Department of Environment and Conservation

Vegetation Health Service

Annual Report 2007-2008 Phytophthora Detection

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Vegetation Health Service – Annual Report 2007-2008 *Phytophthora* Detection

1. Introduction

The **Vegetation Health Service (VHS)** provides a dedicated, specialist scientific service for the detection and identification of *Phytophthora* species from samples associated with the management of the State's forest and conservation estate, logging and mining activities, private industry and research. The VHS is funded by DEC's Sustainable Forest Management Division (90%) and Nature Conservation Division (10%). It is run by Science Division staff based at DEC's Kensington Research Centre. Its services are offered free of charge to all DEC sections and personnel. The service is also available to external clients at a standard fee of \$77 per sample, with special discounted rates applying to samples collected for Alcoa World Alumina Australia and the Forest Products Commission (FPC), and for Natural Heritage Trust projects. No other *Phytophthora* testing service is available on this scale in WA.

Samples received by the VHS in most cases include a mixture of soil and plant-root material, which is baited for *Phytophthora* using the *Eucalyptus sieberi* cotyledon baiting method. Bait material is plated to selective agar medium for incubation, and any possible *Phytophthora* colonies that emerge are then isolated to pure culture for identification to species. Where isolation of the pathogen from specific host-plant tissue is required, roots are surface-sterilised and direct-plated to selective agar medium for *Phytophthora* isolation. All isolates are identified to species in the VHS from their morphological characters, and DNA sequencing is used for selected isolates through the service provided by the CPSM (Murdoch University).

Results are supplied to clients as soon as possible. All results (both *Phytophthora*-positive and negative) are added to the **VHS database**, along with details of sampling location, land tenure, etc. This database now contains 31,578 records (at 30th June 2008), and this information resource is made available to land managers and researchers as required. Representative *Phytophthora* cultures are added to the **VHS Culture Collection** (see **Section 3** below), which now contains 1,155 cultures. Cultures from the Collection are made available to researchers, both in DEC and in other institutions, on request.

The VHS also provides advice to assist Departmental staff, and also the public, with other plant disease problems in forests, plantations, parks and reserves, and nurseries.

2. Annual summary – samples processed

During the 2007-2008 financial year the VHS received 2,353 samples for testing for the presence of *Phytophthora* (**Table 1** and **Figure 1**). This was well above the previous year's total of 1,696 and is the largest annual total processed to date (see **Table 4** and **Figure 4**).

The sources of these samples are as follows:

DEC – samples sent by **DEC Dieback Interpreters** (or by contractors to DEC), and **District staff**, in fulfilment of DEC's forest and land management responsibilities.

BCI – samples sent by DEC personnel associated with the **Biodiversity Conservation Initiative**.

ALCOA – samples sent directly by **Alcoa World Alumina Australia** (or by contractors to Alcoa) in fulfilment of Alcoa's forest management responsibilities prior to, during and after mining activities, on the DEC estate.

PRIVATE – samples sent directly by external clients (land managers or private contractors).

RECOUP – samples for external clients sent by DEC dieback interpreters.

FPC – samples sent directly by, or for, the **Forest Products Commission**, including FPC Nurseries.

NHT – samples associated with projects funded by the Natural Heritage Trust.

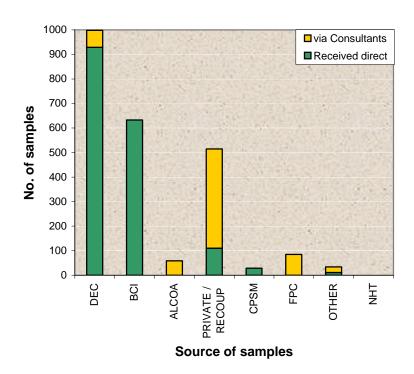
CPSM – samples associated with projects carried out at the **Centre for** *Phytophthora* **Science and Management** at Murdoch University.

The majority of samples processed by the VHS are collected by, or for, DEC. These include samples sent by FMB Dieback Interpreters from DEC-managed forest being monitored for *Phytophthora* in conjunction with logging by the FPC. Whilst all sampling of Forest Blocks due for logging by FPC was previously done by FMB Dieback Interpreters, some has been carried out this year by consultants and it appears that this trend will continue. Processing of samples collected by DEC in the Fitzgerald River National Park (Bell Track), associated with the **Biodiversity Conservation Initiative (BCI)**, commenced in May 2006. Samples are also received from various DEC District offices (**Table 2** and **Figure 2**). An increased number of Consultants – Glevan Consulting, and Dieback Treatment Services (as in previous years), and in addition Cardno BSD, NPC Consulting, Moore Mapping, RPS Environmental, Coffey Environments, and GHD – supplied 641 samples from various sources including DEC land and Alcoa (**Table 1** and **Figure 1**). No samples were received from NHT projects in 2007-08.

VHS facilities have been made available for the processing of samples for **other DEC research projects** associated with the BCI (Dr Chris Dunne). Assistance was provided to a PhD student at UWA, Carly Bishop, for processing samples collected in the Walpole district; and to Vicki Stokes (then with DEC) for processing samples from the Black Gravel project. These sets of samples for other projects are not included in the VHS data in this report, although VHS staff may have assisted with *Phytophthora* identification.

Table 1 and Figure 1. Numbers of samples received from major sources, including consultants, in 2007-2008. Total numbers of samples received from the same sources in 2006-2007 are given in the Table for comparison.

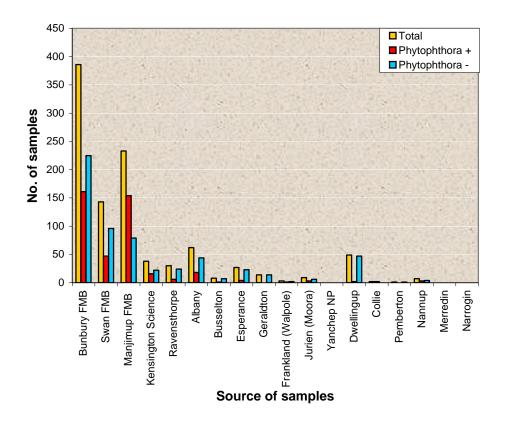
Source	No. of samples				
	Received direct	Received via Consultants	Total 2007-2008	Total 2006-2007	
DEC	929	69	998	964	
BCI	633	0	633	247	
ALCOA	0	59	59	121	
PRIVATE / RECOUP	110	405	515	207	
CPSM	29	0	29	150	
FPC	0	85	85	3	
OTHER	11	23	34	4	
NHT	0	0	0	0	
TOTAL	1712	641	2353	1696	



Details of the sources (by District) of DEC samples received for the year are shown in **Table 2** and **Figure 2** (including Recoup samples, but excluding BCI samples and DEC samples collected by consultants. The locations where DEC and other samples were collected are shown on the attached **Map**, with results broken down to distinguish *P. cinnamomi*, Other *Phytophthora* spp. (including undescribed new *Phytophthora* taxa), and Negative.

Table 2 and Figure 2. Numbers of samples received from DEC offices in 2007-2008, and numbers giving positive or negative recoveries of *Phytophthora*. Total numbers of samples received from the same sources in 2006-2007 are given in the Table for comparison. DEC samples received from consultants, and BCI samples, are not included.

DEC Office	No. of Samples				
SEO OTTION	Total received 2007-2008	<i>Ph</i> . Positive 2007-2008	<i>Ph</i> . Negative 2007-2008	Total received 2006-2007	
Bunbury FMB	386	161	225	313	
Swan FMB	143	47	96	127	
Manjimup FMB	233	154	79	298	
Kensington Science	38	16	22	90	
Ravensthorpe	30	6	24	247	
Albany	62	18	44	43	
Busselton	8	1	7	7	
Esperance	27	4	23	10	
Geraldton	14	0	14	0	
Frankland (Walpole)	3	1	2	4	
Jurien (Moora)	9	3	6	23	
Yanchep NP	0	0	0	1	
Dwellingup	49	2	47	0	
Collie	2	2	0	0	
Pemberton	1	0	1	2	
Nannup	7	3	4	0	
Merredin	0	0	0	2	
Narrogin	0	0	0	2	
TOTAL	1012	418	594	1169	



3. Phytophthora species

Based upon the traditional methods of identification of *Phytophthora* isolates in pure culture by microscopic examination of their morphological characters, the following six species (in addition to *P. cinnamomi*) were previously known to be present in WA natural ecosystems: *P. citricola, P. megasperma, P. cryptogea, P. drechsleri, P. nicotianae* (Stukely *et al.*, 1997), and *P. boehmeriae* (D'Souza *et al.*, 1997).

In addition to the above species, *P. inundata* had been identified by DNA sequencing prior to June 2007 (Stukely et al., 2007b). Testing of new and historical isolates by this technique since 2005 has also led to the discovery of as many as ten **new and undescribed** *Phytophthora* taxa, designated "P.spp.1–11", from dying native vegetation in WA (see Section 3.1) (Stukely et al., 2007c; Stukely et al., 2008).

During 2007-2008, *Phytophthora* was isolated from a total of 650 samples (561 in 2006-2007) (**Table 3, Figure 3**). *Phytophthora cinnamomi* was, as usual, the species most frequently isolated (573 samples). *P. cryptogea* was isolated from eight samples, *P. inundata* from five, and *P. nicotianae* from three.

The putative new species **Psp4**, closely related to *P. citricola* (and morphologically indistinguishable from it), was isolated from 10 samples and identified by DNA sequencing. An additional 36 isolates were identified as "*P. citricola*" on morphology only, and it is likely that these are also Psp4 (see **Section 3.1**). Thirteen samples gave isolates that were shown to be members of other new (undescribed) *Phytophthora* taxa by DNA sequencing (see **Section 3.1**): **Psp 1** (four isolates), **Psp2** (four), **Psp3** (three), **Psp10** (one), and **Psp11** (one).

One sample yielded both *P. cinnamomi* and Psp4, and one had both *P. cinnamomi* and "*P. citricola*" (probably also Psp4). Another two *Phytophthora* isolates, from some of the most recent samples tested, were still to be fully identified to species.

The locations where DEC and other samples were collected are shown on the attached **Map**, with results broken down into three categories to distinguish a). *P. cinnamomi*, b). Other *Phytophthora* spp. (including the undescribed new *Phytophthora* taxa), and c). Negative.

Representative pure cultures of all of the *Phytophthora* species isolated, and cultures of individual species representing different geographic locations, ecosystems, or host plants, or morphological types, were added to the permanent **VHS Culture Collection**. These cultures are maintained in a pure and viable condition by periodically sub-culturing, checking their purity and establishing fresh storage cultures. There are now 1,155 cultures stored in the VHS Collection. In addition, the VHS holds *Phytophthora* cultures from the following earlier collections that were stored by researchers and consultants: MJS (M Stukely), DCE (E Davison), TCH (T Hill) and HSA (Hart, Simpson and Associates). Cultures from the Collection are made available to researchers within DEC and in other institutions on request. This culture collection is a unique, and very valuable, scientific resource.

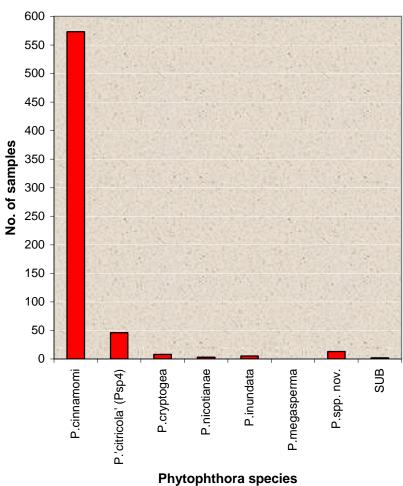
DNA sequencing of historical isolates from the Collection has continued (see Section 3.1).

Table 3 and Figure 3. Numbers of samples from which Phytophthora species were isolated by the VHS in 2007-2008.

Phytophthora	No. of samples with positive Phytophthora recovery		
species	Primary result	Second species ¹	
P. cinnamomi	573		
'P. citricola' (Psp4)	46	2	
P. cryptogea	8		
P. nicotianae	3		
P. inundata	5		
P. megasperma	0		
P. spp. nov. ²	13		
SUB ³	2		
TOTAL	650	2	

¹Second species – in addition to *P. cinnamomi*, these samples yielded a second Phytophthora species as shown.

³SUB – these *Phytophthora* cultures are in the process of being identified or are unable to be fully identified.



²P. spp. nov. – these isolates represent undescribed new *Phytophthora* taxa P.spp.1-11(see Section 3.1), excluding Psp4 'P. citricola'.

3.1. New *Phytophthora* records, and undescribed *Phytophthora* taxa in WA

The adoption and use of DNA sequencing techniques for the identification of *Phytophthora* species is causing something of a revolution in *Phytophthora* taxonomy, worldwide. The availability of these techniques in WA, through the **Centre for** *Phytophthora* **Science and Management** (CPSM) at Murdoch University, has enabled the routine testing of some WA isolates that could not be identified satisfactorily by traditional morphological examination.

<u>DNA sequencing and data analysis:</u> DNA was extracted from pure cultures of *Phytophthora* grown on cornmeal agar, and the Internal Transcribed Spacer (ITS) regions of the rDNA were amplified using primers ITS6 and ITS4. BLASTn searches of sequence data were conducted in GenBank to determine the most closely related *Phytophthora* spp. Sequences were then aligned and parsimony and distance analyses conducted in PAUP. **Phylogenetic trees** were constructed (see **Attachment**).

Very significant progress has been made in the last year. We have now received ITS rDNA sequence data for a total of over 200 recent and historical isolates of *Phytophthora*, mostly obtained from natural ecosystems in WA. Sequences of representative isolates of all undescribed taxa and newly recorded species from WA were deposited on GenBank. [GenBank is a large, international, public domain database of DNA sequences that was created in 1988.]

In brief:

- ➤ There are now known to be as many as **ten undescribed new species of** *Phytophthora* present in natural ecosystems in WA (designated "P. spp. 1 11").
- ➤ The WA Phytophthoras show a very high level of diversity, representing eight of the ten phylogenetic clades (groups) of *Phytophthora* that are known worldwide. See the **phylogenetic tree** (**Attachment**); explanatory notes are given in **Section 9** (p. 11).
- ➤ In addition to the above ten new species (for each of which there are multiple isolates), there are **five unique** *Phytophthora* **isolates** from natural ecosystems in WA.
- ➤ Only a **single isolate** of the many identified earlier as *P. megasperma* (based on their morphology) had a 100% match to sequences for *P. megasperma* on GenBank. The others represent several diverse new taxa.
- None of the many isolates identified earlier as *P. citricola* (based on their morphology) had a 100% match to sequences for *P. citricola* on GenBank. These isolates all represent new taxa, mostly Psp4 and some Psp2 (the latter has a distinct morphology).
- Many of the ten new taxa, and all five unique isolates, have so far been recorded only in WA. A few of the new taxa are a close match to undescribed isolates found overseas.
- All but one (Psp8) of the ten undescribed *Phytophthora* species have been found associated with dying plants in native forest or heath-land in WA.
- > Several of the new *Phytophthora* taxa are associated with deaths of plants representing multiple families.
- ➤ The number of plant species associated with the new Phytophthoras is still increasing rapidly, as new isolates are found.
- ➤ Two isolates of each of *P. asparagi* and the undescribed *P.* taxon 'niederhauseria' were identified by DNA sequencing. These represent new records of these species from natural vegetation in WA. They were all isolated from samples collected in 2006-2007.
- An undescribed isolate collected near Busselton in November 2005, designated Psp6 and lodged on GenBank, was found in 2008 to be a 100% match to a pathogen of tobacco in crops in the USA. It has been provisionally named there as *P*. taxon 'personii'.
- ➤ Isolates previously identified as *P. gonapodyides* are now believed to align with the undescribed *P.* taxon 'PgChlamydo'.
- The large group of isolates designated earlier as Psp5 are now included as *P. cryptogea* (as per their morphological identification), as greater numbers of sequences with a wider range of variation for this species have been added to GenBank from other sources.

3.2 Management implications – the need for further investigation of new taxa

Many of the undescribed new *Phytophthora* taxa (reported in **Section 3.1**) are genetically quite distinct from the other Phytophthoras found to date in natural ecosystems in WA, despite some of them having some strong physical similarities in culture to the known *Phytophthora* species (eg *P. citricola, P. megasperma, P. drechsleri*). These genetic differences are cause for concern. They suggest that the 'new' Phytophthoras may have a different set of capabilities, strengths and vulnerabilities, and may pose different potential and immediate levels of threat to biodiversity, which must now be investigated.

It is imperative that these undescribed Phytophthoras are all properly described and documented as separate, individual components of our biodiversity.

The undescribed Phytophthoras, and the newly recorded ones, are all potential pathogens, as indicated by their associations with dying native flora that have already been recorded. It must be remembered that no *Phytophthora* has yet been documented as a "benign" organism – all are pathogens, with host specificities varying from a single plant species (eg *P. sojae*) to over 1,000 (*P. cinnamomi*). Detailed investigations must therefore be conducted on each new species to determine its host range, pathogenicity, distribution, ease of spread, preferred environmental conditions, and other characteristics such as its response to the inhibitor, phosphite.

It is not known whether these new taxa are **indigenous or introduced**, or if **hybrids** may be involved. Some of them (eg Psp4, formerly identified as 'P. citricola'), <u>but not all</u>, are widespread and associated mostly with low-impact dieback sites. **However, it is important at this stage that they should <u>not</u> all be treated as indigenous (and hence "not threatening"), until they are investigated further.** It appears that they are not particularly new introductions, with some isolates of most of them having been obtained from samples collected in WA in the 1980s and 1990s, as well as more recently.

It is likely that the 'new' Phytophthoras will all have at least some level of pathogenic ability, and so it can be expected that they will damage native vegetation under some circumstances. It is known, for example, that the "P. megasperma" group causes most damage following summer rainfall in coastal heath-land. Clearly, this high impact occurs at irregular intervals. It is also likely that increased levels of human activity in and around infested areas in the short term, together with the consequences of climate change and other stressors in the longer term, will exacerbate the effects of at least some of these Phytophthoras on natural ecosystems.

The undescribed Phytophthoras should therefore now all be regarded as a threat, and managed accordingly, until it is proven otherwise. Consistent, active efforts should be made to prevent their spread to non-infested areas. This is clearly an issue that now needs to be addressed by land managers with a well-considered, adaptive management approach.

New research projects (Section 6) will provide answers to some of the key questions above. An estimate of the level of threat that each *Phytophthora* poses to our biodiversity is required, so decisions can then be made, based on sound science, as to whether specific management strategies involving intervention are appropriate or necessary. Controls applied currently for *Phytophthora cinnamomi* (such as phosphite application) may not always be appropriate, nor directly applicable without modification, for managing the 'new' *Phytophthora* species.

Intervention (if deemed appropriate) at the earliest possible opportunity, to confine and possibly eradicate small *Phytophthora* infestations in priority areas, can be expected to pay very large dividends in future nature conservation efforts, and could also benefit the commercial sector. This applies to all *Phytophthora* species.

4. Historical record of VHS operations and samples processed

Since the Vegetation Health Service laboratory was established at Kensington Research Centre in 1992 (initially as the Dieback Detection Service), a total of 24,911 samples have been processed for *Phytophthora* detection (to 30th June 2008) (**Table 4** and **Figure 4**).

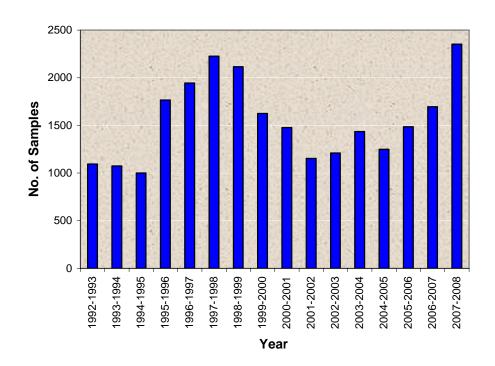
Prior to 1992, the routine sample processing was carried out at Dwellingup Research Centre. Between 1984 and 1992 this was done by Janet Webster. All identification of *Phytophthora* species isolated from these samples between 1985 and 1992 was completed by Mike Stukely at Kensington. Records from these earlier years, for which map references are available, have been included in the **VHS database** which now has a total of 31,578 records.

The VHS at Kensington was managed between 1992 and 2001 by Francis Tay, and from 2001 by Mike Stukely. Currently, Technical support (1 FTE) is provided on a time-share basis by Janet Webster and Juanita Ciampini. The long-term involvement by individual staff members in this specialised laboratory role, and in assisting VHS clients, is a definite advantage.

Since 2005 a strong collaborative relationship has been developed with the **Centre for Phytophthora Science and Management** (CPSM) at Murdoch University (Director: Associate Professor Giles Hardy). This has enabled the testing by DNA sequencing of new as well as historical *Phytophthora* isolates, which has led to the discovery of the new, undescribed taxa, of new records for WA of several known species and of undescribed species known from elsewhere (**Section 3**). A student project investigating the new Phytophthoras has commenced at Murdoch University under joint supervision by Prof. Hardy and M Stukely (**Section 6**).

Table 4 and Figure 4. Numbers of samples processed by the VHS for *Phytophthora* detection from 1992-93 to 2007-08.

Year	No. of Samples	Year	No. of Samples
1992-1993	1095	2000-2001	1477
1993-1994	1075	2001-2002	1154
1994-1995	1001	2002-2003	1210
1995-1996	1767	2003-2004	1435
1996-1997	1944	2004-2005	1250
1997-1998	2227	2005-2006	1486
1998-1999	2115	2006-2007	1696
1999-2000	1626	2007-2008	2353
		TOTAL	24,911



5. Concluding Comment

Land managers are encouraged to make full use of the services provided by the VHS for the detection of *Phytophthora*. The sample-processing service is provided free of charge to all DEC personnel and sections. No other service, capable of processing the required numbers of samples, is available in WA.

The implementation of **Best Practice methods and standards** for managing *Phytophthora* dieback is based upon the key steps of detection, diagnosis, demarcation and mapping of infested areas, and hence the identification of uninfested areas and those that are protectable (CALM, 2002). The VHS laboratory's testing of samples for the presence of *Phytophthora* is an integral part of this process.

It is important to recognise that areas of land must be **regularly re-assessed and re-tested** for *Phytophthora* infestation, since with time the pathogen will continue to spread from its known, established foci. This spread may be autonomous (by root-to-root contact between host plants, and through dispersal of zoospores in water), or through the activity of vectors such as native and feral animals, and people with their vehicles and machinery.

The **appropriate frequency of re-assessment and re-testing** for a given area of land will depend upon several factors:

- > the **values** associated with that area,
- > the **likelihood** or **level of risk** of introduction of **any** *Phytophthora* into that area, and
- the **consequences** of the introduction of **any** *Phytophthora* species to the ecosystem.

Information on the distribution of <u>all Phytophthora</u> species must be up-to-date, for land management to be most effective.

6. Collaborations and Student Projects (Co-supervised)

a. PhD project (Alex Rea) – Classical and molecular taxonomy and pathogenicity testing of *Phytophthora* species – commenced in April 2007 at Murdoch University.

7. Recent Publications and Presentations by VHS staff

- Research paper on the undescribed *Phytophthora*, "P.sp.2" published. Stukely *et al.* (2007a): **A new homothallic** *Phytophthora* **from the jarrah forest in Western Australia**. *Australasian Plant Disease Notes* **2:** 49-51.
- Research paper on a new *Phytophthora* record for WA published. Stukely *et al.* (2007b): *Phytophthora inundata* from native vegetation in Western Australia. *Australasian Plant Pathology* **36:** 606-608.
- ➤ Oral Presentation to WA Dieback Information Group (DIG) meeting, 13 July 2007 (also a presentation to DIG on 4 July 2008). Stukely: **New Phytophthora** species in WA.
- ➤ Poster presented at international conference. Stukely *et al.* (2007c): **Molecular testing uncovers new** *Phytophthora* **taxa from natural ecosystems in Western Australia.** 4th IUFRO Meeting on Phytophthoras in Forests and Natural Ecosystems, Monterey, California, USA, 26-31 August 2007.
- ➤ Internal DEC report a comprehensive review of the role of the VHS, its outputs, staff and facilities. Stukely (2008): The Science Division's Vegetation Health Service A Review, April 2008.
- Paper prepared for international workshop. Stukely *et al.* (2008): **Molecular re**evaluation of *Phytophthora* taxa collected over the past three decades from natural

- **ecosystems in Western Australia**. 3rd International *Phytophthora* and *Pythium* Workshop: "Integration of traditional and modern approaches for investigating the taxonomy and evolution of *Phytophthora*, *Pythium* and related genera" 23-24 August 2008, Turin, Italy (associated with the **International Congress of Plant Pathology**).
- ➤ Paper in preparation for international journal. Burgess *et al.*: Molecular re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia.

8. References Cited

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9. Attachments, notes and acknowledgments

1. Map showing results of *Phytophthora* sample testing by DEC Vegetation Health Service, 2007-2008.

Phytophthora cinnamomi (CIN), Other Phytophthora species (PSP), and Negative (NEG) recoveries are shown separately.

The map was produced by Naiem Babaii (DEC Geographic Information Services).

- 2. Phylogenetic trees (based on ITS rDNA sequences) showing a limited selection of the nearly 100 known species of *Phytophthora*, with the ten undescribed WA taxa, and five additional unique isolates in Group 6 (indicative only, July 2008).
 - <u>Tree 1:</u> The ten undescribed taxa isolated from WA samples ("**P. spp. 1-11**") are highlighted red. The species groupings proposed by Cooke *et al.* (2000) (Groups 1-9) are shown at the right.

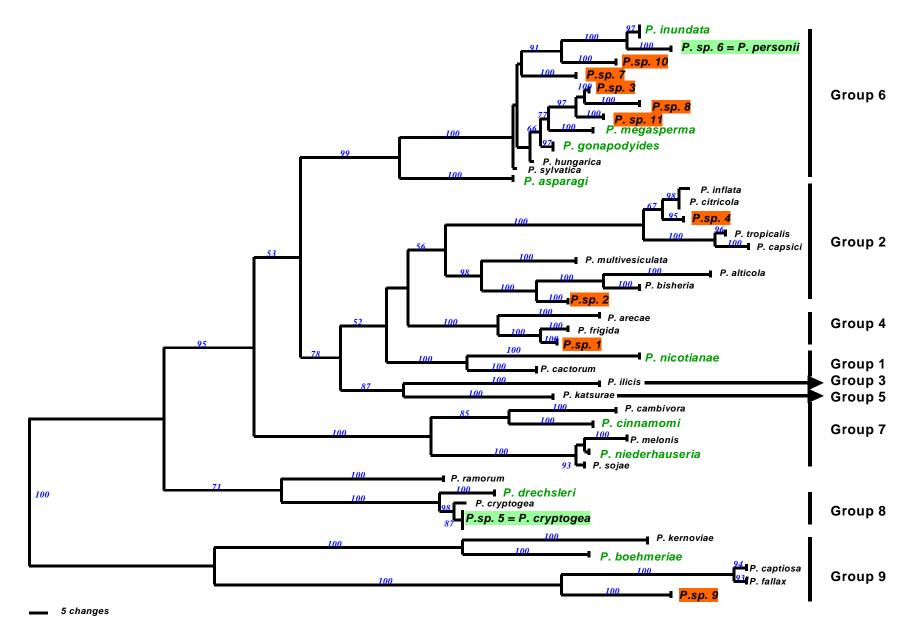
Phytophthora species shown in green text are those known previously to be present in WA natural ecosystems, based on the traditional morphological identification of pure cultures (Stukely *et al.* 1997; D'Souza *et al.* 1997).

The other species shown include all of those most closely related to each of the 'new' WA taxa, based on analysis of their ITS rDNA sequences, from the **GenBank database**.

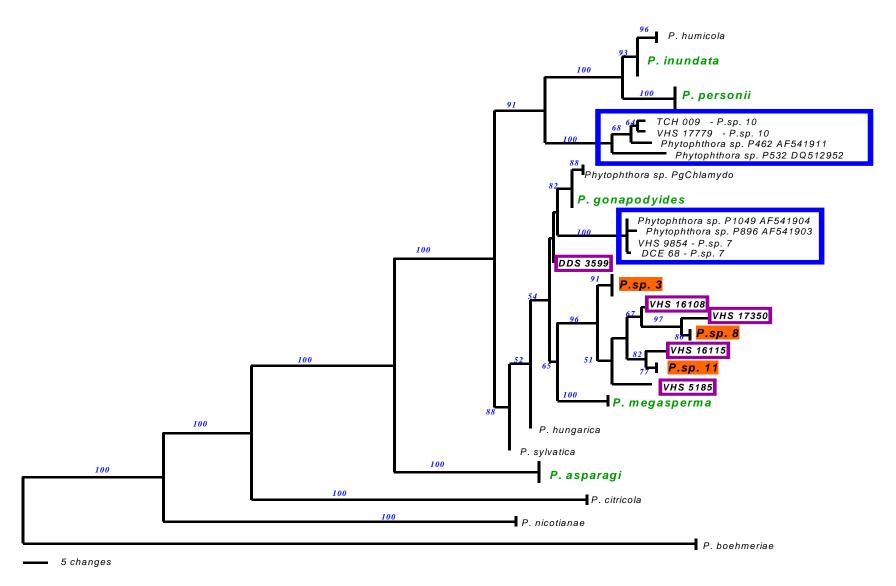
Four newly-described *Phytophthora* species causing declines of eucalypts, in South Africa (*P. alticola, P. frigida* – Maseko *et al.* 2007) and New Zealand (*P. captiosa, P. fallax* – Dick *et al.* 2006), are also shown.

<u>Tree 2:</u> Detail of the new WA taxa in Cooke's **Group 6** (P.spp. 10, 7, 3, 8, 11), with their closest known relatives (see blue boxes). The five **unique** WA isolates are shown in purple boxes. These isolates do not match any others on the GenBank world database, so far.

Analyses of rDNA sequence data and the construction of the phylogenetic trees were carried out by Dr Treena Burgess (Centre for *Phytophthora* Science and Management, Murdoch University).



TREE 1 – Phytophthora Groups 1-9: New taxa 'P.spp. 1-11' are highlighted red.



TREE 2 – *Phytophthora* Group 6: New taxa P.spp. 10, 7, 3, 8, 11; and five unique isolates (see purple boxes). VHS Annual Report 2007-2008.

