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Phytophthora inundata from native vegetation in Western Australia

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Abstract. *Phytophthora inundata* was found associated with a dead *Xanthorrhoea preissii* in *Eucalyptus marginata* (jarrah) forest in the south-west of Western Australia in January 2005. The isolate was sterile in pure culture and when paired with tester isolates of A1 and A2 mating types of other *Phytophthora* species. Mature sporangia were produced in 4 hours and discharged zoospores very soon after maturation. Some hyphal swellings were observed. Another isolate of *P. inundata* was isolated in 2005 from soil from Dandaragan on the sandplains north of Perth. *Phytophthora* isolates collected from dying native vegetation near the South Coast in 1986 and near Mount Barker in 1997 were also shown to be *P. inundata*. This is the first record of *P. inundata* from Australian natural ecosystems.

Additional keywords: DNA sequencing, *Phytophthora cinnamomi.*

An important element of the management of the jarrah (*Eucalyptus marginata*) forest, and of other native forest, woodland and heathland ecosystems in Western Australia, is the mapping of the extent of *Phytophthora* dieback disease, caused by the water-mould *Phytophthora cinnamomi* (Shearer and Tippett 1989). Mapping of diseased forest is based on shadowless colour aerial photography that is validated by testing soil samples collected from beneath dying *Phytophthora* sensitive 'indicator species' for the presence of the pathogen. Other *Phytophthora* species have been isolated from soil and diseased plants in the south-west, including *P. citricola*, *P. megasperma*, *P. cryptogea*, *P. drechsleri* and *P. nicotianae* in descending order of frequency (Stukely *et al*. 1997). *P. boehmeriae* has been recovered (D'Souza *et al*. 1997) at one location. *Phytophthora* species have been isolated alone, or in the same samples as *P. cinnamomi*. Some *Phytophthora* isolates have remained unidentified because the reproductive structures required for their identification to species by morphological characters alone were not produced in culture.

A *Phytophthora* species (cultures VHSC 13920, WAC 12880, and WAC 12881) was isolated by baiting a sample of soil plus roots that was collected during routine soil testing in January 2005. The sample was taken from beneath a dead grasstree, *Xanthorrhoea preissii*, within jarrah forest in the Blackwood River catchment in the south-west of Western Australia. The *X. preissii* specimen was located ~50 m from the edge of the forest, in a gully with a seasonal stream flowing into the forest from cleared farmland on the opposite side of a road.

The sample was baited with *Eucalyptus sieberi* cotyledons (Marks and Kassaby 1974), which were plated onto NARPH selective agar (Hüberli *et al*. 2000) after 5 days and from which a pure culture was then isolated. Colonies on cornmeal agar (BBL, Becton, Dickinson & Co., North Ryde, NSW) had a uniform to slightly stellate growth pattern, but on carrot agar they were stellate. Mean radial growth on carrot agar in darkness was 6.9 mm/day at 25°C and 3.3 mm/day at 36°C, which is similar to the rates reported for *P. inundata* by Brasier *et al*. (2003). A mean growth rate of 0.9 mm/day (averaged over 22 days) was recorded when plates were refrigerated at –2 to 6°C. The upper and lower limits for growth are yet to be determined.

Production of sporangia was induced by suspending freshly colonised squares of 10% V8 juice agar in non-sterile soil extract, at 25°C in daylight. After ~24 h sporangia were formed in small numbers. However, when squares were cut from a 3-week-old culture on V8 agar and treated similarly, the first mature sporangia were observed in only 4 h, with zoospore release commencing less than an hour later. Fifty recently formed but mature sporangia were measured. Sporangia dimensions (length × breadth) were 33.7–72.8 × 19.5–52.0 µm

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(mean 55.7 × 36.1 µm), which are generally smaller than those reported for *P. inundata* by Brasier *et al*. (2003). The mean length : breadth ratio of sporangia was 1.54, whereas Brasier *et al*. (2003) reported this ratio to be in the range 1.2–1.5 for nine isolates. Sporangia were consistently terminal, persistent, ovoid-obpyriform, and non-papillate (exit pore average 12.6 µm diam.), as described by Brasier *et al*. (2003). Internal proliferation was observed following the release of zoospores from sporangia, and some nested sporangia were observed. Occasionally, immature sporangia germinated directly by a single apical germ tube. Sporangiophores were single, with lengths in the range from 50 to over 210 µm. Brasier *et al*. (2003) reported sporangiophores single or sympodial, with lengths commonly in the range 150–850 µm. Intercalary hyphal swellings were formed on hyphae growing from colonised 10% V8 juice agar squares suspended in non-sterile soil extract, but not on the original agar plates.

Oogonia and oospores were not formed in pure culture. Pairing of isolate VHSC 13920 on 10% V8 juice agar with *P. cinnamomi* type A1 (DCE 25) and type A2 (DCE 60) tester isolates failed to produce oogonia. Additional pairings were carried out using *P. drechsleri* A1 (DCE 444, IMI 329666), *P. cryptogea* A2 (VHS 1136, IMI 329673), *P. cambivora* A1 (DCE 530), and *P. cambivora* A2 (DCE 532) testers, but no oogonia were produced after 1 month. Brasier *et al*. (2003) reported abundant production of oogonia when all *P. inundata* isolates were paired with *P. drechsleri* A1 or A2 testers, but pairings between *P. inundata* isolates were less consistently fertile. Further investigation of the breeding system of our isolates is required.

A subculture grown on cornmeal agar at 25°C for 8 days was used for DNA extraction and sequencing. DNA was extracted using the method described by Graham *et al*. (1994) and the Internal Transcribed Spacer (ITS) regions of the rDNA were amplified using primers ITS6 and ITS4 (Cooke *et al*. 2000). ITS regions were sequenced using the same primers and BigDye 3.1 technology (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions.

Sequence comparison with GenBank accessions (Altschul *et al*. 1997) showed 100% homology between 816 nucleotides of sequence from VHSC13920 and the ITS1, ITS2 and 5.8s rDNA sequences from four isolates of *P. inundata* (GenBank accessions AF266791 and AF541912-AF541914). Sequences with 100% homology with VHSC13920 included that from a paratype of *P. inundata* (AF266791, from isolate IMI389751/P246b; in Brasier *et al*. 2003).

P. inundata was formally described by Brasier *et al*. (2003), although it had earlier been included as a separate taxon in the *Phytophthora* 'O' group by Brasier *et al*. (1993). The earliest isolates of this taxon were recorded in 1970 and 1972 in the UK, and its known distribution includes countries in northern and southern Europe as well as South America (Brasier *et al*. 2003). The first reported isolations of *P. inundata* in Australia (APPD/VPRI 32407a, APPD/VPRI 32408a) were made from pear-baits from soil within carrot and parsley crops in the Cranbourne area of Victoria in mid 2005 (Cunnington *et al*. 2006). They were not associated with disease.

Another isolate of *P. inundata* was recovered in Western Australia from the northern sandplains, from a soil sample collected from the TiWest Joint Venture Cooljarloo minesite, Dandaragan (30°40′S, 115°28′E) in October 2005. It was identified by DNA sequencing, and has been deposited as WAC 12864/VPRI 32531. In this isolate also, it was noted that the sporangia discharged very soon after they matured, and some hyphal swellings formed in water.

DNA sequence testing has revealed that an unidentified sterile *Phytophthora* isolate (DDS 1540) that we obtained from a soil sample collected beneath dying native vegetation near Stokes Inlet on the South Coast in 1986 is also *P. inundata*, as is an isolate collected from a swampy area near Mount Barker in 1997 (VHSC 2910). This species has not previously been recorded in a natural ecosystem in Australia.

P. inundata is associated with root and collar rots in hardwood trees and shrubs, including *Aesculus*, *Olea*, *Salix*, *Prunus*, and *Vitis*, especially after flooding or on very wet soils (Brasier *et al*. 2003). The pathogenicity of *P. inundata* to native flora in the jarrah forest and elsewhere in Western Australia, and indeed Australia as a whole, is unknown. In addition to native flora, it may pose a threat to commercially cultivated species under suitable conditions, based on its known host range. The extent of its distribution here is also unclear at this stage. It is expected that the application of DNA sequence data to check the identification of stored cultures will result in further records of this and other new *Phytophthora* taxa.

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389–3402. doi: 10.1093/nar/25.17.3389

Brasier CM, Hamm PB, Hansen EM (1993) Cultural characters, protein patterns and unusual mating behaviour of *Phytophthora gonapodyides* isolates from Britain and North America. *Mycological Research* **97**, 1287–1298.

Brasier CM, Sanchez-Hernandez E, Kirk SA (2003) *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. *Mycological Research* **107**, 477–484. doi: 10.1017/S0953756203007548

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. doi: 10.1006/fgbi.2000.1202

Cunnington JH, Jones RH, de Alwis S, Minchinton EJ (2006) Two new *Phytophthora* records for Australia. *Australasian Plant Pathology* **35**, 383–384. doi: 10.1071/AP06036

D'Souza NK, Webster JL, Tay FCS (1997) *Phytophthora boehmeriae* isolated for the first time in Western Australia. *Australasian Plant Pathology* **26**, 204. doi: 10.1071/AP97034

Graham GC, Meyers P, Henry RJ (1994) A simplified method for preparation of fungal DNA for PCR and RAPD analyses. *BioTechniques* **16**, 48–50.

Hüberli D, Tommerup IC, Hardy GEStJ (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* **29**, 164–169. doi: 10.1071/AP00029

Marks GC, Kassaby FY (1974) Detection of *Phytophthora cinnamomi* in soils. *Australian Forestry* **36**, 198–203.

Shearer BL, Tippett JT (1989) Jarrah dieback: the dynamics and management of *Phytophthora cinnamomi* in the jarrah (*Eucalyptus marginata*) forest of south-western Australia. Research Bulletin No.3, Department of Conservation and Land Management, Como, Western Australia.

Stukely MJC, Shearer BL, Tay FCS, Hart RM, Hart RP (1997) *Phytophthora* species in natural vegetation in Western Australia. In 'Programme and summaries – 11th biennial conference, Australasian Plant Pathology Society, Perth, Western Australia'. p. 199.

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