

On-site real-time PCR detection of *Phytophthora ramorum* causing dieback of *Parrotia persica* in the UK

K. J. D. Hughes^{a*}, P. M. Giltrap^a, V. C. Barton^a, E. Hobden^a, J. A. Tomlinson^a and P. Barber^b

^aCentral Science Laboratory (CSL), Sand Hutton, York, YO41 1LZ; and ^bDepartment for Environment, Food and Rural Affairs (Defra), Plant Health and Seeds Inspectorate (PHSI), Suite B, Tile Works Office Block, Whitestone Business Park, Withington, Hereford HR1 3SE, UK

In Europe *Phytophthora ramorum* mainly causes dieback of *Rhododendron* and *Viburnum*, but in the UK it has also been reported on other ornamentals including *Hamamelis* (Giltrap *et al.*, 2004) as well as on a limited number of tree species (Brasier *et al.*, 2004).

In November 2004, Defra's PHSI collected samples from a public garden in south Wales where *P. ramorum* was under eradication. Each sample was tested on-site by CSL using real-time (TaqMan®) PCR for *P. ramorum* on a Cepheid SmartCycler (Tomlinson *et al.*, 2005). This identified *P. ramorum* on *Parrotia persica* (Persian ironwood; Hamamelidaceae), which was causing necrotic leaf lesions and twig dieback. Duplicate material was also sent to CSL where *P. ramorum* was consistently isolated from both stem and leaf tissue following surface decontamination and isolation onto semi-selective medium (Lane *et al.*, 2002). An ITS sequence was obtained from a culture of *P. ramorum* isolated from *P. persica* (GenBank DQ066919) and this was identical to other *P. ramorum* isolates on GenBank. Pathogenicity of the isolate was confirmed by wound-inoculating healthy leaves of *P. persica* with mycelial plugs and incubating these in a damp chamber at room temperature (*c.* 20°C) in the laboratory for six days. Extensive lesions developed on the leaves and the pathogen was re-isolated from the leading edge; thus completing Koch's postulates. Healthy wounded leaves, inoculated with agar alone, did not develop symptoms.

This is the first report of *P. ramorum* affecting *P. persica*. The infected plant was destroyed and measures were taken to eradicate the pathogen according to European Union phytosanitary legislation and the EU was notified.

Acknowledgements

We would like to thank Mr T. Davies for his help in confirming the identity of the host and for technical assistance with sampling. Funding for this work was provided by Defra's Plant Health Division and quarantine material was processed and held under Defra licence PHL 251A5016 (02/2005).

References

- Brasier CM, Denman S, Rose J, Kirk SA, Hughes KJD, Griffin RL, Lane CR, Inman AJ, Webber JF, 2004. First report of ramorum bleeding canker on *Quercus falcata*, caused by *Phytophthora ramorum*. *Plant Pathology* 53, 804.
- Giltrap PM, Inman AJ, Barton VC, Barnes AV, Lane CR, Hughes KJD, Tomlinson J, Dean ML, Izzard K, 2004. First report of ramorum dieback (*Phytophthora ramorum*) on *Hamamelis virginiana* in the UK. *Plant Pathology* 53, 526.
- Lane CR, Beales PA, Hughes KJD, Griffin RL, Munro D, Brasier CM, Webber JF, 2002. First outbreak of *Phytophthora ramorum* in England, on *Viburnum tinus*. *Plant Pathology* 52, 414.
- Tomlinson JA, Boonham N, Hughes KJD, Griffin RL, Barker I, 2005. On-site DNA extraction and real-time PCR for detection of *Phytophthora ramorum* in the field. *Applied and Environmental Microbiology* 71, 6702–10.

*E-mail: k.hughes@csl.gov.uk. Accepted 20 March 2006 at www.bspp.org.uk/ndr where figures relating to this paper can be viewed.

© 2006 HM Government
Journal compilation © 2006 BSPP

First report of *Phytophthora boehmeriae* on black wattle in Brazil

A. F. Dos Santos^a, E. D. M. N. Luz^b and J. T. De Souza^{b,c*}

^aEMBRAPA Florestas, Caixa Postal 319, 84311-000, Colombo, PR; ^bCEPLAC, CEPEC, Caixa Postal 07, 45600-970, Itabuna, BA, Brazil; and ^cMars Inc., Hackettstown, NJ, 07840, USA

Black wattle (*Acacia mearnsii*) is an Australian tree species cultivated in several countries. In Brazil, particularly in Rio Grande do Sul State (RS), approximately 140 000 ha are cultivated for tannin extraction (Dos Santos *et al.*, 2005).

The gummosis complex, which has *Phytophthora nicotianae* as one of the causal agents, is the main disease of black wattle in Brazil and is characterised by lesions at the trunk base with gum exudation (Dos Santos *et al.*, 2005). Different symptoms were observed in plantations at the municipality of Piratini, RS (southern Brazil) and were characterised by dark lesions without gum exudation on trunks of 3- to 4-year-old wattle trees up to 10 m height. Isolates of *Phytophthora* spp. were obtained from the lesions and deposited in the Brazilian collection of *Phytophthora* species, under accession numbers CBP 307, 308 and 309. Sporangia of all isolates were ovoid to spherical, papillate and caducous, measuring $35 \mu\text{m} \pm 1.42 \times 30 \mu\text{m} \pm 1.37$, with a length/width ratio of 1.16:1, mean depth of papillae of $4.83 \mu\text{m} \pm 0.04$, and pore exit of $4.69 \mu\text{m} \pm 0.04$. The isolates were homothallic, forming plerotic oospores with smooth walls and amphigynous antheridia. The ITS sequences obtained for isolates CBP 307 (AY428533), CBP 308 (AY428534), and CBP 309 (AY428535) were identical and most closely matched those of two isolates of *P. boehmeriae* KACC40173 (AY228076) from Korea and SCRP23 (DQ297406) from China. This and the morphological similarity (Erwin & Ribeiro, 1996) suggest that these isolates are *P. boehmeriae*. However, seven clear single base pair differences were noted between the Brazilian and other *P. boehmeriae* isolates. This, combined with isozyme variation (Oudemans &

Coffey, 1991) suggests that further studies are needed to confirm the taxonomic status of *P. boehmeriae*.

Pathogenicity tests were done by inoculating five 1-year-old wattle plants with 7 mm mycelial discs of 5-day old cultures of the three isolates used in this study. The mycelial discs were placed in 7 mm diameter holes made in the bark with a cork borer, at 5 cm above the soil. Plants were maintained at approximately 25°C and were assessed 45 days after inoculation. All three isolates were pathogenic to black wattle and were re-isolated from the lesions.

Phytophthora boehmeriae was reported as one of the causal agents of the gummosis complex on black wattle in South Africa (TPCP, 2004) and is of quarantine importance for the citrus industry in Brazil. This is the first report of the involvement of *P. boehmeriae* in the aetiology of the gummosis complex of black wattle in Brazil.

References

- Dos Santos AF, Luz EDMN, De Souza JT, 2005. *Phytophthora nicotianae*: agente etiológico da gomose da acácia negra no Brasil. *Fitopatologia Brasileira* 30, 81–4.
- Erwin DC, Ribeiro OK, 1996. *Phytophthora Diseases Worldwide*. St. Paul, MN, USA: APS Press.
- Oudemans P, Coffey MD, 1991. A revised systematics of twelve papillate *Phytophthora* species based on isozyme analysis. *Mycological Research* 95, 1025–46.
- TPCP, 2004. Black butt of *Acacia mearnsii*. University of Pretoria, Pretoria, South Africa. <http://fabinet.up.ac.za/tpcp/pamphlets/pdf/blackbutt.pdf>.

*E-mail: jorgetdes@yahoo.com.br. Accepted 2 May 2006 at www.bspp.org.uk/ndr where figures relating to this paper can be viewed.

© 2006 The Authors
Journal compilation © 2006 BSPP