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Molecular diagnosis of *Phytophthora lateralis* in trees, water, and foliage baits using multiplex polymerase chain reaction

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Summary

A polymerase chain reaction (PCR)-based protocol for detection of *Phytophthora lateralis* in plant tissues and water is described. Base-pair (bp) deletions in both of the ribosomal DNA internal transcribed spacer (ITS) regions in *P. lateralis* were used to design complementary PCR primer sequences that amplify a 738 bp fragment only if *P. lateralis* DNA is present in the sample. Universal control primers based on conserved sequences of the nuclear ribosomal small subunit are included in a multiplexed reaction, providing an internal check on the procedure. The universal primers amplify an approximately 550 bp fragment that is common to plants, protists, and true fungi. The procedure reliably detects *P. lateralis* in cedar stem tissues and in roots. Positive reactions were obtained with as few as 200 *P. lateralis* zoospores in water.

1 Introduction

Since the 1920s, *Phytophthora lateralis* Tuck. and Milb. has been killing Port-Orford-cedar [POC, *Chamaecyparis lawsoniana* (A. Murr.) Parl.] trees in the Pacific North-west (HANSEN et al. 2000), and the pathogen was recently confirmed from French horticultural nurseries (HANSEN et al. 1999). Intensive efforts are underway to halt further spread in the forests of western North America. It is also a dangerous pathogen of international quarantine concern. However, the diagnosis of the disease in critical situations and specific confirmation of the pathogen have both been difficult.

The disease was first reported in nurseries growing POC for ornamental plantings near Seattle Washington (ZOBEL et al. 1985). *Phytophthora lateralis* continues to spread and kill in the old hedgerows and landscape trees along the West Coast, and POC is no longer an important part of the nursery trade. In 1952 *P. lateralis* was first reported near Coos Bay in south-west Oregon, in the native POC range (ROTH et al. 1957). Within a few years dead cedars were appearing throughout the lowland forests of southern Oregon and northern California. The spread into the mountains was slower, but as road building and timber harvest accelerated in the 1960s and 1970s, *P. lateralis* followed.

Zoospores initiate infection in the fine roots, and the hyphae then grow up the roots in the inner bark. The pathogen kills the root as it advances. Trees are colonized rapidly, and typically exhibit an advancing margin of red-brown necrotic phloem extending 50 cm or more above ground. Seedlings are killed within weeks of infection, and large forest trees are usually dead within a year of first crown symptoms. The infected trees are colonized by secondary fungi and bark beetles within the season of death, however, and the distinctive necrotic phloem margin is no longer visible.

Although *P. lateralis* may persist in roots and root fragments for years after a tree is killed, direct isolation is usually unsuccessful after 1 year (HANSEN and HAMM 1996). Population levels of the pathogen in the soil are very low (< 2 propagules/g), and it can not be recovered by normal soil dilution plating procedures. However, baiting of the organic

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fraction of infested soil using cedar foliage baits may be successful (OSTROFSKY et al. 1977). The pathogen grows very slowly in culture (about 2 mm/day) and is sensitive to hymexazol, the antibiotic commonly used to inhibit faster growing *Pythium* species in selective media.

At present, the field diagnosis of cedar root disease relies on the fact that there are few other lethal pathogens of POC active in the forest (HEPTING 1971). Symptoms are distinctive only in dying or very recently dead trees where the margin between living and dead phloem tissue can be seen. Confirmation that *P. lateralis* killed long dead trees, or is present in soil, water or on equipment, is hampered by difficulties in direct isolation, small sample size, and detection methods that rely on time-consuming bioassays of unknown sensitivity. Other pathogens may be involved in horticultural situations, notably *P. cinnamomi* (TORGESON et al. 1954), and *Phytophthora gonapodyides* is widely present in forest streams, although not associated with any disease (E. M. HANSEN, unpublished data). Even when isolated into culture, identification can be difficult for those unfamiliar with the distinctive features of *P. lateralis*. For these reasons we have developed a rapid, specific, sensitive molecular diagnostic protocol for identification of *P. lateralis* in various substrates.

Many species of *Phytophthora* can be identified from unique DNA sequences in the internal transcribed spacer (ITS) regions of their nuclear ribosomal DNA (COOKE and DUNCAN 1997). In this assay base-pair (bp) deletions in both of the ITS regions in *P. lateralis* were used to design complementary polymerase chain reaction (PCR) primer sequences that amplify a 738 bp fragment only if *P. lateralis* DNA is present in the sample. This approach has previously been demonstrated to detect *P. citricola* in European forest trees (SCHUBERT et al. 1999). Because the lack of a 738 bp PCR product in our test is taken as evidence that *P. lateralis* DNA is not present within the limits of detectability, endogenous control primers were included as a check on the success or failure of the PCR reaction. The control primers are based on conserved sequences of the nuclear ribosomal small subunit and amplify an approximately 550 bp fragment of this region that is common to plants, protists, and true fungi. This paper reports the initial tests of the *P. lateralis* primer set to demonstrate its specificity and ability to detect the pathogen in various substrates.

2 Materials and methods

2.1 Primer design and specificity

Isolates from the Oregon State University (OSU) *Phytophthora* collection were grown on corn meal agar and mycelium was scraped from the agar surface. The collected mycelium was placed into 2 ml microfuge tubes with two 5 mm glass beads, frozen in liquid nitrogen, and pulverized in a Mini-Beadbeater (Biospec Products, Bartlesville, OK, USA) for 30 s at 4200 r.p.m. After pulverization, the samples were incubated in CTAB extraction buffer (2% (w/v) CTAB (cetyltrimethylammonium bromide), 100 mM Tris, pH 8.0, 20 mM Na_2EDTA pH 8, 1.4 M NaCl, 1% (w/v) polyvinylpolypyrrolidone, 0.1% (v/v) 2-mercaptoethanol) at 65°C for 30 min. The DNA was purified in 24 : 1 chloroform : isoamyl alcohol and precipitated from the aqueous phase by the addition of isopropanol. After centrifugation, the DNA pellet was washed in 70% ethanol and resuspended in TE (5 mM Tris, pH 8.0, 0.5 mM Na_2EDTA). The PCR was performed in 50 μl reaction volumes (1 × enzyme buffer, 200 μM dNTP, 0.4 μM ITS primer sets ITS4 and ITS5 (WHITE et al. 1990), 2.5 U RedTaq DNA polymerase (Sigma, St. Louis, MO, USA), and 1 μl template (DNA). The reaction conditions were 35 cycles of 60 s at 94°C for denaturing, 60 s at 55°C for annealing and 60 s at 72°C for extension. After

amplification, PCR products were prepared for direct sequencing by isopropanol precipitation. Cycle sequencing in both 5′ to 3′ and 3′ to 5′ directions with primers ITS5 and ITS4 were performed using dye-terminator chemistry on an ABI model 377 fluorescent sequencer (PE Applied Biosystems, Inc., Foster City, CA, USA). Contigs were assembled and the overlapping sequences edited using the Staden package (STADEN 1996).

Complete ITS1, 5.8S, and ITS2 sequences of several *Phytophthora* species (Table 1) were aligned using CLUSTALX (THOMPSON et al. 1997) and compared for regions unique to *P. lateralis*. ITS regions from five *P. lateralis* isolates from different locations were sequenced to assess variability within the species. Forward and reverse primers (Platf: 5′-TTA GTT GGG GGC TTC TGT TC-3′, Platr: 5′-AGC TGC CAA CAC AAA TTT C-3′) were designed to span deletions in the *P. lateralis* ITS1 and ITS2 regions, respectively. These deletions were not present in the other *Phytophthora* species included in the alignment.

2.2 Sample collection

We tested the assay in POC stem phloem, roots, surrounding soil, and in water. The lower stem bark was scraped from living cedar trees in order to find the margin between healthy and diseased tissue in the phloem. The trees were infected with *P. lateralis* or

Table 1. **Isolates and ITS DNA sequences used to design *P. lateralis* specific primers**

Phytophthora species	Source[3]	Accession number
P. gonapodyides	OSU-Oregon	107
P. 'europaea'[1]	OSU-France	2ae2
P. citricola	OSU-France	2ae5
P. megasperma complex	OSU-France	8carp
P. citricola	OSU-France	Ab2
P. gonapodyides	OSU-France	Ab4
P. megasperma complex	OSU-France	Aecjvi
P. megasperma	OSU-France	Bk1
P. species unknown[2]	OSU-France	C32b
P. species unknown[2]	OSU-France	Edr6
P. cactorum	GenBank	L41357
P. cambivora	GenBank	AJ007040
P. cinnamomi	GenBank	L41374
P. citricola	GenBank	L41375
P. cryptogea	GenBank	L41376
P. fragariae fragariae	GenBank	Y08665,Y0866
P. gonapodyides	GenBank	AJ007368
P. megasperma	GenBank	L41381
P. nicotiana	GenBank	L41383
P. palmivora	GenBank	L41384
P. syringae	GenBank	L41386
P. vignae	GenBank	L41388
P. lateralis	OSU-Oregon	Pc95
P. lateralis	OSU-France	Plat1
P. lateralis	OSU-Oregon	Plat19
P. lateralis	OSU-Oregon	Plat20
P. lateralis	OSU-France	Plat9800

[1]Undescribed species, related to *P. cambivora*.
[2]Unidentified *Phytophthora* species.
[3]'OSU' indicates that isolates from the country named are in the OSU collection.

P. cinnamomi, as confirmed by direct isolation onto *Phytophthora* selective medium. Bark samples were collected from above and below the margin of infection. Portions about 2 mm × 3 mm were cut out for DNA extraction. POC seedlings were inoculated with *P. lateralis* by dipping their roots in a suspension of zoospores. After 5 weeks incubation in the greenhouse, approximately 1 g of fibrous roots were cut from each potted seedling.

Soil samples were collected from beneath dead and dying POC trees in the forest. The trees were still green and exhibited a clear margin between infected and healthy tissues, or had been dead for 1 or 2 years as judged by foliage deterioration. As we have so far been unsuccessful at extracting *P. lateralis* DNA directly from soils (data not shown) we used a baiting technique to trap the fungus (OSTROFSKY et al. 1977). After fresh POC foliage segments about 2 cm long were floated on flooded soil samples for 3–7 days, the baits were removed and prepared for DNA extraction. Water samples were collected from forest streams. Some samples were spiked with known numbers of *P. lateralis* zoospores. These were obtained by growing the fungus for 7 days in pea broth, rinsing the colonies in distilled water then reflooding them with filtered stream water, and incubating them for an additional 24–48 h. Water samples with and without the addition of *P. lateralis* were filtered through 3 μm filters (Millipore Corp., Bedford MA, USA) to collect zoospores and other propagules and the filters were then extracted or plated on *Phytophthora*-selective medium. The volume of water filtered varied; the results are presented as number of zoospores per filter unless otherwise noted.

2.3 DNA extraction

All samples were extracted immediately after collection. To extract DNA, foliage baits were frozen in liquid nitrogen, ground in a beadmill, and incubated at 65°C for 1 h in CTAB extraction buffer. After chloroform extraction, the DNA was precipitated with isopropanol, and resuspended in TE. DNA extractions directly from POC root and bark samples were performed similarly, except that they were further purified to reduce PCR inhibitors by passing the extract over QiaAmp Spin Columns (Qiagen Inc., Valencia, CA, USA). Membranes from water samples were frozen in liquid nitrogen, ground in a beadmill, and boiled in 200 μl of Instagene Matrix (BioRad, Hercules, CA, USA).

2.4 Multiplex PCR

The PCR was performed in 15 μl multiplex reactions (1 × enzyme buffer, 200 μM dNTP, 0.4 μM *P. lateralis* primers (Platf and Platr), 0.1 μM universal internal control primers (NS1 and NS2, WHITE et al. 1990), 0.8 U RedTaq DNA polymerase, and 1 μl template (DNA). The thermal cycler was programmed for 30 s annealing at 52°C and 1 min extension at 72°C. The PCR products were electrophoresed on 2% (w/v) agarose gels in Tris-Borate-EDTA buffer (pH 8.6) and visualized with ethidium bromide under UV light. The fragment sizes were estimated by comparison with a 100 bp DNA ladder (MBI Fermentas Inc., Amherst, NY, USA).

3 Results

3.1 *Phytophthora lateralis* ITS sequence

The ITS1, 5.8S, and ITS2 sequences were identical in all five *P. lateralis* isolates. Sequence data for isolate Plat9800 were submitted to the GenBank library under the accession number AF287256.

3.2 Specificity

The expected 550 bp band from the universal primer set used as an endogenous control was present in all lanes (Fig. 1), but the 738 bp *P. lateralis* band was present only in DNA amplified from known cultures of *P. lateralis*. The 738 bp band was absent in the other species tested, including those most likely to be found with *P. lateralis*. The 'universal band' was often faint where DNA of *P. lateralis* was present at high concentration, as in isolation from mycelium, due to primer-limited competition for the substrate in the PCR reaction.

3.3 Detection and sensitivity

Phytophthora lateralis was detected in POC bark samples collected from areas of the stem exhibiting the characteristic stain associated with *P. lateralis* infection, but not from unstained samples collected beyond the advancing front of colonization in the phloem (Fig. 2). Only the endogenous control band was visible from trees infected with *P. cinnamomi*. Seedling roots (Fig. 3) were always positive for the endogenous control band, indicating a successful PCR reaction. The infected roots from inoculated seedlings showed the *P. lateralis* band, but roots from healthy seedlings did not.

As we have not been able to recover *P. lateralis* DNA directly from soil samples, we baited soils with POC foliage and tested the baits for the pathogen. *P. lateralis* mycelium,

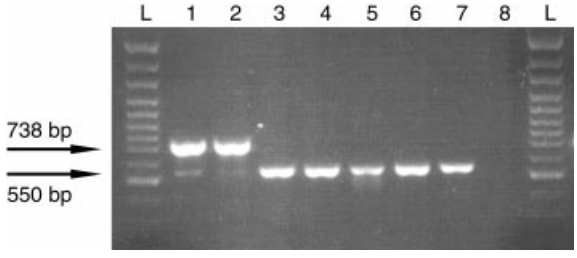

Fig. 1. Specificity. Multiplex PCR demonstrating specificity of the *P. lateralis*-specific primers (738 bp band) and universal amplification of the endogenous control primers (550 bp band). Lanes (1–2) *P. lateralis*; (3) *P. drechsleri*; (4) *P. cryptogea*; (5) *P. gonopodyides*; (6) *P. fragariae*; (7) *P. cinnamomi*; (8) water control; (L) 100 bp ladder

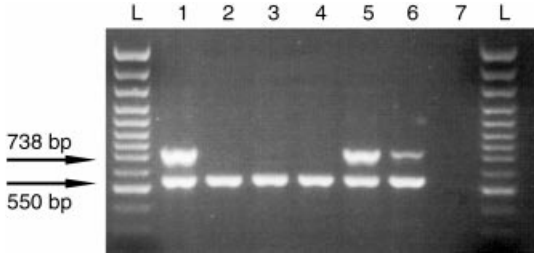

Fig. 2. Tree bark. Multiplex PCR detection of *P. lateralis* (738 bp band) and endogenous control (550 bp band) in DNA extracted from POC stem tissue. Lane (1) *P. lateralis* control mycelium; (2) uninfected POC bark; (3–4) bark infected with *P. cinnamomi*; (5–6) bark infected with *P. lateralis*; (7) water control; (L) 100 bp DNA ladder

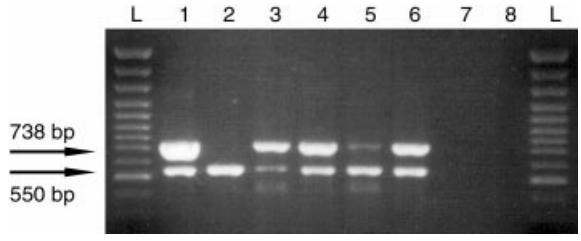

Fig. 3. Seedling roots. Multiplex PCR detection of *P. lateralis* (738 bp band) and endogenous control (550 bp band) in DNA extracted from POC seedling roots. Lane (1) *P. lateralis* control mycelium; (2) uninfected seedling roots; (3–6) seedling roots infected with *P. lateralis*; (7–8) water control; (L) 100 bp ladder

healthy POC foliage, and sterile water were used as assay controls. The endogenous control band was present in all lanes except the water control and lane 4 (Fig. 4). In the latter case, the missing endogenous control band indicated that the PCR reaction failed, probably due to the presence of PCR inhibitors in the DNA sample. Positive *P. lateralis* bands resulted from soil samples collected beneath symptomatic and recently dead trees, but not from the soil sample from beneath a tree that had been dead for 2 years.

Zoospore infection is an important means of pathogen dispersal and so two experiments were designed to test the PCR assay on water samples. Zoospores were captured on filters and the filters were subjected to the PCR assay (Figs 5 and 6). DNA samples that had been extracted from mycelium of *P. lateralis* and *P. gonapodyides* were included as controls. In the first demonstration (Fig. 5), zoospore dilutions were added to sterile water and filtered. As few as 340 zoospores per filter gave positive reactions for the *P. lateralis* band, whereas samples with fewer zoospores were negative. The second test (Fig. 6) was conducted on stream water collected from a source known to contain *P. gonapodyides* and other *Phytophthora* species, but not *P. lateralis*. Filters plated directly on selective media revealed about 25 *Phytophthora* propagules per litre; *P. lateralis* was not cultured. The weak endogenous control bands resulted from other microorganisms present in the non-sterile stream water and indicated successful reactions in all stream samples. Zoospore dilutions were added to stream water to determine the limits of detectability of the PCR assay in stream water. The *P. lateralis* band was present only in samples spiked with 200 or more *P. lateralis* zoospores.

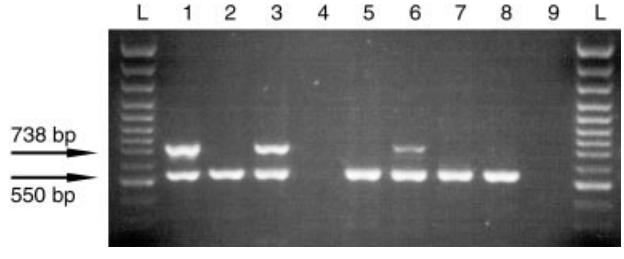

Fig. 4. Foliage baits. Multiplex PCR detection of *P. lateralis* (738 bp band) and endogenous control (550 bp band) in DNA extracted from POC foliage baits floated on flooded soil samples. Lane (1) *P. lateralis* control mycelium; (2) uninfected foliage; (3–4) soil collected from root zone of dying tree; (5–6) soil collected from root zone of tree that had been dead for 1 year; (7–8) soil collected from root zone of tree that had been dead for 2 years; (9) water control; (L) 100 bp ladder. Lane 4 is a failed PCR reaction

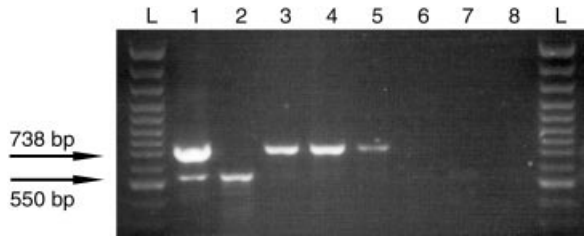

Fig. 5. Distilled water. Multiplex PCR detection of *P. lateralis* (738 bp band) and endogenous control (550 bp band) in DNA extracted from membrane filters used to trap propagules from distilled water spiked with zoospores. Lane (1) *P. lateralis* control mycelium; (2) *P. gonopodyides* control mycelium; (3) 34 000 zoospores per filter; (4) 3400 zoospores; (5) 340 zoospores; (6) 34 zoospores; (7) 3.4 zoospores; (8) water control; (L) 100 bp ladder

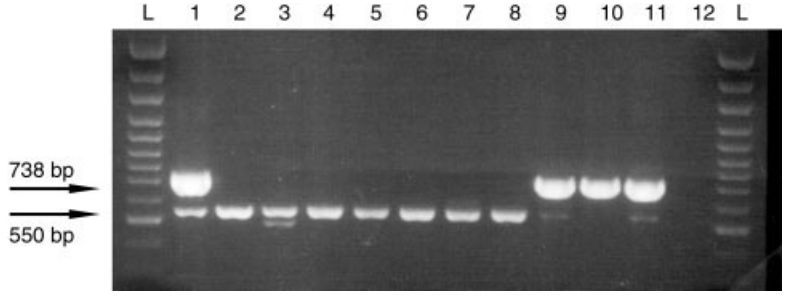

Fig. 6. Stream water. Multiplex PCR detection of *P. lateralis* (738 bp band) and endogenous control (550 bp band) on membrane filters used to trap propagules from stream water and stream water spiked with zoospores. Lane (1) *P. lateralis* control mycelium; (2) *P. gonopodyides* control mycelium; (3–4) unaltered stream water; (5–6) two zoospores per filter; (7–8) 20 zoospores; (9) 200 zoospores; (10) 2000 zoospores; (11) 20 000 zoospores; (12) water control; (L) 100 bp ladder

4 Discussion

The PCR method described here allows the rapid identification of *P. lateralis* in culture and in POC stem and root tissue, as well as in soils that were baited with cedar foliage. The assay should also be useful for confirming the presence of *P. lateralis* in other situations where direct isolation is difficult, such as from the fine fibrous roots of POC seedlings and the very thin bark of Pacific yew, which is also a host to *P. lateralis* in the forest (DeNitto and Kliejunas 1991; Murray and Hansen 1997). The present method does not work reliably on soils, but perhaps this could be remedied with further developmental research. For example, new soil DNA extraction kits are now available from several molecular biology companies. The method does provide rapid detection of *P. lateralis* in the foliage baits currently used to detect the pathogen in soil. This approach reduces the time for soil tests relying on culturing the fungus by several days, and increases the detection limits as fast-growing fungi will not interfere with the PCR.

The assay detects zoospores of *P. lateralis* in water that have been concentrated on membrane filters. In preliminary trials (data not shown), however, the pathogen has not been detected from forest streams flowing through stands of dead and dying POC. Presumably the concentration of *P. lateralis* propagules in the stream was below the detection limits for the assay.

The specificity of the assay has been demonstrated with a limited number of other *Phytophthora* species, including those that have been encountered with *P. lateralis* in nature. In addition, published ITS sequences of other *Phytophthora* species differ in the diagnostic region and these species will not react with the *P. lateralis* primers [See note added in proof, below].

Although the PCR approach is more sensitive than direct plating in some situations, it still suffers from the challenge posed by the small amount of tissue that can be analysed in any one sample. In cases where the number of propagules is low, as in soil or trees that have been dead for several years, it may take multiple samples to reveal the pathogen. Alternatively, propagules may be concentrated by baiting, or filtering larger volumes of water. Confidence in the methodology is gained by adding the universal primer in a multiplex PCR reaction. Future adaptation of the primers to real-time PCR will allow quantitative measurements of *P. lateralis* in various substrates, and may increase the sensitivity of the assay.

[Note added in proof] The primers and protocol described here are also useful for detection of the undescribed species of *Phytophthora* that causes 'sudden oak death' (SOD) in California. The aetiology of this new disease was unknown when the *P. lateralis* diagnosis work was carried out. Now that it is known that the new *Phytophthora* is closely related to *P. lateralis*, with a very similar ITS sequence (Rizzo and Garbelotto, unpublished data. See http://camfer.CNR.Berkeley.EDU/oaks/). The diagnostic protocol gives a positive reaction for pure cultures of the SOD *Phytophthora*, and detects the pathogen in bark samples taken from lesions on naturally infected tanoak (*Lithocarpus densiflorus*) trees (data not shown). This expanded specificity demonstrates a theoretical limitation to the diagnostic protocol for *P. lateralis*, but since the SOD *Phytophthora* does not cause expanding lesions on POC, there should be no confusion in results so long as hosts are specified. Further, the diagnostic test may prove valuable for detection and management efforts with SOD.

Résumé

Diagnostic moléculaire par PCR multiplex pour détecter Phytophthora lateralis dans les arbres, l'eau et le feuillage utilisé comme piège

Un protocole basé sur la PCR est décrit pour détecter *Phytophthora lateralis* dans les tissus végétaux et l'eau. Des délétions de paires de bases dans chacune des régions ITS de l'ADN ribosomal de *P. lateralis* ont été utilisées pour définir des amorces de PCR qui n'amplifient un fragment de 738 paires de bases que si l'ADN de *P. lateralis* est présent dans l'échantillon. Des amorces universelles basées sur des régions conservées de la petite sous-unité de l'ADN ribosomal nucléaire ont été incluses dans une réaction de PCR multiplex, fournissant ainsi un témoin interne de la réaction. Ces amorces universelles amplifient un fragment de 550 pb qui est commun aux plantes, aux protistes et aux champignons vrais. Ce protocole permet la détection de *P. lateralis* dans les tiges et dans les racines du *Chamaecyparis*. Des réactions positives ont été obtenues avec seulement 200 zoospores de *P. lateralis* dans l'eau.

Zusammenfassung

Molekulare Diagnose von Phytophthora lateralis in Bäumen, Wasser und als Köder benutzten Blättern mittels Multiplex-PCR

Eine auf der PCR beruhende Methode zum Nachweis von *Phytophthora lateralis* in Pflanzengeweben und Wasser wird beschrieben. Deletionen in den beiden ITS Regionen der ribosomalen DNA von *P. lateralis* wurden zur Synthese von PCR-Primern ausgenutzt, die ein 738 Basenpaare langes Fragment nur dann amplifizieren, wenn *P. lateralis* in der Probe vorhanden ist. Universelle Primer, die konservierten Sequenzen der kleinen Unterheit der ribosomalen Kern-DNA entsprechen, wurden als interne Kontrollen in die Multiplex-PCR miteinbezogen. Diese Primer amplifizieren ein ungefähr 550 Basenpaare langes Fragment, das sowohl bei Pflanzen als auch bei Protisten und höheren Pilzen vorkommt. Mit der Methode liess sich *P. lateralis* im Stamm und in den Wurzeln von Lawsons Scheinzypresse verlässlich nachweisen. Für den Nachweis von *P. lateralis* im Wasser waren mindestens 200 Zoosporen nötig.

References

COOKE, D. E. L.; DUNCAN, J. M., 1997: Phylogenetic analysis of *Phytophthora* species based on ITS 1 and ITS 2 sequences of the ribosomal RNA gene repeat. Mycol. Res. **101,** 667–677.

DENITTO, G.; KLIEJUNAS, J. T., 1991: First report of *Phytophthora lateralis* on Pacific yew (Abstract). Plant Dis. **75,** 968.

HANSEN, E. M.; HAMM, P. B., 1996: Survival of *Phytophthora lateralis* in infected roots of Port-Orford cedar. Plant Dis. **80,** 1075–1078.

HANSEN, E. M.; DELATOUR, C.; STREITO, J.-C., 1999: *Phytophthora lateralis* in Europe. Plant Dis. **83,** 587.

HANSEN, E. M.; GOHEEN, D. J.; JULES, E. S.; ULLIAN, B., 2000: Managing Port-Orford-cedar and the introduced pathogen *Phytophthora lateralis*. Plant Dis. **84,** 4–14.

HEPTING, G. H., 1971: Diseases of Forest and Shade Trees of the United States. USDA Forest Service Agric. Handbook No. 386. Washington, DC.

MURRAY, M. S.; HANSEN, E. M., 1997: Susceptibility of Pacific yew to *Phytophthora lateralis*. Plant Dis. **81,** 1400–1404.

OSTROFSKY, W. D.; PRATT, R. G.; ROTH, L. F., 1977: Detection of *Phytophthora lateralis* in soil organic matter and factors that affect its survival. Phytopathology **67,** 79–84.

ROTH, L. F.; TRIONE, E. J.; RUHMANN, W. H., 1957: *Phytophthora*-induced root rot of native Port-Orford-cedar. J. For. **55 (April),** 294–298.

SCHUBERT, R.; BAHNWEG, G.; NECHWATAL, J.; JUNG, T.; COOKE, D. E. L.; DUNCAN, J. M.; MULLER-STARCK, G.; LANGEBARTELS, C.; SANDERMANN. H. Jr; OßWALD, W., 1999: Detection and quantification of *Phytophthora* species which are associated with root-rot diseases in European deciduous forests by species-specific polymerase chain reaction. Eur. J. For. Path. **29,** 169–188.

STADEN, R., 1996: The Staden sequence analysis package. Mol. Biotechnol. **5,** 233–241.

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. Nucl. Acids Res. **24,** 4876–4882.

TORGESON, D. C.; YOUNG, R. A.; MILBRATH, J. A., 1954: *Phytophthora* Root Rot Diseases of Lawson Cypress and Other Ornamentals. Agric. Exp. Stat. Oregon State College. Station Bull. No. 537. Corvallis, OR.

WHITE, T. J.; BRUNS, T.; LEE, S.; TAYLOR, J., 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a Guide to Methods and Applications. Ed. by INNIS, M. A.; GELFAND, D. H.; SNINSKY, J. J.; WHITE, T. J. New York: Academic Press Inc. pp. 315–321.

ZOBEL, D. B.; ROTH, L. F.; HAWK, G. M., 1985: Ecology, Pathology, and Management of Port-Orford-cedar (*Chamaecyparis lawsoniana*). USDA Forest Service Gen. Tech. Rep. No. PNW-184. Portland, OR.