For. Path. 35 (2005) 305–314

Differentiating the two closely related species, *Phellinus weirii* and *P. sulphurascens*

By Y. W. Lim[1], Y. C. A. Yeung[1], R. Sturrock[2], I. Leal[2] and C. Breuil[1]

[1]Department of Wood Science, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada. E-mail: colette.breuil@ubc.ca; [2]Canadian Forest Service, Pacific Forestry Centre, Victoria, BC, Canada

Summary

Phellinus weirii s.l., an aggressive root rot pathogen, causes extensive wood losses and lowers the productivity of western red cedar (WRC, *Thuja plicata*), Douglas fir (*Pseudotsuga menziesii*) and other conifers. This fungus has been recognized as a cedar form (*P. weirii* s.s.) and a non-cedar form (*P. sulphurascens*). Differentiating the two species is difficult because their fruiting bodies and cultural morphologies are very similar. However, differences in growth rate and colony morphology were observed when they were grown on malt extract agar with WRC feeder strips. In addition, different restriction fragment length polymorphism patterns were obtained using (i) the internal transcribed spacer (ITS) region cut with the restriction enzyme *Rsa*I, and (ii) the partial large subunit ribosomal DNA region cut with *Age*I and *Nci*I. Furthermore, a new specific primer set was designed from the ITS region of *P. weirii* s.s. and was used to differentiate it from *P. sulphurascens* and other decay fungi that are frequently found in coniferous trees. These species-specific primers will facilitate the detection of *P. weirii* in standing trees well before visible signs of infection are apparent.

1 Introduction

Phellinus weirii (Murr.) Gilb. is an aggressive root rot pathogen that causes butt rot of western red cedar (WRC, *Thuja plicata* Donn.) and laminated root rot of several conifers including Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Larsen et al. 1994; Thies and Sturrock 1995). The geographical range of this root rot pathogen stretches along the Pacific coast from northern California to south-eastern Alaska. It has also been reported in Japan, Siberia and China (Aoshima 1953; Parmasto and Parmasto 1979; Dai 2004). The fungus causes extensive wood losses and reduces forest productivity (Hansen and Goheen 2000). Its predominant mode of spread is by way of vegetative mycelia, which initially grow along the root surfaces and move from infected to healthy roots when the roots come in contact (Wallis and Reynolds 1965). After penetration of sound or injured root bark, the fungus kills phloem and cambial tissues and initiates decay of xylem tissues. Infected trees with such weakened root systems are susceptible to wind-throw and bark beetle attack (Hansen and Goheen 2000). The longevity of the fungus on infested sites is enhanced by its ability to survive up to 50 years in stumps of fallen or cut trees (Hansen 1979).

Phellinus weirii was first described in 1914 on WRC (Murrill 1914). Subsequently, the host range of the fungus was extended to other coniferous species including Douglas fir (Larsen and Cobb-Poulle 1990; Allen et al. 1996). Since 1940, mycologists have recognized two different forms of the fungus: a cedar form and a non-cedar form. The cedar form causes root and butt rot, particularly in old-growth cedar; however, trees are rarely killed by this fungus (Allen et al. 1996). In contrast, the non-cedar form usually

Received: 26.11.2004; accepted: 22.02.2005; editor: O. Holdenrieder

kills its hosts. The cedar form has been named *P. weirii*, perennial *P. weirii*, and Thuja form. The non-cedar form has been known as annual *P. weirii*, *P. sulphurascens* Pilat, Douglas fir form, and non-Thuja form (Mounce et al. 1940; Buckland et al. 1954; Clark 1958; Lowe 1966; Kotlaba and Pouzar 1970; Larsen and Cobb-Poulle 1990; Banik et al. 1993; Larsen et al. 1994). Wagner and Fischer (2002) suggested new combination of *Phellinidium weirii* and *Phellinidium sulphurascens* based on the large subunit (LSU) phylogeny. However, the two species did not group with the other *Phellinidium* species in their phylogenetic tree. Therefore, we followed the recommendation of Larsen and Cobb-Poulle (1990) and used *P. weirii* for the cedar form and *P. sulphurascens* for the non-cedar form in this paper.

Although they have different ecological preferences and cause somewhat different pathological symptoms, the differentiation of these two species can be difficult. Fruiting bodies, which could be a key element in the identification of these fungi, are relatively rare and do not necessarily, occur each year nor at exactly the same time of year. Previous investigators have developed various techniques including morphological, physiological, serological and molecular methods to assist differentiating the two forms (Buckland et al. 1954; Clark 1958; Angwin 1989; Angwin and Hansen 1993; Banik et al. 1993; Bae et al. 1994; Larsen et al. 1994; Hansen et al. 1998). However, these methods do not always give clear answers and can be time consuming, labour intensive, and technically difficult.

Alternative procedures such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the ribosomal DNA (rDNA) region and PCR using species-specific primers have been used very effectively for detecting or identifying fungal pathogens both in laboratory cultures and in plant tissues (Willits and Sherwood 1998; Turenne et al. 1999; Hamelin et al. 2000; Germain et al. 2002). In this paper, we developed PCR-RFLP and a multiplex PCR using both fungal universal primer and specific primers to *P. weirii* and compared these methods to a biological assay.

2 Materials and methods

2.1 Fungal strains and culture on WRC feeder strips

Western red cedar wood for preparation of feeder strips was taken from a 60-year-old tree. The feeder strips ($5 \times 3 \times 0.2$ cm) were taken from the same location in the outer heartwood. To minimize volatilization or alteration of the extractives, feeder strips were sterilized by γ-radiation. The eight *P. sulphurascens* and eight *P. weirii* strains used in this study are listed in Table 1. All strains of *P. sulphurascens* and *P. weirii* were cultured on 2% malt extract agar (MEA). Each agar disk (5 mm in diameter) was taken from the edge of a freshly grown colony, and placed near the rim of a 2% MEA plate. A total of four plates per strain were obtained. When growth was first observed, feeder strips were placed opposite to the plug on the agar in two of the four plates per strain. Controls were plates with no feeder strips. All plates were incubated at 20°C in the dark. Measurements were taken 10 days after inoculation. The growth of each culture was measured from the inoculums to the growing edge of the colony. Two measurements were taken from each strain and averaged. Growth rates between the controls and experimental plates for each strain were compared.

2.2 DNA extraction, PCR amplification and sequencing

Total DNA was extracted from mycelia grown on MEA plates as described by Lim et al. (2005). Extracted DNA was electrophoresed through a 0.7% agarose gel in TAE buffer, stained with ethidium bromide and visualized with UV light. The internal transcribed spacer (ITS) and LSU regions were amplified using the primer sets, ITS5/ITS4 (White

Table 1. **List of strains used in this work and their host and locality**

Fungi	Source[1]	Host	Locality
Phellinus weirii	CFS-504	*Thuja plicata*	Salmon Arm, BC, Canada
	CFS-545	*T. plicata*	Slocan, BC, Canada
	CFS-549	*T. plicata*	Fauquier, BC, Canada
	CFS-550	*T. plicata*	Fauquier, BC, Canada
	CFS-586	*T. plicata*	Smithers, BC, Canada
	Forintek-129A	*T. plicata*	Valley, BC, Canada
	Forintek-175	*T. plicata*	Thompson River, BC, Canada
	CFS-501	*T. plicata*	Wap Lake, BC, Canada
P. sulphurascens	CFS-511	*Pseudotsuga menziesii*	Chilliwack, BC, Canada
	CFS-543	*P. menziesii*	Nelson, BC, Canada
	CFS-556	*P. menziesii*	Kamloops, BC, Canada
	CFS-581	*P. menziesii*	Cowichan Lake, BC, Canada
	CFS-600	*P. menziesii*	Campbell River, BC, Canada
	CFS-538	*Pinus contorta*	Boston Bar, BC, Canada
	CFS-503	*Pinus monticola*	Cowichan Lake, BC, Canada
	CFS-509	*Tsuga heterophylla*	Chilliwack, BC, Canada
P. viticola	CBS381.82	*Picea abies*	Germany
P. ferreus	CBS444.48	*P. menziesii*	BC, Canada
P. ferrugineofuscus	CBS299.33		UK
P. nigrolimitatus	CBS214.48		Canada
P. pini	UAMH8177	*Picea glauca*	BC, Canada
Antrodia carbonica	BUBC1001	*T. plicata*	Vancouver, BC, Canada
Athelia sp.	BUBC1002	*T. plicata*	Vancouver, BC, Canada
Perenniporia subacida	BUBC1003	*T. plicata*	Maple Ridge, BC, Canada

[1]CFS, Canadian Forest Service, Pacific Forestry Centre, Victoria, British Columbia, Canada; Forintek, Forintek Canada Corp.; CBS, the culture collection of Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; UAMH, the University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Canada; BUBC, Colette Breuil's Culture Collection, the University of British Columbia, Canada.

et al. 1990) and LROR/LR3 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR amplification was performed as described by Lee et al. (2000). The PCR product sizes were determined in 0.5% agarose gels by comparison to a 1 kb DNA marker (Gibco BRL, Rockville, MD, USA). The PCR products were purified using a Qiaquick PCR Purification Kit (Qiagen Inc., Mississauga, ON, Canada). Sequencing was performed on an ABI 3700 automated sequencer (Perkin-Elmer Inc., Foster City, California, USA) at the DNA synthesis and Sequencing Facility, Macrogen (Seoul, Korea). The sequences were proofread, edited, and merged into comparable sequences using the PHYDIT program version 3.2 (http://plasza.snu.ac.kr/~jchun/phydit/). The sequences were submitted to GenBank. The accession numbers for the ITS are AY829341 (CFS-504), AY829342 (CFS-586), AY829343 (CFS-511) and AY829344 (CFS-543). The accession numbers for the LSU rDNA are AY829345 (CFS-504), AY829346 (CFS-586), AY829347 (CFS-511) and AY829348 (CFS-543).

2.3 RFLP marker to distinguish *Phellinus weirii* and *Phellinus sulphurascens* and *Phellinus weirii*-specific primers amplification

The ITS and partial LSU rDNA region sequences were analysed using Gene Tool (http://tools.neb.com/NEBcutter2/index.php) to search for a potential RFLP marker. After restriction maps of the sequences were defined, *Rsa*I from ITS region, and *Age*I and *Nci*I from LSU were selected as they can differentiate *P. weirii* from *P. sulphurascens*. For each

strain, 10 μl of the amplified products were digested with each restriction enzyme as described by the manufacturer. The restriction fragments were separated on a 2% agarose gel stained with ethidium bromide and determined by comparison to a 1 kb DNA molecular marker.

A specific primer set for *P. weirii* was designed from the ITS region. They were named PW164 with the sequence, 5′-GCT TCC ATT TTT CTT AGG-3′ (forward) and PW659 with the sequence, 5′-TCA AAA GGG CGT ATT AAT G-3′ (reverse). With these primers, a 495-bp fragment is expected to be amplified. PCR amplifications with the specific primers were carried out in a thermal cycler (MJ Research Inc., Watertown, USA) as follows: 4 min at 94°C, 25 cycles of denaturation at 94°C for 50 s, annealing at 50–60°C for 40 s, extension at 72°C for 50 s, and final extension at 72°C for 10 m. To examine the specificity of the primer set, closely related species including *P. ferreus*, *P. ferrugineofuscus*, *P. nigrolimitatus*, *P. pini*, *P. viticola* and other decay fungi that were frequently isolated from WRC such as *Perenniporia subacida*, *Antrodia carbonica*, *Athelia* sp. were also tested.

For the detection of *P. weirii* in forest trees, decay samples from WRC (16 samples), Douglas fir (eight samples), and lodgepole pine (five samples) trees were tested. After surface sterilization by rapid flaming, small woodchips taken from cross-sections of decayed wood samples were placed into microtubes for DNA extraction, and also onto MEA plates for culturing. DNA extraction was the same as described above. We performed a multiplex PCR using PW164/PW659 and LROR/LR5 to detect *P. weirii* from decayed woods. Amplification was programmed with an initial denaturing at 96°C for 4 min, followed by 30 cycles of 94°C for 50 s, 56°C for 40 s, and 72°C for 50 s, with a final extension at 72°C for 10 min. Plates for culturing the microorganisms were incubated for 1 week at room temperature.

3 Results

3.1 RFLP marker and specific PCR amplification

A single specific product was obtained from each of the eight strains of *P. sulphurascens* and *P. weirii* following PCR amplifications of either ITS or LSU rDNA regions. The amplicons of *P. sulphurascens*/*P. weirii* were 731/737 bp for the ITS region and 661/661 bp for the LSU rDNA region. Two strains of each species were sequenced in order to find the enzymatic restriction sites. Because of the high sequence similarity of the two fungal species, 98.76% for ITS and 98.94% for LSU rDNA, only a small number of endonucleases could be tested to differentiate these two species. In *P. sulphurascens* ITS, a single recognition site was digested by *Rsa*I resulting in two distinct bands of approximately 454 and 277 bp, while *P. weirii* ITS was not digested giving a single band of 737 bp. For the LSU rDNA region, *P. weirii* had one recognition site for *Age*I that produced two bands of 175 and 468 bp, whereas *P. sulphurascens* had no recognition site. LSU rDNA digested with *Nci*I generated three bands of 71, 218 and 372 bp for *P. weirii*, and two bands of 71 and 590 bp for *P. sulphurascens*. The results are shown in Fig. 1.

Even though the ITS sequences for *P. weirii* and *P. sulphurascens* were very similar, a region containing an insertion or deletion was identified in the ITS1 and ITS2 regions and was used to design *P. weirii*-specific primers PW164 and PW659. The resulting PCR products of 495 bp were observed only in *P. weirii* isolates. The specificity of the PW164/PW659 primers also was assessed on 24 strains of 10 species (Table 1). Occasionally, non-targeted fragments at about 150 bp were observed for *P. pini* at an annealing temperature of 50°C. However, these non-specific bands were absent when higher annealing temperatures (52–58°C) were used (Fig. 2).

Finally, the specific primers were used to detect the presence of *P. weirii* in 29 decayed wood samples including 16 from WRC, eight from Douglas fir, and five from lodgepole

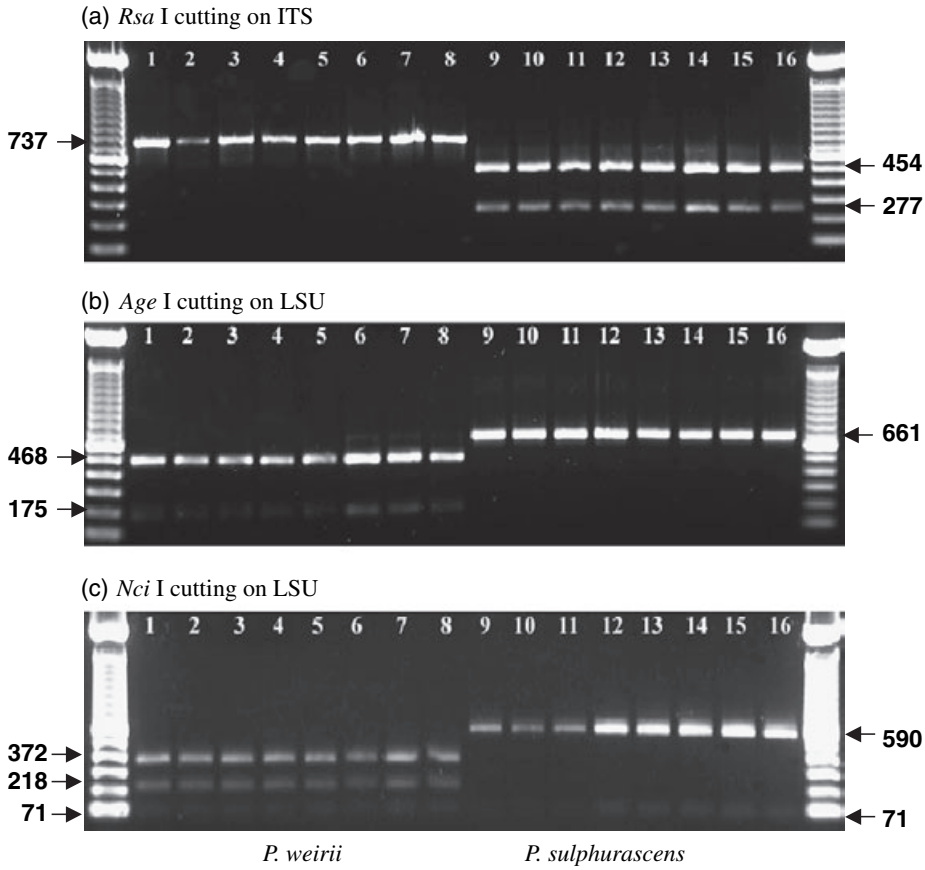

Fig. 1. PCR-RFLP profiles generated by digestion of ITS region with *Rsa*I (a), and LSU rDNA region with *Age*I (b) and *Nci*I (c). Electrophoresis was performed on 2% agarose gels at 3 V/cm. Both ends of lanes, 100-bp ladder; lanes 2–8, *Phellinus weirii*; lanes 9–16, *P. sulphurascens*

pine trees. The PCR fragments were amplified from three of 16 DNA samples extracted from decayed WRC trees. However, the extracted DNAs were amplified from all the wood samples when fungal universal primers of partial LSU region (LROR and LR5) were used. These primers serve as positive controls and demonstrate the presence of fungal DNA suitable for PCR (Fig. 2). The three PCR fragments obtained with the specific primers were purified and sequenced. The nucleotide sequences were the same as those of the *P. weirii* isolates. Wood samples from the same wood chips that were used for DNA extractions, were placed on MEA plates to recover the microorganisms present in the wood. However, we were unable to recover the slow-growing *P. weirii*, which was out competed by fast-growing fungal species like *Trichoderma* and *Penicillium* as well as other decay fungi.

3.2 Growth on WRC feeder strips

Average growth rates after 10 days for strains of *P. weirii* and *P. sulphurascens* on 2% MEA without WRC feeder strips were similar (Fig. 3a); 47.7 mm (±2.24) for *P. weirii* and

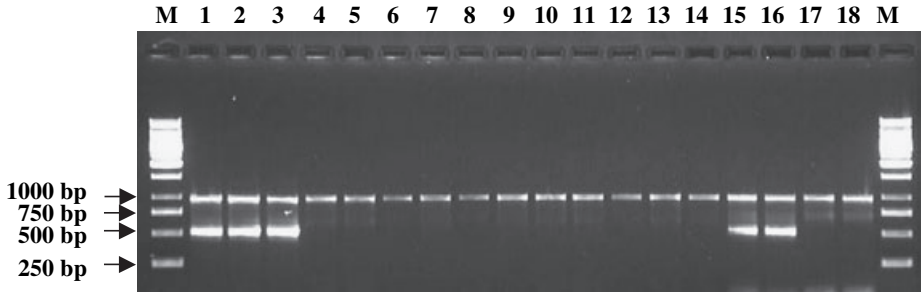

Fig. 2. A multiplex PCR amplification of ca. 950 bp using LROR/LR5 (top bands) and 495 bp DNA fragment using the *P. weirii*-specific primers PW164/PW659 (lower bands) on DNA of fungal cultures (lanes 1–14) and wood samples (lanes 15–18). Lane M: 100 bp ladder, 1–3: *P. weirii*, 4–6: *P. sulphurascens*, 7: *P. ferreus*, 8: *P. ferrugineofuscus*, 9: *P. nigrolimitatus*, 10: *P. pini*, 11: *P. viticola*, 12: *Antrodia carbonica*, 13: *Athelia* sp., 14: *Perenniporia subacida*; two examples of decayed WRC trees (15–16) and decayed Douglas fir trees (17–18)

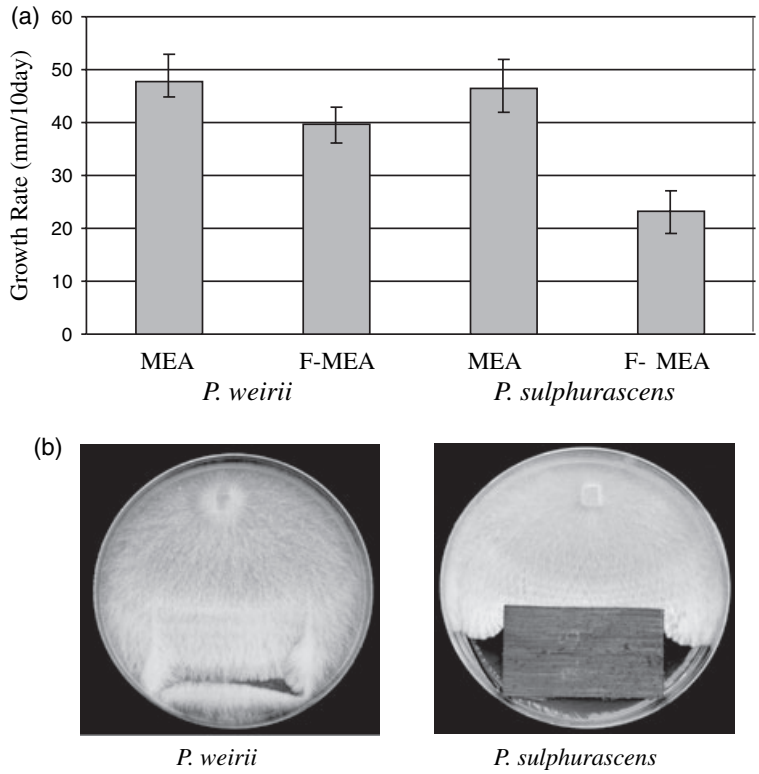

Fig. 3. A comparison of growth rates on MEA (control) *vs.* WRC feeder strip/MEA (F-MEA) (a) and biological test (b) of 17 days growth on WRC feeder strips. *Phellinus weirii* is able to grow on WRC, *P. sulphurascens* is not

46.5 mm (±2.58) for *P. sulphurascens*. When grown with WRC feeder strips, both strains showed a reduction in growth in comparison with controls, with *P. sulphurascens* growth being the most affected. With WRC feeder strips, the average growth of *P. weirii* strains was 39.8 mm (±2.19); while that of *P. sulphurascens* was 23.2 mm (±2.48). Differences in colony morphology were also observed when the feeder strip was introduced in the media (Fig. 3b). The colour of the agar changed from a light straw colour to a darkened reddish brown colour. As the cultures approached the feeder strips, the growth of *P. weirii* cultures remained steady and extended onto and covered the feeder strip. In contrast, the growth of *P. sulphurascens* was slowed and the fungus did not grow onto the strip.

4 Discussion

Our unambiguous differentiation of *P. weirii* from *P. sulphurascens* from a wide range of geographical regions demonstrated the effectiveness of PCR-RFLP analysis. PCR-RFLP analysis is more effective than classical methods. It is also more effective than existing molecular fingerprinting techniques such as isozyme banding patterns, mitochondrial and M13 fingerprinting (Bae et al. 1994). This is especially true when large numbers of samples need to be examined. It can also differentiate closely related fungal species in different taxonomic groups (Turenne et al. 1999; Lee et al. 2003). The assay can be completed within a day. The RFLP patterns can be observed visually in agarose gels without the sophisticated equipment often required for other DNA fingerprinting methods (Förster et al. 1989; Bae et al. 1994; Hansen et al. 1998). It is advantageous to amplify the ITS and LSU rDNA regions as rDNA genes occur in high copy numbers in fungal cells (Scherer and Magee 1990). Further, large numbers of sequences for these regions are available in public databases such as GenBank.

An alternative method to PCR-RFLP is the PCR amplification of species-specific sequences. This method permits rapid diagnosis of pathogens collected over large geographical areas. Simultaneous detection of important pathogens may be possible by the concomitant use of several species- or genus-specific primer pairs in the same PCR assay (Jones and Bej 1994). Species-specific primers are frequently designed from ITS sequences (Chen et al. 1996; Lecomte et al. 2000; Bahnweg et al. 2002; Germain et al. 2002). ITS regions show high levels of interspecific polymorphisms, and multiple copies are present in fungal cells. This permits their amplification from minute amounts of DNA (Gardes and Bruns 1993). The ITS-specific primers designed in this work amplified only *P. weirii* strains. No cross-reactions were observed using this PCR assay with phylogenetically related *Phellinus* species and with other decay fungi frequently isolated from WRC. For example, even though *P. ferreus*, *P. ferrugineofuscus*, *P. nigrolimitatus* and *P. viticola* are closely related to *P. weirii* and *P. sulphurascens* based on their 28S rDNA phylogeny (Wagner and Fischer 2002), they could not be amplified using our *P. weirii*-specific primers. In the work reported here, these specific primers were also used very effectively to detect *P. weirii* in wood decay samples highly infested by other fungal species. When grown in artificial media these fungal species normally out-compete *P. weirii*, thereby preventing its detection using cultural methods.

Although molecular analytic methods are becoming common practice in many laboratories, there are still facilities without such capabilities. Researchers in such laboratories may be able to use other biological tests to differentiate species. For example, Buckland et al. (1954) observed that isolates of *P. sulphurascens* from Douglas fir seldom attacked wood blocks of WRC *in vitro*, in contrast to the active growth of *P. weirii* isolates on similar wood blocks. Our growth results on MEA containing WRC feeder strips were consistent with Buckland's work. The growth rate of *P. weirii* on MEA was less affected by feeder strips than that of *P. sulphurascens*, and *P. weirii* covered the strips while *P. sulphurascens* only grew around the strips. It is likely that the extractives in WRC

heartwood have a stronger fungicidal effect on *P. sulphurascens*, especially given that this fungus is not pathogenic to WRC (Wethern 1959). The fungicidal effect of WRC feeder strips will be a function of the quality and quantity of extractives present in them. About 10% of the total WRC heartwood consists of extractives (Gardner 1963) and up to 23% extractive content has been found in some portions of WRC wood (MacLean and Gardner 1956). Consequently, if using a bioassay such as WRC feeder strips to differentiate between *P. sulphurascens* and *P. weirii*, diagnosticians need to account for variability in wood extractives. This could be done using either chemical analysis or the use of test strains from both species with known reaction.

In conclusion, although a biological assay can be used to differentiate *P. sulphurascens* and *P. weirii*, it has limitations and takes several weeks. Using molecular tools, the differentiation of the two *Phellinus* species can be carried out in a day. *Phellinus weirii* detection using specific primers is even quicker and cheaper as the assay involves fewer steps. Not only can the assay differentiate and identify *P. weirii* from other *Phellinus* species but it also can detect *P. weirii* in wood collected from decayed WRC trees. We anticipate that our *P. weirii*-specific primer test will also be a useful tool for detecting the fungus directly from roots or stems. This will enable forest managers to better predict and monitor the presence and dissemination of this pathogen in both forest plantations and natural stands.

Acknowledgements

This work was supported by funding from the Natural Sciences and Engineering Research Council of Canada and the Canadian Biotechnology Strategy Fund (as Canadian Forest Service Genomics funds to R. Sturrock).

Résumé

Différenciation des deux espèces apparentées Phellinus weirii et P. sulphurascens

Phellinus weirii s.l., est un agent pathogène des racines à forte agressivité, causant d'importantes pertes de bois et de production chez *Thuja plicata*, le Douglas (*Pseudotsuga menziesii*) et d'autres conifères. Deux formes du champignon ont été reconnues, sur Thuya (*P. weirii* s.s.) et sur les hôtes autres que Thuya (*Phellinus sulphurascens*). La différenciation des deux espèces est difficile car les carpophores et la morphologie en culture sont très similaires. Toutefois, des différences de taux de croissance et de morphologie des colonies ont été observées en culture sur extrait de malt gélosé avec des fragments de *Thuja plicata*. De plus, différents profils RFLP ont été obtenus en utilisant a) la région ITS et l'enzyme de restriction *Rsa*I, et b) une partie de la région ADN codant pour la grosse sous-unité ribosomique ((LSU rDNA) et les enzymes *Age*I et *Nci*I. Enfin, un nouveau couple d'amorces spécifique a été développé dans la région ITS de *P. weirii* s.s, permettant de différencier cette espèce de *P. sulphurascens* et d'autres agents de pourriture fréquemment rencontrés chez les conifères. Ces amorces spécifiques faciliteront la détection de *P. weirii* dans les arbres sur pied bien avant l'apparition des premiers signes d'infection.

Zusammenfassung

Eine Methode zur Unterscheidung der zwei nahe verwandten Arten Phellinus weirii und Phellinus sulphurascens

Phellinus weirii s.l. ist ein aggressiver Wurzelfäuleerreger, der grosse Holzverluste bei *Thuja plicata*, *Pseudotsuga menziesii* und anderen Koniferen verursacht. Von diesem Pilz sind zwei Formen bekannt, eine davon kommt auf *Thuja* vor (*P. weirii* s.s.) und die zweite (*P. sulphurascens*) auf anderen Nadelgehölzen. Die Unterscheidung dieser zwei Arten ist schwierig, da ihre Fruchtkörper und auch die Kulturmorphologie sehr ähnlich sind. Bei Kultur auf Malzagar mit Thujaholz wurden jedoch Unterschiede in der Wachstumsrate und der Myzelmorphologie beobachtet. Ausserdem wurden unterschiedliche RFLP-Muster erhalten, wenn a) die ITS-Region mit dem Restriktionsenzym *RSA*I verdaut wurde und b) die LSU-rDNA-Region mit *Age*I und *Nci*I geschnitten wurde. Aus der ITS-Region von *P. weirii* s.s. wurde ein neues, spezifisches Primerset konstruiert, das zur Unterscheidung

dieses Pilzes von *P. sulphurascens* und anderen häufigen Fäuleerregern an Koniferen dient. Diese artspezifischen Primer erleichtern den Nachweis von *P. weirii* s.s. in stehenden Bäumen, bevor erkennbare Symptome der Infektion auftreten.

References

Allen, E.; Morrison, D.; Wallis, G., 1996: Common tree diseases of British Columbia. Victoria (BC), Canada: Natural Resources Canada, Canadian Forest Service, pp. 20–23.

Angwin, P. A., 1989: Genetics of sexuality and population genetics of *Phellinus weirii*. PhD Dissertation, Oregon State University, Corvallis, OR, 175 pp.

Angwin, P. A.; Hansen, E. M., 1993: Pairing tests to determine mating compatibility in *Phellinus weirii*. Mycol. Res. **97,** 1469–1475.

Aoshima, K., 1953: Wood rotting *Poria* from Japan II. Govt. Forest Exp. Sta. Bul. **59,** 57–64.

Bae, H.; Hansen, E. M.; Strauss, S. H., 1994: Restriction fragment length polymorphisms demonstrate single orgin of infection centers in *Phellinus weirii*. Can. J. Bot. **72,** 440–447.

Bahnweg, G.; Möller, E. M.; Anegg, S.; Langebartels, C.; Wienhaus, O.; Sandermann, H., 2002: Detection of *Heterobasidion annosum* s.l. [(Fr.) Bref.] in Norway spruce by polymerase chain reaction. J. Phytopathol. **150,** 382–389.

Banik, M. T.; Paul, J. A.; Burdsall, H. H.; Cook, M. E., 1993: Serological differentiation of two forms of *Phellinus weirii*. Mycologia **85,** 605–611.

Buckland, D. C.; Molnar, A. C.; Wallis, G. W., 1954: Yellow laminated root-rot of Douglas-fir. Can. J. Bot. **32,** 69–81.

Chen, W.; Gray, L. E.; Grau, C. R., 1996: Molecular differentiation of fungi associated with brown stem rot and detection of *Phialophora gregata* in resistant and susceptible soybean cultivars. Phytopathology **86,** 1140–1148.

Clark, J. W., 1958: The occurrence and investigations of physiological strains of *Poria weirii* Murr. PhD Dissertation, Yale University, New Haven, CT, 85 + 14pl pp.

Dai, Y. C., 2004: First report of laminated root rot on *Sabina przewalskii* caused by *Phellinus weirii sensu stricto* in China. Plant Dis. **88,** 573.

Förster, H.; Kinscherf, T. G.; Leong, S. A.; Maxwell, D. P., 1989: Restriction fragment length polymorphisms of the mitochondrial DNA of *Phytophthora megasperma* isolated from soybean, alfalfa, and fruit trees. Can. J. Bot. **67,** 529–537.

Gardes, M.; Bruns, T. D., 1993: ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. **2,** 113–118.

Gardner, J. A. F., 1963: The Chemistry and Utilization of Western Red Cedar. Canadian Department of Forest Publication 1023, Ottawa, 26 pp.

Germain, H.; Laflamme, G.; Bernier, L.; Boulet, B.; Hamelin, R. C., 2002: DNA polymorphism and molecular diagnosis in *Inonotus* spp. Can. J. Plant Pathol. **24,** 194–199.

Hamelin, R. C.; Mourassa, M.; Rail, J.; Dusabenyagasani, M.; Jacobi, V.; Laflamme, G., 2000: PCR detection of *Gremmeniella abietina*, the causal agent of *Scleroderris* canker of pine. Mycol. Res. **104,** 527–532.

Hansen, E. M., 1979: Survival of *Phellinus weirii* in Douglas-fir stumps after logging. Can. J. For. Res. **9,** 484–488.

Hansen, E. M.; Goheen, E. M., 2000: *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in Western North America. Annu. Rev. Phytopathol. **38,** 515–539.

Hansen, E. M.; Angwin, P. A.; Dreisbach, T. A.; Gernandt, D.; McWilliams, M. G., 1998: Species limits for *Phellinus weirii*. In: Root and butt rots. Proc. 9th Int. Conf. Root and Butt Rots, Carcans-Maubuisson Sept. 1–7, 1997. Ed. by. Delatour, C.; Guillaumin, J. J.; Lung-Escarmant, B.; Marçais, B., Paris: INRA Editions (France) Les Colloques No. 89, pp. 119–127.

Jones, D.; Bej, A. K., 1994: Detection of flood-borne microbial pathogens using polymerase chain reaction methods. In: PCR Technology, Current Innovations. Ed. by Griffin, H. G.; Griffin, A. M. London, UK: CRC Press, pp. 341–365.

Kotlaba, F.; Pouzar, Z., 1970: Revision of the original material of *Phellinus sulphurascens* Pil., *Xanthochrous glomeratus* ssp. *heinrichii* and *Polyporus rheades* Pers. (Hymenochaetaceae). Česká Mykol. **24,** 146–152.

Larsen, M. J.; Cobb-Poulle, L. A., 1990: *Phellinus* (Hymenochaetaceae). A survey of the world taxa. Synopsis Fungorum **3,** 1–206.

Larsen, M. J.; Lombard, F. F.; Clark, J. W., 1994: *Phellinus sulphurascens* and the closely related *P. weirii* in North America. Mycologia **86,** 121–130.

Lecomte, P.; Péros, J.-P.; Blancard, D.; Bastien, N.; Délye, C., 2000: PCR assays that identify the grapevine dieback fungus *Eutypa lata*. App. Env. Microbiol. **66,** 4475–4480.

Lee, J. S.; Ko, K. S.; Jung, H. S., 2000: Phylogenetic analysis of *Xylaria* based on nuclear ribosomal ITS1-5.8S-ITS2 sequences. FEMS Microbiol. Lett. **187,** 89–93.

Lee, S.; Kim, J.-J.; Fung, S.; Breuil, C., 2003: A PCR-RFLP marker distinguishing *Ophiostoma clavigerum* from morphologically similar *Leptographium* species associated with bark beetles. Can. J. Bot. **81,** 1104–1112.

Lim, Y. W.; Kim, J.-J.; Chedgy, R.; Morris, P. I.; Breuil, C., 2005. Fungal diversity from western red cedar fences and their resistance to β-thujaplicin. Antonie van Leeuwenhoek **87,** 109–117.

Lowe, J. L., 1966: The Polyporaceae of North America. The genus *Poria*. Techn. Publ. N. Y. State Univ. Coll. Forest. **90,** 1–183.

MacLean, H.; Gardner, J. A. F., 1956: Distribution of fungicidal extractives (thujaplicin and water-soluble phenols) in western red cedar heartwood. For. Prod. J. **6,** 510–515.

Mounce, I.; Bier, J. E.; Nobles, M. K., 1940: A root-rot of Douglas-fir caused by *Poria weirii*. Can. J. Res. **18,** 522–533.

Murrill, W. A., 1914: An enemy of western red cedar. Mycologia **6,** 93–94.

Parmasto, E.; Parmasto, I., 1979: The xanthochroic reaction in Aphyllophorales. Mycotaxon **8,** 201–232.

Scherer, S.; Magee, P. M., 1990: Genetics of *Candida albicans*. Microbial. Rev. **54,** 226–241.

Thies, W. G.; Sturrock, R. N., 1995. Laminated Root Rot in Western North America. Portland, OR: USDA Forest Service, 32 pp. PNW-GTR-349.

Turenne, C. Y.; Sanche, S. E.; Hoban, D. J.; Karlowsky, J. A.; Kabani, A. M., 1999: Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. J. Clin. Microbiol. **37,** 1846–1851.

Wagner, T.; Fischer, M., 2002: Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. Mycologia **94,** 998–1016.

Wallis, G. W.; Reynolds, G., 1965: The initiation and spread of *Poria weirii* root rot of Douglas-fir. Can. J. Bot. **43,** 1–9.

Wethern, J. D., 1959: Pulp and chemical potential for western red cedar utilization. For. Prod. J. **9,** 308–313.

White, T. J.; Bruns, T. D.; Lee, S. B.; Taylor, J. W., 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.; Sninisky, J. J.; White, T. J. New York: Academic Press, pp. 315–322.

Willits, D. A.; Sherwood, J. E., 1998: Polymerase chain reaction detection of *Ustilago hordei* in leaves of susceptible and resistant barley varieties. Phytopathology **89,** 212–217.