait plant tests at Rothamsted increased the detection of PCNs by 8.7% to 64.3%. These results strongly suggest that many PCN infestations are cryptic, due to inadequacies of conventional sampling and extraction methods, and detection can only be improved if testing beyond the routine counts is carried out. It has been estimated that the incidence of PCNs in ware-growing areas ranged from 22% in Scotland,[30] through 26% in Northern Ireland[36] to 67% in England and Wales.[37] In our study, 64.3% of the fields tested contained PCNs.

All of the methods were laborious, but all were, of course, much faster than a bait plant test. However, PCR and IEF results can be obtained in the same day, whereas ELISA results are only obtained the next day.

The discrepancy between the PCR results from using different sets of primers requires further study. Only when this problem has been resolved and a quantitative procedure devised will PCR become an important diagnostic tool. It is certainly sufficiently sensitive to be important, but, as yet, identifications based on PCR have to be treated with caution. The promise of the ELISA-based system may be increased if better, more specific antibodies are found, perhaps by exploiting the phage display system. This would eliminate some of the uncertainties over cyst identification and thereby increase sensitivity. Also, the potential for automation is enormous and a phage display library targeted at plant parasitic nematodes would allow antibodies that recognise other common soil-dwelling nematodes to be found.

ACKNOWLEDGEMENTS

The authors acknowledge support from Dow Agro-

Sciences, the Ministry of Agriculture, Fisheries and Food, British Potato Council and Zeneca. IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

REFERENCES

1 Haydock PPJ and Evans K, Management of potato cyst nematodes in the UK: an integrated approach? *Outlook on Agriculture* **27**:253–260 (1998).

2 Whitehead AG, Nichols AJF and Senior JC, The control of potato pale cyst-nematode (*Globodera pallida*) by chemical and cultural methods in different soils. *J Agric Sci, Cambridge* **123**:207–218 (1994).

3 Anon, Quarantine procedure No 30, *Globodera pallida* and *G rostochiensis*, Soil sampling methods. *EPPO Bulletin* **21**:233–240 (1991).

4 Stone AR, Co-evolution of potato cyst nematodes and their hosts: implications for pathotypes and resistance. *EPPO Bulletin* **15**:131–137 (1985).

5 Golden AM, Morphology and identification of cyst nematodes, in *Cyst Nematodes*, ed by Lamberti F and Taylor CE, pp 23–45 (1986).

6 Bakker J and Gommers FJ, Differentiation of potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida* and two *Globodera rostochiensis* pathotypes by means of two-dimensional electrophoresis. *Proc Koninklijke Nederlandse Akademie van Wetenschappen C* **85**:309–314 (1982).

7 Fleming CC and Marks RJ, A method for the quantitative estimation of *Globodera rostochiensis* and *Globodera pallida* in mixed-species samples. *Records of Agricultural Research of the Department of Agriculture for Northern Ireland* **30**:67–70 (1982).

8 Ibrahim SK and Rowe JA, Use of isoelectric focusing and polyacrylamide gel electrophoresis of non-specific esterase phenotypes for the identification of cyst nematodes *Heterodera* species. *Fund Appl Nemat* **18**:189–196 (1995).

9 Fox PC and Atkinson HJ, Immunochemical studies on pathotypes of the potato cyst nematodes, *Globodera rostochiensis* and *G pallida*. *Parasitology* **90**:471–483 (1985).

10 Schots A, Hermsen T, Schouten S, Gommers FJ and Egberts E, Serological differentiation of the potato cyst nematodes *Globodera pallida* and *G rostochiensis*. II. Preparation and characterisation of species specific monclonal antibodies. *Hybridoma* **8**:401–413 (1989).

11 Curtis RHC, Dunn J, Yeung M, Robinson MP, Martins F and Evans K, Serological identification and quantification of potato cyst nematodes from clean cysts and processed soil samples. *Ann Appl Biol* **133**:65–79 (1998).

12 Ibrahim SK, Perry RN, Burrows PR and Hooper DJ, Differentiation of species and populations of *Aphelenchoides* and of *Ditylenchus angustus* using a fragment of ribosomal DNA. *J Nematol* **26**:412–421 (1994).

13 Mulholland V, Carde L, O'Donnel KJ, Fleming CC and Powers TO, Use of the polymerase chain reaction to discriminate potato cyst nematode at the species level, in *Diagnostics in Crop Production*, BCPC Symposium Proceedings, Vol 65, pp 247–252 (2000).

14 Bulman SR and Marshall JW, Differentiation of Australasian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR). *NZJ Crop and Hort Sci* **25**:123–129 (1997).

15 Ibrahim SK, Saad AT, Haydock PPJ and Al-Masri Y, Occurrence of the potato cyst nematode *Globodera rostochiensis* in Lebanon. *Nematology* **2**:125–128 (2000).

16 Burrows PR and Perry RN, Two cloned DNA fragments which differentiate *Globodera pallida* from *G rostochiensis*. *Rev Nématol* **9**:199–200 (1988).

17 Blok VC, Phillips MS and Harrower BE, Comparison of British populations of potato cyst nematodes with populations from continental Europe and South America using RAPDs. *Genome* **40**:286–293 (1997).

18 Curran J, Baillie DL and Webster JM, Use of genomic DNA restriction length differences to identify nematode species. *Parasitology* **90**:137–144 (1985).

19 Stratford R, Shields R, Goldsbrough AP and Fleming CC, Analysis of repetitive DNA sequences from potato cyst nematodes and their use as diagnostic probes. *Phytopathology* **82**:881–886 (1992).

20 Ibrahim SK, Baldwin JG, Roberts PA and Hyman BC, Genetic variation in *Nacobbus aberrans*: An approach toward taxonomic resolution. *J Nematol* **29**:241–249 (1997).

21 Evans K, Curtis RH, Robinson MP and Yeung M, The use of monoclonal antibodies for the identification and quantification of potato cyst nematodes. *Bulletin OEPP/EPPO Bulletin* **25**:357–365 (1995).

22 Minnis ST, Haydock PPJ, Ibrahim SK, Evans K, Russell MD and Grove I, Occurrence and distribution of potato cyst nematodes in England and Wales. *Asp Appl Biol* **59**:1–9 (2000).

23 Fenwick DW, Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *J Helminthology* **18**:155–172 (1940).

24 White TJ, Burns T, Lee S and Taylor J, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in *PCR protocols. A guide to methods and applications*, ed by Innes MA, Gelfard D, Sninsky JJ and White TJ, Academic Press, San Diego, pp 315–322 (1990).

25 Oakley BR, Kirsch DR and Morris R, A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Annal Biochem* **105**:361–363 (1980).

26 Statsoft, Inc, STATISTICA for Windows (Computer Program Manual), Tulsa, USA (1999).

27 Crawley MJ, *GLIM for Ecologists*, Blackwell Scientific Publications, Oxford, pp 297–300 (1993).

28 Fleming CC, Turner SJ, Powers TO and Szalansky AL, Diagnostics of cyst nematodes: use of the polymerase chain reaction to determine species and estimate population levels. *Asp Appl Biol* **52**:372–382 (1998).

29 Zijlstra C, A fast PCR assay to identify *Meloidogyne hapla*, *M. chitwoodi* and *M fallax*, and to sensitively differentiate them from each other and from *M incognita* in mixtures. *Fund Appl Nematol* **20**:505–511 (1997).

30 Evans KA, Harling R and Dubickas A, Application of a PCR-based technique to speciate potato cyst nematodes and determine the distribution of *Globodera pallida* in ware growing areas. *Asp Appl Biol* **52**:345–354 (1998).

31 Fleming CC and Marks RJ, The identification of the potato cyst nematodes *Globodera rostochiensis* and *G pallida* by isoelectric focusing of proteins on polyacrylamide *gels*. *Ann Appl Biol* **103**:277–281 (1983).

32 Burrows PR, Halford PD and Evans K, Estimation of genomic variation between British populations of the potato cyst nematode *Globodera pallida* using RAPD-PCR. *Mol Ecol* **5**:697–701 (1996).

33 Phillips MS, Blok VC, Ploeg A and Harrower BE, Studies on an artificially fragmented population of potato cyst nematodes *Globodera pallida*. *Nematologica* **44**:655–666 (1998).

34 Kort J, Ross H, Rumpenhorst HJ and Stone AR, An international scheme for identifiying and classifiying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G pallida*. *Nematologica* **23**:333–339 (2000).

35 Stone AR, Holliday JM, Mathias PL and Parrott DM, A selective survey of potato cyst-nematodes pathotypes in Great Britain. *Plant Pathol* **35**:18–24 (1986).

36 Turner S, Trends in PCN distribution in Northern Ireland, *Potato Cyst Nematode Review Meeting*, 1–2 February 1996, SASA, East Craigs, Edinburgh (1996).

37 Hancock M, Trends in PCN distribution in England and Wales, *Potato Cyst Nematode Review Meeting*, 1–2 February 1996, SASA, East Craigs, Edinburgh (1996).