Specific Polymerase Chain Reaction Primers for the Detection of *Plasmodiophora brassicae* in Soil and Water

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ABSTRACT

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The development of specific oligonucleotide primers for *Plasmodiophora brassicae* has led to a nested polymerase chain reaction (PCR) detection method for *P. brassicae* in soil and water. Initially, the PCR was used to amplify a section of the rDNA repeat. The PCR products were sequenced and the data used to design primers that were directed at the

ribosomal RNA genes and internal transcribed spacer regions. Specificity was tested against more than 40 common soil organisms, host plants, and spore suspension contaminants, as well as *P. brassicae* isolates from around Australia and the world. Sensitivity was determined to be 0.1 femtograms (fg; 10^{-15} g) for pure template and as low as 1,000 spores per g of potting mix. In soil, *P. brassicae* was detected in all soils where the inoculum was sufficient to result in clubroot symptoms. Also outlined is a simple method of DNA extraction from soil.

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is a serious soilborne disease of plants worldwide, capable of severe infection of Chinese cabbage and cauliflower and also of broccoli and cabbage. Other economically important plants affected are brussels sprouts, turnip, canola, mustard, kohlrabi, kales, and bok choy. In Australia, *P. brassicae* is responsible for losses of at least 10% of crucifer crops annually, resulting in more than A\$13 million in lost revenue on domestic and export markets. *P. brassicae* is an obligate biotroph requiring a cruciferous host to complete its life cycle. Affected roots swell into club- and spindle-shaped tumor-like growths, and their capacity for water and nutrient uptake is reduced (12). Resting spores are released into the soil upon the decay of infected roots, leading to the buildup of inoculum in areas that are repeatedly cropped with crucifers. The resting spore stage can survive in soil and plant debris for at least 8 years (12). Effective control methods include soil fumigation, liming, calcium cyanamide treatments, and the use of fungicides such as pentachloronitrobenzene (PCNB) and fluzinam (5,9), but the cost and practicality of these measures can be prohibitive. Therefore, the best means of control is not to plant in clubroot-affected soil.

No reliable and rapid method of detection of this pathogen is available, because *P. brassicae* cannot be cultured. Bait plants currently provide the only sure method of establishing the presence of *P. brassicae* in soils (17). However, this method is generally only reliable when inoculum reaches levels of greater than 1,000 spores per g of dry soil (depending on soil type and environmental conditions) and takes up to 8 weeks, because it relies on observation of gall development (12). The method can be hastened by checking for root-hair infection (15,21), but this is time consuming and laborious. Fluorescence microscopy methods have been developed (2,24), but they require dedicated equipment and a trained eye because debris and other organisms also fluoresce. Binding of resting spores

to soil particles can also bias spore counts, resulting in under-estimations. Serological detection (13,19,25) is difficult because a single root gall can be composed of many different *P. brassicae* races (11) and because sample preparation is a long and complex process. Therefore, a rapid, sensitive, and specific test is required for use by nonspecialist personnel.

Polymerase chain reaction (PCR)-based detection is potentially the solution. It is applicable to soil and small tissue samples, making it an attractive alternative to the limited and time-consuming methods currently in use.

Recently, Ito et al. (10) showed that PCR primers for the isopentenyltransferase gene specifically amplify *P. brassicae* DNA. This might be useful to detect the organism, but its sensitivity is limited because of the low copy number of the gene and the use of only one set of primers, and no method for its application to soil was provided.

The ribosomal repeat and internal transcribed spacer (ITS) regions have proven valuable for detection of other organisms (22) because the rDNA region can be present in up to 200 copies per genome (20). Generally, ITS regions are conserved at the species level but show increased variation in higher taxa. However, in fungi, the base sequence of the ITS region can be highly variable, and this allows for differentiation between and within species (18). The ITS region was, therefore, targeted in this study to identify a unique DNA sequence for PCR-mediated detection of *P. brassicae* in soil, water, and plant debris.

MATERIALS AND METHODS

***P. brassicae* sample collection and preparation.** Eighteen *P. brassicae* isolates were used in this study (Table 1). *P. brassicae* spores were extracted and purified according to the method of Castlebury et al. (7) with the following modifications. Roots containing galls were macerated in sterile distilled water (1:3 wt/vol) and filtered through four layers of cheesecloth. The filtered homogenate was centrifuged at $3,200 \times g$ for 15 min, with the pellet resuspended in sterile distilled water (sdH₂O) and recentrifuged (at least five times) until the supernatant was clear. The spore suspen-

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sion was layered onto a discontinuous gradient of LUDOX HS40 (DuPont Australia, Bayswater, Victoria, Australia), consisting of a 15-ml 50% lower layer and a 10-ml 30% upper layer, and spun at $600 \times g$ for 10 min. The resting spores formed a band near the top of the gradient and were carefully removed with a pipette. Starch grains formed a pellet at the bottom of the gradient and were also removed and used in later experiments. Spores were centrifuged twice in sdH₂O to remove LUDOX and stored at 4°C for up to 18 months. Nucleic acids were extracted from *P. brassicae* spore suspensions by the method of Dellaporta et al. (8) and stored at 4°C.

Soil organism collection and preparation. A variety of soil fungi, bacteria, and plant species were used in this study (Table 2).

Fungal isolates were grown on V8 juice agar (23), and mycelial fragments were used to inoculate 50 ml of nutrient broth in 250-ml flasks. Cultures were incubated on an orbital shaker at room temperature for 7 to 11 days. Mycelia were collected by centrifugation, and genomic DNA was extracted by either the method of Dellaporta et al. (8) or that of Lee and Taylor (14).

Two bacterial species were obtained from a culture collection, and fifteen unidentified species were isolated from *P. brassicae* spore suspensions. Prior to purification, spore suspensions were streaked onto nutrient agar plates and single bacterial colonies were restreaked and grown in liquid nutrient broth. Bacteria were not identified but were distinguished on the basis of morphology (colony characteristics and microscopic shape and size) and Gram

TABLE 1. *Plasmodiophora brassicae* isolates used during development of specific polymerase chain reaction primers

Isolate number	Country of origin	Location (collector)
1	Australia	Werribee, Victoria (E. C. Donald, Agriculture Victoria)
2	Australia	Mornington, Victoria (E. C. Donald, Agriculture Victoria)
3	Australia	Lindenow, Victoria (E. C. Donald, Agriculture Victoria)
4	Australia	Werribee, Victoria field isolate (R. Faggian, RMIT University)
5	Australia	Werribee, Victoria field isolate (R. Faggian, RMIT University)
6	Australia	Mornington, Victoria field isolate (R. Faggian, RMIT University)
7	Australia	Lindenow, Victoria field isolate (R. Faggian, RMIT University)
8	Australia	Manjimup, Western Australia (R. Lancaster, Agriculture Western Australia)
9	Australia	Manjimup, Western Australia (R. Lancaster, Agriculture Western Australia)
10	Australia	Queensland field isolate (P. Stevens, Queensland Dept. Primary Industries)
11	Australia	Queensland field isolate (P. Stevens, Queensland Dept. Primary Industries)
12	Australia	Murray Bridge, South Australia (B. Tadawan, University of Adelaide)
13	Australia	New South Wales field isolate (L. James, New South Wales Agriculture)
14	Australia	Tasmanian field isolate (J. Dennis, Tasmanian Dept. Primary Industries & Fisheries)
15	New Zealand	Christchurch (S. Bulman, New Zealand Institute of Food and Crop Research)
16	New Zealand	Gore (S. Bulman, New Zealand Institute of Food and Crop Research)
17	United Kingdom	(J. Roberts and J. Duncan, Scottish Crop Research Institute)
18	United States	Georgia (J. Braselton and A. Trese, Ohio University)
19	United States	Ohio (J. Braselton and A. Trese, Ohio University)
20	Japan	Hiroshima (S. Orihara, Chugoku National Agricultural Experimental Station)
21	Japan	Osaka (S. Orihara, Chugoku National Agricultural Experimental Station)

TABLE 2. Fungal, bacterial, and plant species used to test the specificity of *Plasmodiophora brassicae* polymerase chain reaction primers

Species ^a	Origin ^b
<i>Aspergillus niger</i> ₁ Tiegh.	RMIT 582 (J. Upsha, Materials Research Laboratory, Dept. of Defence, Australia)
<i>Aspergillus niger</i> ₂ Tiegh.	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Colletotrichum coccodes</i> (Wallr.) S. J. Hughes	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Erysiphe cichoracearum</i> DC.	J. Cunningham (RMIT University)
<i>Fusarium avenaceum</i> (Fr.:Fr.) Sacc.	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Fusarium graminearum</i> ₁ Schwabe	RMIT 604 (A. Hocking, CSIRO Food Research Culture Collection, 2740)
<i>Fusarium graminearum</i> ₂ Schwabe	J. Roberts, J. Duncan, Scottish Crop Research Institute
<i>Fusarium oxysporum</i> Schlechtend.	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Fusarium sulphureum</i> Schlechtend.	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Gliocladium roseum</i> Bainier	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Leptosphaeria maculans</i> (Desmaz.) Ces. & De Not.	B. Howlett (University of Melbourne)
<i>Penicillium chrysogenum</i> ₁ Thom	RMIT 547 (F. M. Cole, Botany Dept., Monash University)
<i>Penicillium chrysogenum</i> ₂ Thom	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Phytophthora cinnamomi</i> Rands	VPRI 20837a (Agriculture Victoria)
<i>Pythium</i> sp.	VPRI 20666a (Agriculture Victoria)
<i>Pythium ultimum</i> Trow	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Rhizoctonia solani</i> ₁ Kühn	VPRI 21150a (Agriculture Victoria)
<i>Rhizoctonia solani</i> ₂ Kühn	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Spongopora subterranea</i> (Wallr.) Lagerh.	D. De Boer (Agriculture Victoria)
<i>Verticillium dahliae</i> ₁ Kleb.	VPRI 20677a (Agriculture Victoria)
<i>Verticillium dahliae</i> ₂ Kleb.	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Verticillium lecanii</i> (A. Zimmerm.) Viégas	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Verticillium tricorpus</i> I. Isaac	J. Roberts, J. Duncan (Scottish Crop Research Institute)
<i>Bacillus subtilis</i>	RMIT 30-7 (RMIT University)
<i>Bradyrhizobium</i> sp.	A. Rawal (RMIT University)
<i>Streptomyces albus</i>	RMIT 346-1 (RMIT University)
<i>Brassica oleracea</i> L.	European Clubroot Differential Series
<i>Brassica napus</i> L.	European Clubroot Differential Series
Unidentified bacterial species ($\times 15$)	<i>Plasmodiophora</i> resting spore suspensions

^a Subscripts 1 and 2 after the species name denotes the same species from a different location; i.e., different isolates.

^b Culture collection numbers given where possible, otherwise location and collector. RMIT = Royal Melbourne Institute of Technology; VPRI = Victorian Plant Research Institute.

reaction. Bacterial DNA was extracted by the genomic DNA mini-prep method of Ausubel et al. (3).

Young leaves or roots of uninfected Chinese cabbage were removed, washed in sterile water, and the DNA extracted by the method of Dellaporta et al. (8), as was that of the starch grains purified from infected roots. Both were used to test primer specificity. DNA was extracted from soil by the method of Wang et al. (26) with the following modifications. Soil samples of approximately 50 to 100 mg were ground in an Eppendorf tube with a small amount of acid-washed sand (Sigma, Castle Hill, New South Wales, Australia), using a tissue homogenization pestle (Crown Scientific, Rowville, Victoria, Australia) in 200 μ l of 0.5 M NaOH (BDH Chemicals, Kilsyth, Victoria, Australia). The sample was centrifuged in a benchtop centrifuge for 5 min at $22,000 \times g$, and 5 μ l of the supernatant was diluted in 500 μ l of Tris-EDTA (TE).

The DNA samples were quantified using a Philips PU 8625 UV/VIS spectrophotometer (Philips, Sydney, Australia) and by the minigel method (16) and then stored at 4°C.

Fig. 1. Diagram of the ribosomal DNA repeat of *Plasmodiophora brassicae* showing the approximate locations of the main primers PbITS1 and PbITS2 and the nested primers PbITS6 and PbITS7.

Fig. 2. Ethidium bromide-stained 1.5% agarose gel with main and nested polymerase chain reaction products from a 10-fold dilution series of purified *Plasmodiophora brassicae* DNA. Main primers PbITS1 and PbITS2 are on the top row and nested primers PbITS6 and PbITS7 are on the bottom row. Lanes are numbered from left to right. Lane 1, *Bacillus subtilis* phage SPP-1 DNA digested with *Eco*RI (SPP/*Eco*RI) molecular weight marker; lanes 2 to 10, 10 ng of *P. brassicae* template DNA to 100 ag, respectively; and lane 11, negative control of sterile distilled water.

PCR reaction conditions. The PCR reactions were performed in a Perkin Elmer 480 version 2.0 thermocycler (Perkin-Elmer, Scoresby, Victoria, Australia) in a 25- μ l volume with the following components: 0.25 U of *Tth Plus* DNA polymerase (1 mM MgCl₂ and 1 $\times$ reaction buffer; Fisher Biotech, Bayswater, Victoria Australia), 5 mM dNTP mix (Fisher Biotech), 2.5% Tween 20 (Ajax Chemicals, Auburn, New South Wales, Australia), 60 ng of forward and reverse primer, and either 10 ng of DNA from spores and cultured organisms or 1 μ l of unquantified DNA from soil and water. Of the first PCR reaction, 1 μ l was used as template for the nested PCR, with the same reaction and cycling conditions outlined above. All components were dispensed with aerosol-resistant tips (Interpath Services, West Heidelberg, Victoria, Australia) in a laminar flow cabinet, and negative controls containing water instead of DNA were included in all PCR reactions. The PCR products were resolved on 1.5% agarose gels run at 90 V, stained with ethidium bromide, and visualized over UV light.

Sequencing and primer design. The ITS1, 5.8S, and part of the 18S and ITS2 regions were amplified by PCR from genomic DNA isolated from *P. brassicae* (isolates 15 and 18) using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (27). The PCR reaction conditions and the sequencing protocol used for this step are described in Bulman and Marshall (6).

Fig. 3. Ethidium bromide-stained 1.5% agarose gel with polymerase chain reaction products from *Plasmodiophora brassicae* isolates and soil fungi. Universal primers ITS1 and ITS4 are on the top row, main primers PbITS1 and PbITS2 are on the middle row, and nested primers PbITS6 and PbITS7 are on the bottom row. Lanes are numbered from left to right. Lane 1, *Bacillus subtilis* phage SPP-1 DNA digested with *Eco*RI (SPP/*Eco*RI); lane 2, *P. brassicae* isolate 2; lane 3, *P. brassicae* isolate 10; lane 4, *P. brassicae* isolate 14; lane 5, *Fusarium sulphureum*; lane 6, *F. oxysporum*; lane 7, *F. avenaceum*; lane 8, *Gliocladium roseum*; lane 9, *Rhizoctonia solani*₂ (the second of two isolates of the same species, each at a different location); lane 10, *Pythium ultimum*; lane 11, *Verticillium tricorpus*; lane 12, *V. lecanii*; lane 13, *Colletotrichum coccodes*; lane 14, negative control of sterile distilled water; lane 15, negative control; and lane 16, SPP/*Eco*RI.

Primers PbITS1 (5'-ACTTGCATCGATTACGTCCC-3') and PbITS2 (5'-GGCATTCTCGAGGGTATCAA-3') were designed using the Primer3 software package (S. Rozen and H. J. Skaletsky, Whitehead Institute for Biomedical Research, Cambridge, MA) and were based on conserved regions found between isolates 15 and 18, using a primer G + C content of approximately 50% and a primer melting temperature of 60°C. The PbITS1 and PbITS2 primers were tested for the amplification of a predicted-size fragment from the rDNA-ITS region from all *P. brassicae* isolates.

The PbITS1 and PbITS2 PCR products from three Australian isolates (isolates 1, 3, and 8) were cleaned using a Qiagen PCR purification kit (Qiagen, Clifton Hill, Victoria, Australia) and sequenced directly using an ABI PRISM Ready Reaction Dye Terminator Cycle Sequencing kit (Perkin-Elmer) on an Applied Biosystems model 373 Automated DNA Sequencer Stretch (Perkin-Elmer). The sequence data were aligned with those of isolates 15 and 18 using the PILEUP program in the GCG package (Wisconsin Package, version 8; Genetics Computer Group, Madison, WI) to identify further conserved regions. From this alignment, the nested primers PbITS6 (5'-CAACGAGTCAGCTTGAATGC-3') and PbITS7 (5'-

TGTTTCGGCTAGGATGGTTC-3') were designed, again using the Primer3 package and the criteria outlined above. Primers PbITS1 and PbITS6 are both complementary to regions within the 18S rDNA fragment, while PbITS2 and PbITS7 are complementary to regions within ITS2 and ITS1, respectively (Fig. 1).

Primer specificity. The specificity of the primer pairs was tested by attempting amplification using DNA from a number of common soil organisms, host plants, and bacterial contaminants found in resting spore suspensions (Table 2). Also, DNA was extracted from different soils that were either artificially inoculated with approximately 10^6 spores per g of soil or soils known to contain *P. brassicae* resting spores up to commercially relevant levels (those that produce sufficient symptoms to reduce yield). These included soils from infested farms, artificially inoculated potting mix, and dam water laden with mud and algae.

Primer sensitivity. Sensitivity was tested by creating 10-fold serial dilutions of purified *P. brassicae* DNA in water, from 10 ng to 0.1 femtograms (fg; 10^{-15} g) ml^{-1} , and checking for amplification products with the nested PCR protocol. Also, 10-fold serial dilutions of *P. brassicae* resting spores in double-autoclaved potting mix was tested, ranging from 10^7 to 0 spores per 100 mg of potting mix. Total potting mix DNA was extracted by the NaOH method outlined earlier and used in the nested PCR protocol.

The PCR detection sensitivity was compared with a bait plant technique. Chinese cabbage plants were grown in steam-sterilized potting mix in controlled temperature glass houses with a 12-h photoperiod. After 14 days, plant roots were inoculated directly with 200- μl suspensions of resting spores in sterile water. Spore concentration ranged from 10^7 to 0 spores per ml in 10-fold serial dilutions. Plants were visually assessed for clubroot symptoms after a further 8 weeks of growth.

RESULTS

Specificity. Amplification of *P. brassicae* DNA from all isolates with primers PbITS1 and PbITS2 resulted in the predicted product size of approximately 1,100 bp (Fig. 2). The nested primers PbITS6 and PbITS7 resulted in a product of the predicted size of approximately 620 bp (Fig. 2). Australian National Genomic Information Service (ANGIS, The University of Sydney, New South Wales, Australia) database sequence comparisons using BLASTN (1) revealed that these fragments matched those of *P. brassicae* (accession no. U18981).

The DNA from host plants and over 40 common soil organisms, root pathogens, and spore suspension contaminants (Table 2) gave

Fig. 4. Ethidium bromide-stained 1.5% agarose gel with polymerase chain reaction products from soil DNA extractions. Main primers PbITS1 and PbITS2 are on the top row and nested primers PbITS6 and PbITS7 are on the bottom row. Lanes are numbered from left to right. Lane 1, *Bacillus subtilis* phage SPP-1 DNA digested with *Eco*RI (SPP/*Eco*RI); lane 2, resting spore suspension; lane 3, rotting root gall; lane 4, artificially inoculated dam water; lane 5, autoclaved potting mix; lane 6, artificially inoculated autoclaved potting mix; lane 7, uninfested soil; lanes 8 to 10, infested farm soil; and lane 11, negative control.

Fig. 5. Ethidium bromide-stained 1.5% agarose gel with nested polymerase chain reaction products from spore dilutions in potting mix. Lanes are numbered from left to right. Lane 1, *Bacillus subtilis* phage SPP-1 DNA digested with *Eco*RI (SPP/*Eco*RI); lane 2 and 3, 10^6 spores per g of potting mix; lanes 4 and 5, 10^5 spores per g of potting mix; lanes 6 and 7, 10^4 spores per g of potting mix; lanes 8 and 9, 10^3 spores per g of potting mix; lanes 10 and 11, 10^2 spores per g of potting mix; lanes 12 and 13, potting mix control; lanes 14 and 15, infested farm soil; and lane 16, positive control.

no amplification products with both the main and nested primers (not all data shown; one gel chosen at random) (Fig. 3). Also, only soils that were artificially inoculated or those known to be infested with *P. brassicae* gave amplification products in the PCR, as did artificially inoculated dam water (Fig. 4). However, DNA from all samples gave products with the universal primers ITS1 and ITS4, indicating the presence of amplifiable DNA.

Sensitivity. The PCR amplification of the dilution series of purified *P. brassicae* DNA resulted in visible product with the main primers (PbITS1 and PbITS2) down to 1 pg of template, and in combination with the nested primers (PbITS6 and PbITS7), the sensitivity was increased to 0.1 fg (Fig. 2). In potting mix, *P. brassicae* resting spores could be detected with bright bands consistently down to levels of 10⁵ spores per g of potting mix, while levels as low as 10³ spores per g could also be detected with fainter bands (Fig. 5). The sensitivity of the PCR matched that of the bait plants, with both methods detecting levels down to 10³ spores per g of potting mix (Table 3).

DISCUSSION

In this study, two pairs of PCR primers have shown potential for use in a diagnostic test for *P. brassicae* in soil, water, and infected plant tissue. Efforts to produce a molecular biology-based assay for the detection of *P. brassicae* have been hampered in the past by several factors. First, the organism is an obligate pathogen. Second, *P. brassicae* is difficult to purify from infected roots and is often contaminated with bacteria and host-plant debris. Finally, *P. brassicae* is known to take up random segments of host plant DNA (4). Every precaution was taken to prevent these factors from affecting the results. The possibility that the organism had taken up plant DNA could not be eliminated; therefore, host-plant DNA was tested against all primers, but proved negative.

The results suggest that the PCR primers PbITS1, PbITS2, PbITS6, and PbITS7 specifically amplify *P. brassicae* DNA, with no amplification from the closely related *Spongospora subterranea*, host plants, or other common soil microorganisms. Isolate origin had no effect, with *P. brassicae* isolates from all over Australia and around the world resulting in PCR amplification.

The high copy number of rDNA, up to 220 tandem repeats in fungi (20), enables high sensitivity of detection. In this case, a single set of primers (PbITS1 and PbITS2) gives a detectable amplification product with 1 pg of purified template DNA. This level is improved to 0.1 pg when the nested primers (PbITS6 and PbITS7) are used. More importantly, this PCR method allows detection of *P. brassicae* resting spores in soil and potting mix at levels similar to that of bait plants. However, the coarse nature of the potting mix hindered DNA extraction procedures, and it is expected that more sensitive detection levels could be achieved in different substrates. For example, amplification of DNA extracted from infested farm soil produced strong bands (Fig. 5); yet, based on plant symptoms observed in the field, spore levels were less than those required to cause loss of yield.

Therefore, the nested PCR assay has potential as a diagnostic test for detection of *P. brassicae* in soil, water, and plant material,

which would be of value not only to farmers but also to seed and seedling producers. The assay is very specific, significantly faster than all other methods used to date (giving a result in 1 day), and does not require complicated preparation of samples, regardless of whether they originate from soil, water, or plant tissue. It is also superior to the assay developed by Ito et al. (10), which has not been tested against isolates of *P. brassicae* from outside Japan, is less sensitive, and has not been used with soil, water, or infected plant samples.

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TABLE 3. Sensitivity of the polymerase chain reaction (PCR) and bait plant methods for detection of *Plasmodiophora brassicae* in soil

Resting spores per g of soil	Bait plant ^a	PCR protocol ^a
10 ⁷	+	+
10 ⁶	+	+
10 ⁵	+	+
10 ⁴	+	+/-
10 ³	+	+/-
10 ²	-	-
10 ¹	-	-
0	-	-

^a + = *P. brassicae* detected; - = *P. brassicae* not detected.

Australasian Plant Pathology Society, Perth.

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