 Doi: 10.1111/j.1365-3059.2006.01442.x

Molecular detection of *Phytophthora capsici* in infected plant tissues, soil and water

Z. G. Zhang, Y. Q. Li, H. Fan, Y. C. Wang* and X. B. Zheng

Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China

A species-specific PCR assay was developed for rapid and accurate detection of the pathogenic oomycete *Phytophthora capsici* in diseased plant tissues, soil and artificially infested irrigation water. Based on differences in internal transcribed spacer (ITS) sequences of *Phytophthora* spp. and other oomycetes, one pair of species-specific primers, PC-1/PC-2, was synthesized. After screening 15 isolates of *P. capsici* and 77 isolates from the Ascomycota, Basidiomycota, Deuteromycota and Oomycota, the PC-1/PC-2 primers amplified only a single PCR band of *c.* 560 bp from *P. capsici*. The detection sensitivity with primers PC-1/PC-2 was 1 pg genomic DNA (equivalent to half the genomic DNA of a single zoospore) per 25-μL PCR reaction volume; traditional PCR could detect *P. capsici* in naturally infected plant tissues, diseased field soil and artificially inoculated irrigation water. Using ITS1/ITS4 as the first-round primers and PC-1/PC-2 in the second round, nested PCR procedures were developed, increasing detection sensitivity to 1 fg per 25-μL reaction volume. The results suggested that the assay detected the pathogen more rapidly and accurately than standard isolation methods. The PCR-based methods developed here could simplify both plant disease diagnosis and pathogen monitoring, as well as guiding plant disease management.

Keywords: molecular detection, nested PCR, oomycetes

Introduction

Phytophthora capsici is known to infect many species of pepper, tomato and other agronomic and ornamental crops of the Solanaceae and Cucurbitaceae families. In pepper fields, the oomycete is soilborne and initial infections of roots, collars and lower leaves occur. The pathogen grows within the host and produces sporangia on the surface of diseased tissue, especially leaves. Sporangia are spread by splashing water from irrigation or rain. With moisture present, zoospores released from sporangia swim for a few minutes to more than an hour before encysting. The pathogen survives in the soil in host debris for months (Zheng, 1997).

Few effective, economical and environmentally safe management options are available for *P. capsici* blight. A major reason for this lack is the inability to detect accurately the presence and identity of the pathogen, especially in plant tissues, soil and irrigation water. Also, *Phytophthora* has been widely acknowledged as a taxonomically 'difficult' genus (Brasier, 1983), as many of the characteristics used for species identification are plastic, highly influenced by environment, show overlap between species, and have unknown genetic basis. Also, plants affected by sudden wilt are generally infected by other genera, including *Pythium*, *Fusarium* and *Rhizoctonia*. Traditional methods to detect or isolate these pathogens involve plating infected plant parts or soil on selective medium and conducting a pathogenicity assay. However, this is limited by its lack of sensitivity and specificity, as *P. capsici* shares similar morphology with certain other *Phytophthora* spp., such as *Phytophthora tropicalis*, when grown on medium (Aragaki & Uchida, 2001). In addition, these laborious, time-consuming methods preclude processing large numbers of samples and require extensive knowledge of fungal taxonomy.

Polymerase chain reaction (PCR) techniques offer advantages over traditional methods of detection and diagnosis because the fungi do not need to be cultured prior to detection by PCR, and the technique is rapid and sensitive (Bonants *et al.*, 1997; Lacourt & Duncan, 1997; Frederick *et al.*, 2002; Ippolito *et al.*, 2002; Kong *et al.*, 2003; Li & Hartman, 2003; Mercado-Blanco *et al.*, 2003; Hayden *et al.*, 2004; Silvar *et al.*, 2005; Zhang *et al.*, 2005). In this study, PCR primers derived from ITS sequences were developed for the specific detection of *P. capsici*. The specificity and sensitivity of the reaction were tested on a range of wild *Phytophthora* species and on representatives of three fungal divisions, as well as of the host plants. The PCR protocols were tested for their ability to detect

*E-mail: wangyc@njau.edu.cn

Accepted 10 March 2006

© 2006 The Authors
Journal compilation © 2006 BSPP

P. capsici in naturally diseased plant tissues, artificially inoculated irrigation water, and diseased soil samples collected in the field.

Materials and methods

Source of isolates

Table 1 lists the 15 *P. capsici* isolates and other isolates used in this study. All isolates were stored either on lima bean agar slants at 10°C (*Phytophthora* spp. and *Pythium* spp.) or on potato dextrose agar at 4°C (other species).

Mycelium and zoospore preparation

For genomic DNA extraction, mycelia were collected on filter paper and stored at –70°C until use, according to Zhang *et al*. (2005). Zoospores were produced by an isolate (Weijiao1) of *P. capsici* recovered from a diseased pepper (*Capsicum annuum*) plant. Sample preparation

Table 1 Isolates of fungi and oomycetes used to screen primer specificity

Species	Host	Source	No. of isolates	Amplification with primers PC-1/PC-2[a]
Phytophthora capsici	*Capsicum annuum*	Jiangsu	3	+
	Cucumis sativus	Jiangsu	8	+
	Lycopersicon esculentum	Jiangsu	4	+
Phytophthora boehmeriae	*Gossypium hirsutum*	Jiangsu	2	–
Phytophthora cinnamomi	Unknown	W. H. Ko	1	–
Phytophthora cryptogea	*Gerbera jamesonii*	Jiangsu	3	–
Phytophthora nicotianae	*Nicotiana tabacum*	Yunnan	3	–
Phytophthora tropicalis	*Theobroma cacao*	W. H. Ko	2	–
Phytophthora colocasiae	*Colocasia esculenta*	Hainan	1	–
Phytophthora drechsleri	*L. esculentum*	Jiangsu	1	–
Phytophthora sojae	*Glycine max*	Heilongjiang	1	–
Phytophthora syringae	Unknown	UK	1	–
Phytophthora quercina	Unknown	Hungary	1	–
Phytophthora phaseoli	Unknown	Unknown	1	–
Phytophthora idaei	Unknown	UK	1	–
Phytophthora fragariae var. *rubi*	Unknown	UK	1	–
Phytophthora erythroseptica	Unknown	Ireland	1	–
Phytophthora cambivora	Unknown	France	1	–
Phytophthora palmivora	*Ficus carica*	Jiangsu	1	–
Peronophythora litchii	*Litchi chinensis*	Guangdong	1	–
Pythium aphanidermatum	Unknown	Jiangsu	1	–
Pythium vexans	Soil	W. H. Ko	2	–
Pythium splendens	Soil	W. H. Ko	1	–
Tilletia indica	*Triticum aestivum*	J. H. Peng	2	–
Tilletia walkeri	*T. aestivum*	J. H. Peng	1	–
Tilletia controversa	*T. aestivum*	B. S. Hu	1	–
Tilletia caries	*T. aestivum*	B. S. Hu	1	–
Ustilago nuda	*T. aestivum*	Jiangsu	1	–
Ustilago nuda	*T. aestivum*	Heilongjiang	1	–
Ustilago maydis	*Zea mays*	Jiangsu	1	–
Alternaria solani	*L. esculentum*	Jiangsu	1	–
Alternaria sp.	Unknown	Jiangsu	1	–
Alternaria longipes	*N. tabacum*	Fujiang	1	–
Ascochyta fabae	*Vicia faba*	Q. H. Chen	1	–
Botrytis cinerea	*L. esculentum*	Jiangsu	1	–
Botrytis cinerea	*Lactuca scariola*	Fujian	1	–
Botrytis cinerea	*Vitis vinifera*	Fujian	1	–
Colletotrichum orbiculare	*Cucumis sativus*	Jiangsu	1	–
Colletotrichum orbiculare	*Citrullus lanatus*	Jiangsu	1	–
Colletotrichum higgisianum		Jiangsu	1	–
Colletotrichum gloeosporioides	*Diospyros kaki*	Q. H. Chen	1	–
Colletotrichum truncatum	*G. max*	Q. H. Chen	1	–
Colletotrichum capsici	*C. annuum*	Jiangsu	1	–
Fusarium equiseti	Unknown	CGMCC[b]	1	–
Fusarium avenaceum	Unknown	CGMCC	1	–
Fusarium nivale	Unknown	CGMCC	1	–
Fusarium sambucinum	Unknown	CGMCC	1	–

Table 1 *Continued*

Species	Host	Source	No. of isolates	Amplification with primers PC-1/PC-2[a]
Fusarium culmorum	Unknown	CGMCC	1	–
Fusarium oxysporum f.sp. *vasinfectum*	*Gossypium hirsutum*	Jiangsu	1	–
F. oxysporum f.sp. *cucumerinum*	*C. sativus*	Jiangsu	1	–
F. oxysporum f.sp. *niveum*	*C. lanatus*	Jiangsu	1	–
F. oxysporum f.sp. *cubense*	*Musa sapientum*	Fujian	1	–
Fusarium graminearum	*T. aestivum*	Jiangsu	1	–
Fusarium moniliforme	*Oryza sativa*	Jiangsu	1	–
F. moniliforme	*G. hirsutum*	Jiangsu	1	–
Fusarium solani	Unknown	Jiangsu	1	–
Fusarium sp.	*Alternanthera philoxeroides*	Jiangsu	1	–
Fusarium sp.	Soil	Jiangsu	5	–
Macrophoma kawatsukai	*Malus pumila*	Jiangsu	1	–
Rhizoctonia solani	*G. hirsutum*	Jiangsu	1	–
Rhizoctonia solani	*Cucumis melo*	Fujian	1	–
Verticillium albo-atrum	*Medicago schischkinii*	Xinjiang	1	–
Verticillium dahliae	*G. hirsutum*	Jiangsu	1	–
Verticillium fungicola	Unknown	CGMCC	1	–
Verticillium lecanii	Unknown	CGMCC	1	–
Verticillium psalliotae	Unknown	CGMCC	1	–
Verticillium nigrescens	Unknown	CGMCC	1	–
Mycosphaerella melonis	*C. lanatus*	Shanghai	1	–

[a]+, 560-bp product amplified by primers PC-1/PC-2; –, no amplified products.
[b]China General Microbiological Culture Collection.

and labelling were performed according to Zhang *et al.* (2004). The zoospore suspension was diluted to 1×10^4 zoospores mL^{-1}.

Soil preparation and inoculation

To detect pathogens in soil samples, 100-μL *P. capsici* zoospore suspensions containing 100 zoospores were inoculated into 0·5 g twice-autoclaved soil substrate in 1·5-mL conical tubes. The tubes were vortexed at maximum speed for 1 min, freeze-dried (–40°C) for 2–3 days, ground in liquid nitrogen to produce a fine powder, and stored at –70°C prior to DNA extraction.

DNA extraction

DNA was prepared according to Zhang *et al*. (2004). All DNA preparations were kept at –70°C. DNA was extracted from infected plant tissues according to Tooley *et al*. (1997). A 10-mg sample of diseased tissue (stem or leaf) was cut from each plant, placed into 10 μL freshly prepared 0·5 M NaOH, and macerated with a plastic pestle. After the tubes were centrifuged at 12 000 ***g*** for 5 min, 5 μL of supernatant was removed and immediately diluted with 195 μL 100 mM Tris pH 8·0. The samples were then either used immediately for PCR (1 μL per 25-μL reaction mixture) or frozen at –20°C for later use.

DNA was extracted from soil samples directly according to the method of Li & Hartman (2003).

To extract DNA from artificially inoculated irrigation water samples collected from Nanjing, 2-mL aliquots of *P. capsici* zoospore suspension were added to 28-mL samples of irrigation water in 50-mL conical tubes. The mixtures were then centrifuged at 12 000 ***g*** for 20 min to pellet propagules for DNA extraction after the tubes had been vortexed at maximum speed for 1 min. Water (60 μL ddH_2O) and silica (0·5 g) were added and the mixture was vortexed for 1 min, after which suspension solutions were added to PCR reactions.

Primer design and PCR amplification

Specific primers for *P. capsici* (PC-1, 5′-GTCTTGTACCCTATCATGGCG-3′ and PC-2, 5′-CGCCACAGCAGGAAAAGCATT-3′) were designed to amplify PCR products of 560 bp by comparison of the ITS of 46 different *Phytophthora* sequences in GenBank (Table 2).

Each PCR reaction volume of 25 μL contained 1 μl genomic DNA, 0·5 μM primers, 50 μM of each dNTP, 2·5 μL 10 × PCR buffer, 2 mM Mg^{2+}, 2·5 μL 1% bovine serum albumin, 0·25 μL Tween-20 and 1·25 U Taq DNA polymerase (Promega). Amplification was performed with a PE2400 PCR System DNA thermal cycler (Perkin-Elmer Applied Biosystems) programmed for one cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 70°C for 30 s and 72°C for 30 s. A 7-min extension at 72°C completed the programme. Nested PCR included two rounds of amplification using the universal primers ITS1/ITS4 for the first round and the *P. capsici*-specific primers PC-1/PC-2 for the second round. Negative controls lacking template DNA were performed in each experiment to test for contaminated reagents.

Table 2 GenBank accession numbers and *Phytophthora* species of sequences compared to develop species-specific primers PC-1 and PC-2 for *P. capsici*

Accession no.	Species	Accession no.	Species
AF266781	*P. arecae*	AF266784	*P. botryosa*
AF266772	*P. cactorum*	AF266765	*P. cajani*
AF266763	*P. cambivora*	AF266787	*P. capsici*
AF266764	*P.cinnamomi*	AF266788	*P. citricola*
AF266785	*P. citrophthora*	AJ131989	*P. clandestine*
AF266786	*P. colocasiae*	AF266796	*P. cryptogea*
AF266798	*P. drechsleri*	AF266797	*P. erythroseptica*
AF266761	*P. fragariae* var. *rubi*	AF266762	*P. fragariae* var. *fragariae*
AF266793	*P. gonapodyides*	AF266770	*P. heveae*
AF266792	*P. humicola*	AF266773	*P. idaei*
AJ131990	*P. ilicis*	AF266779	*P. infestans*
AF266789	*P. inflata*	AF271222	*P. insolita*
AJ131987	*P. iranica*	AF266771	*P. katsurae*
AF266804	*P. lateralis*	AF266799	*P. medicaginis*
AF266782	*P. megakarya*	AF266794	*P. megasperma*
AF266767	*P. melonis*	AF266777	*P. mirabilis*
AF266790	*P. multivesiculatae*	AF266776	*P. nicotianae*
AF266780	*P. palmivora*	AF266778	*P. phaseoli*
AF266801	*P. porri*	AF266802	*P. primulae*
AF266774	*P. pseudotsugae*	AJ131986	*P. quercina*
AF271221	*P. richardiae*	AF266768	*P. sinensis*
AF266769	*P. sojae*	AF266803	*P. syringae*
AF266800	*P. trifolii*	AF266766	*P. vignae*

Figure 1 Agarose gel electrophoresis of PCR-amplified products using the specific primers PC-1/PC-2. Lane 1, 2000-bp DNA marker; lane 2, negative control; lanes 3–17, *Phytophthora capsici* isolates; lanes 18–24, other fungal and oomycete isolates. The same results were obtained in four replicates.

Results and discussion

Specificity and sensitivity of PCR amplification

Specificity is essential for detecting *P. capsici*. The primer set PC-1/PC-2 was able to amplify a unique DNA fragment of *c*. 560 bp (Fig. 1) from all *P. capsici* isolates tested from different Chinese host plants. However, 77 isolates of other oomycetes and fungi tested yielded no amplification product. All fungal and oomycete isolates tested gave a positive PCR reaction using the ITS universal primers ITS1/ITS4 (data not shown).

The sensitivity of PCR assays is an important concern in the molecular detection of plant pathogens in field soil. In a 25-μL reaction volume assaying *P. capsici*, conventional PCR was able to detect 1 pg of pure genomic DNA (Fig. 2a). In contrast, the limit of detection for *P. capsici* in a previous study was 5 pg DNA (Silvar *et al*., 2005). In a 25-μL reaction volume using conventional PCR, the detection level for *Phytophthora nicotianae* and *Phytophthora citrophthora* was 10 pg from a pure template of total genomic DNA of the pathogen (Ippolito *et al*., 2002). For another primer set, PNIC1/PNIC2, the detection level for *P. nicotianae* was 2·5 pg (Grote *et al*., 2002). The molecular detection sensitivity of one *P. nicotianae* assay was 80–800 fg DNA μL^{-1} (Kong *et al*., 2003). This higher sensitivity may have resulted not only from the high copy number of the ITS target sequence in the *Phytophthora* genome (Cooke *et al*., 2000), but also from the design of the primers.

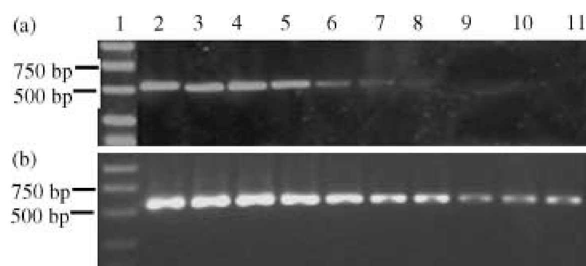

Figure 2 Sensitivity of (a) PCR with primers PC-1/PC-2 using different concentrations of DNA; (b) nested PCR using primers ITS1/ITS4 for the first round of amplification and primers PC-1/PC-2 for the second, for the detection of *Phytophthora capsici*. Lane 1, 2000-bp DNA marker; lane 2, positive control; lanes 3–10, amplified products using DNA at concentrations of 1 μg, 10 μg, 100 μg, 1 fg, 10 fg, 100 fg, 1 pg, 10 pg, 100 pg in a 25-μL PCR reaction volume. The same results were obtained in three replicates.

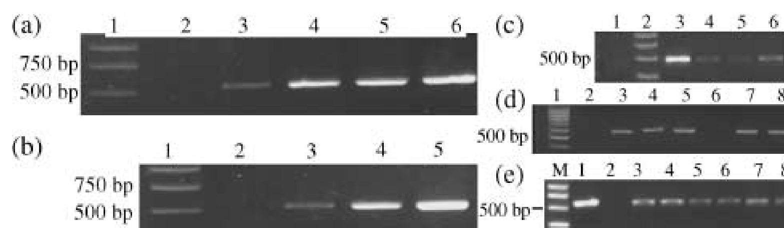

Figure 3 (a) Sensitivity of PCR for detection of *Phytophthora capsici* zoospores. Lane 1, 2000-bp DNA marker; lane 2, negative control; lanes 3–6, amplified products using DNA from 0·5, one, 1·5 and two zoospores, respectively. (b) Products amplified from zoospore DNA extracted from soil samples; lane 1, 2000-bp DNA marker; lane 2, control; lanes 3–5, amplified products using DNA from one, two and four zoospores, respectively. (c) PCR amplification of DNA extracted from diseased plants; lane 1, negative control; lane 2, 2000-bp DNA marker; lane 3, positive control; lanes 4–6, amplified products using DNA from diseased plants. (d) Products amplified from DNA extracted from soil samples; lane 1, 100-bp DNA ladder marker; lane 2, negative control; lanes 3–8, amplified products using DNA from diseased soil samples. (e) Products amplified from DNA from irrigation water; M, 2000-bp marker; lane 1, positive control; lane 2, negative control; lanes 3 and 4, 30 000 zoospores L^{-1}; lanes 5 and 6, 3000 zoospores L^{-1}; lanes 7 and 8, 300 zoospores L^{-1}.

The present study also developed nested PCR to increase the sensitivity of molecular assays. This included two rounds of amplification, first using universal primers (ITS1/ITS4) to increase the target DNA templates, then using the *P. capsici*-specific primers PC-1/PC-2 for the second round. The nested PCR method in this study provided consistent and reproducible results. Nested PCR has been reported to increase detection sensitivity by factors of 10–1000 (Faggian *et al*., 1999; Judelson & Tooley, 2000; Grote *et al*., 2002; Li & Hartman, 2003). Here, nested PCR increased the sensitivity of the primers at least 1000-fold to 1 fg per 25-μL reaction volume (Fig. 2b). Nested PCR assay therefore has potential as a diagnostic tool for detecting and surveying pathogens in diseased plants and soil. The sensitivity of the primer pair PC-1/PC-2 varied from half the genomic DNA of a single zoospore (Fig. 3a) to one zoospore g^{-1} soil in the 25-μL reaction volume (Fig. 3b).

Detection in plant tissues

A single PCR product of *c*. 560 bp was detected from blight-infected pepper samples from Jiangsu Province in 2003 (Fig. 3c). The presence of *P. capsici* in the diseased plants was confirmed by isolating the oomycete from the tissue to a pure culture. In contrast, no PCR product was amplified from healthy pepper tissues as assessed by the selective medium. Two randomly picked plant samples from which 560-bp PCR products were amplified by PC-1/PC-2 also had DNA sequences 100% identical to those of *P. capsici* in this study. This indicates that the DNA from diseased plants that was amplified in PCR assays by primer sets PC-1/PC-2 was derived from *P. capsici*.

Detection in diseased field soil and artificially inoculated irrigation water

Agricultural field soil is a complex ecosystem with a diverse microbial community (Torsvik & Øvreås, 2002). For example, many hundreds of different species of *Phytophthora*, *Pythium*, *Fusarium*, *Verticillium* and *Rhizoctonia*, in addition to various bacteria and nematodes, have been found in field soil. Thus it is important to distinguish *P. capsici* from the other microbes in the soil. DNA extracted from field soil samples collected from six diseased pepper fields in Jiangsu was subjected to PCR using primers PC-1/PC-2. The DNA samples extracted from 1 g soil were suspended in 9 μL ultrapure water used for PCR amplification. Five of the six samples provided 560-bp PCR products amplified by PC-1/PC-2 (Fig. 3d). One randomly chosen soil sample, from which 560-bp PCR products were amplified by PC-1/PC-2, also had DNA sequences 100% identical with those of *P. capsici* in this study. This confirmed that the PC-1/PC-2-amplified PCR products in soil DNA were from *P. capsici* isolates.

Zoospores are another important target for molecular detection, because they spread disease in irrigation water. Their numbers can reach 400 L^{-1} in recirculated irrigation water (MacDonald *et al*., 1994). PCR with the primer set PC-1/PC-2 yielded the 560-bp band in irrigation water artificially inoculated with as few as 300 zoospores L^{-1} (Fig. 3e), indicating that the method used in this study can detect the pathogen in water.

At least 2 weeks are required to detect *P. capsici* from soil by traditional isolation methods, which can delay disease-management decisions. The PCR detection method reported here can provide a definitive diagnosis of the pathogen in soils, plants and irrigation water within hours, and can be used to survey more accurately the occurrence and distribution of the pathogen in soil and irrigation water. This method is very easy to use and requires minimal training.

Acknowledgements

We thank Dr W. H. Ko, Dr H. H. Ho, G. H. Chen, Dr B. S. Hu, Dr J. H. Peng and the China General Microbiological Culture Collection (CGMCC) for providing some isolates. This work was supported in part by the National Natural Science Foundation of China (grant no. 30300228, Z. G. Zhang).

References

Aragaki M, Uchida JY, 2001. Morphological distinctions between *Phytophthora capsici* and *P. tropicalis* sp. nov. *Mycologia* **93**, 137–45.

Bonants P, Hagenaar-de WM, van Gent-Pelzer M, Lacourt I, Cooke D, Duncan J, 1997. Detection and identification of *Phytophthora fragarie* Hickman by the polymerase chain reaction. *European Journal of Plant Pathology* **103**, 345–55.

Brasier CM, 1983. Problems and prospects in *Phytophthora* research. In: Erwin DC, Bartnicki-Garcia S, Tsao PH, eds. Phytophthora*: its Biology, Taxonomy, Ecology and Pathology*. St Paul, MN, USA: APS Press, 351–64.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30**, 17–32.

Faggian R, Bulman SR, Lawrie AC, Porter IJ, 1999. Specific polymerase chain reaction primers for the detection of *Plasmodiophora brassicae* in soil and water. *Phytopathology* **89**, 392–7.

Frederick RD, Snyder CL, Peterson GL, Bonde MR, 2002. Polymerase chain reaction assays for the detection and discrimination of the soybean rust pathogens *Phakopsora pachyrhizi* and *P. meibomiae*. *Phytopathology* **92**, 217–27.

Grote D, Olmos A, Kofoet A, Tuset JJ, Bertolini E, Cambra M, 2002. Specific and sensitive detection of *Phytophthora nicotianae* by simple and nested-PCR. *European Journal of Plant Pathology* **108**, 197–207.

Hayden KJ, Rizzo D, Tse J, Garbelotto M, 2004. Detection and quantification of *Phytophthora ramorum* from California forests using a real-time polymerase chain reaction assay. *Phytopathology* **94**, 1075–83.

Ippolito A, Schena L, Nigro F, 2002. Detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soils by nested PCR. *European Journal of Plant Pathology* **108**, 855–68.

Judelson HS, Tooley PW, 2000. Enhanced polymerase chain reaction methods for detecting and quantifying *Phytophthora infestans* in plants. *Phytopathology* **90**, 1112–9.

Kong P, Hong CX, Jeffers SN, Richardson PA, 2003. A species-specific polymerase chain reaction assay for rapid detection of *Phytophthora nicotianae* in irrigation water. *Phytopathology* **93**, 822–31.

Lacourt I, Duncan JM, 1997. Specific detection of *Phytophthora nicotianae* using the polymerase chain reaction and primers based on the DNA sequences of its elicitin gene *ParaA1*. *European Journal of Plant Pathology* **103**, 73–83.

Li S, Hartman GL, 2003. Molecular detection of *Fusarium solani* f.sp. *glycines* in soybean roots and soil. *Plant Pathology* **52**, 74–83.

MacDonald JD, Ali-Shtaeyh MS, Kabashima J, Stites J, 1994. Occurrence of *Phytophthora* species in recirculated nursery irrigation effluents. *Plant Disease* **78**, 607–11.

Mercado-Blanco J, Collado-Romero M, Parrilla-Araujo S, Rodríguez-Jurado D, Jiménez-Díaz RM, 2003. Quantitative monitoring of colonization of olive genotypes by *Verticillium dahliae* pathotypes with real-time polymerase chain reaction. *Physiological and Molecular Plant Pathology* **63**, 91–105.

Silvar C, Duncan JM, Cooke DEL, Williams NA, Díaz J, Merino F, 2005. Development of specific PCR primers for identification and detection of *Phytophthora capsici* Leon. *European Journal of Plant Pathology* **112**, 43–52.

Tooley PW, Bunyard BA, Carras MM, Hatziloukas E, 1997. Development of PCR primers from internal transcribed spacer region 2 for detection of *Phytophthora* species infecting potatoes. *Applied and Environmental Microbiology* **63**, 1467–75.

Torsvik V, Øvreås L, 2002. Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology* **5**, 240–5.

Zhang ZG, Zhang JY, Zheng XB, Wang YW, Ko WH, 2004. Molecular distinctions between *Phytophthora capsici* and *P. tropicalis* based on ITS sequences of ribosomal DNA. *Journal of Phytopathology* **152**, 358–64.

Zhang ZG, Zhang JY, Wang YC, Zheng XB, 2005. Molecular detection of *Fusarium oxysporum* f.sp. *niveum* and *Mycosphaerella melonis* in infected plant tissue and soil. *FEMS Microbiology Letters* **249**, 39–47.

Zheng XB, 1997. Phytophthora *and Methods in* Phytophthora. Beijing, China: Agricultural Press.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.