

**Department of Environment and Conservation**

Vegetation Health Service

**Annual Report 2006-2007**

***Phytophthora* Detection**

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Department of Environment and Conservation  
**Vegetation Health Service – Annual Report 2006-2007**  
*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 and FPC sections and personnel. The service is also available to external clients at a standard fee of \$77 (incl. GST) per sample, with special discounted rates applying to Alcoa World Alumina Australia and to Natural Heritage Trust projects.

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 29,293 records (at 30<sup>th</sup> June 2007), 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 1,157 cultures. These cultures 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 2006-2007 financial year the VHS received 1,696 samples for testing for the presence of *Phytophthora* (**Table 1** and **Figure 1**). This was well above the previous year's total of 1,489.

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 the **Forest Products Commission**, including FPC Nurseries.

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

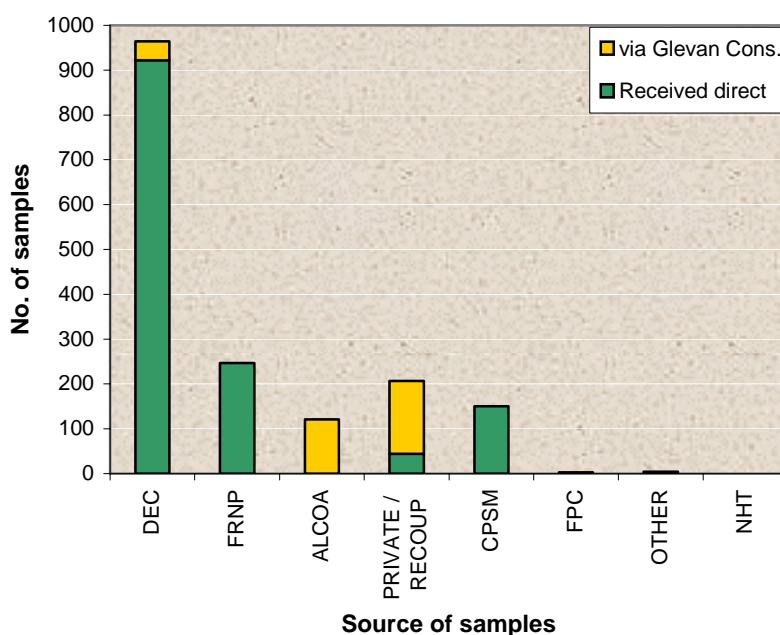
**CPSM** – samples associated with projects carried out at the **Centre for *Phytophthora* Science and Management** at Murdoch University.

The majority of samples processed by the VHS belong to DEC. These include samples sent by DEC Dieback Interpreters from DEC-managed forest being monitored for *Phytophthora* in conjunction with logging by the FPC. 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**). Consultants (Glevan Consulting, and Dieback Treatment Services) supplied 330 samples from various sources including DEC land and Alcoa (**Table 1** and **Figure 1**). No samples were received from NHT projects in 2006-07.

VHS facilities have been made available for the processing of samples for **other research projects** associated with the BCI (Dr Chris Dunne). Samples from a PhD student project being carried out at Murdoch University (Peter Scott) were processed in the VHS, under the Department's partnership with the **Centre for *Phytophthora* Science and Management**. These samples were collected in the tuart forest (Yalgorup National Park). Assistance was also provided to a PhD student at UWA, Carly Bishop, for processing samples collected in the Walpole district. 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 (Glevan Consulting, and Dieback Treatment Services), in 2006-2007. Total numbers of samples received from the same sources in 2005-2006 are given in the Table for comparison.

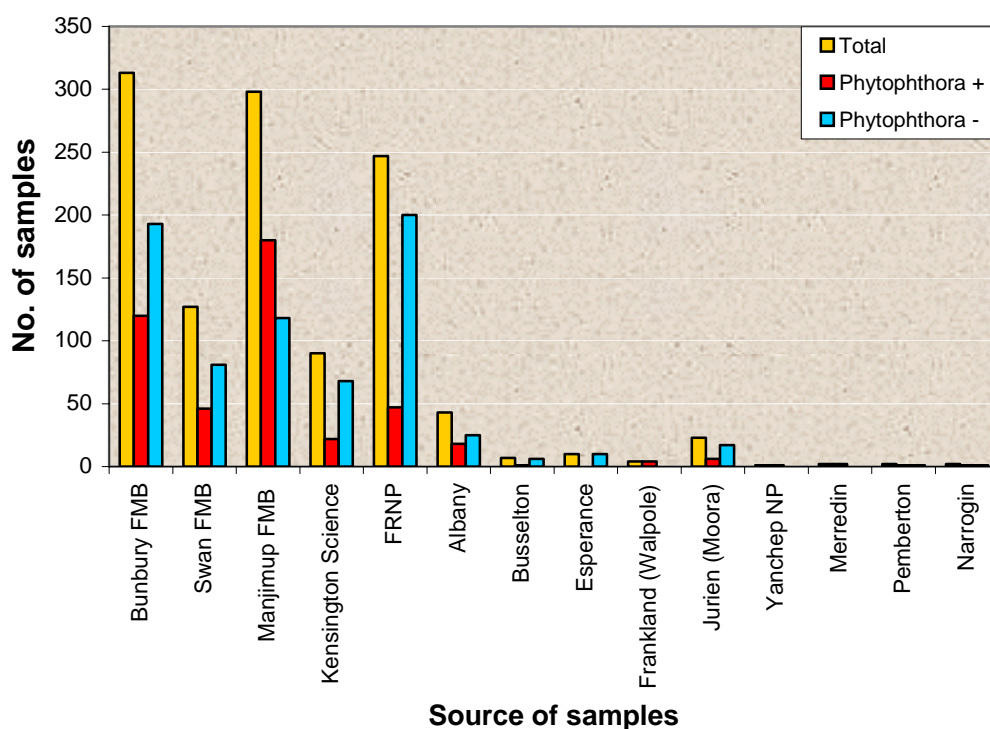
Source	No. of samples			
	Received direct	Received via Glevan Cons.	Total 2006-2007	Total 2005-2006
DEC	922	42	964	674
FRNP	247	0	247	195
ALCOA	0	121	121	263
PRIVATE / RECOUP	44	163	207	182
CPSM	150	0	150	149
FPC	3	0	3	23
OTHER	0	4	4	0
NHT	0	0	0	0
<b>TOTAL</b>	<b>1366</b>	<b>330</b>	<b>1696</b>	<b>1486</b>



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 Glevan). 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 2006-2007, and numbers giving positive or negative recoveries of *Phytophthora*. Total numbers of samples received from the same sources in 2005-2006 are given in the Table for comparison. DEC samples received from Glevan Consulting are not included.

DEC Office	No. of Samples			
	Total received 2006-2007	Ph. Positive 2006-2007	Ph. Negative 2006-2007	Total received 2005-2006
Bunbury FMB	313	120	193	96
Swan FMB	127	46	81	164
Manjimup FMB	298	180	118	223
Kensington Science	90	22	68	19
FRNP	247	47	200	0
Albany	43	18	25	59
Busselton	7	1	6	24
Esperance	10	0	10	17
Geraldton	0	0	0	1
Mandurah	0	0	0	23
Mundaring	0	0	0	2
Frankland (Walpole)	4	4	0	18
Jurien (Moora)	23	6	17	1
Yanchep NP	1	1	0	1
Crawley	0	0	0	1
Merredin	2	2	0	0
Pemberton	2	1	1	0
Narrogin	2	1	1	0
<b>TOTAL</b>	<b>1169</b>	<b>449</b>	<b>720</b>	<b>649</b>



### 3. *Phytophthora* species

Based upon the traditional methods of identification of *Phytophthora* isolates in pure culture from 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).

During 2006-2007, *Phytophthora* was isolated from a total of 561 samples (**Table 3, Figure 3**). [The total number of samples giving positive *Phytophthora* recoveries in 2005-2006 was 657.] *Phytophthora cinnamomi* was, as usual, the species most frequently isolated (476 samples). A probable new species (*P. sp. 4*) closely related to (and morphologically indistinguishable from) *P. citricola* was isolated from 39 samples; *P. nicotianae* was isolated from four; *P. inundata* (2); and *P. megasperma* (1).

Thirty-one samples gave isolates that were shown to be members of **new (undescribed) *Phytophthora* taxa** by DNA sequencing (see **Section 3.1**). This total excludes *P. sp. 4*, noted above (another 39 isolates). Four samples yielded both *P. cinnamomi* and '*P. citricola*' (*P. sp. 4*), and one had both *P. cinnamomi* and another *P. sp.* Twelve *Phytophthora* isolates ("SUB") 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 to distinguish *P. cinnamomi*, Other *Phytophthora* spp. (including the undescribed new *Phytophthora* taxa), and 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 **Culture Collection**. These cultures are maintained in a pure and viable condition by periodically sub-culturing, checking their purity and establishing fresh storage cultures. The VHS now has 1,157 cultures stored in the Collection. Cultures are made available to researchers within DEC and in other institutions on request. This culture collection is a unique, and very valuable, resource.

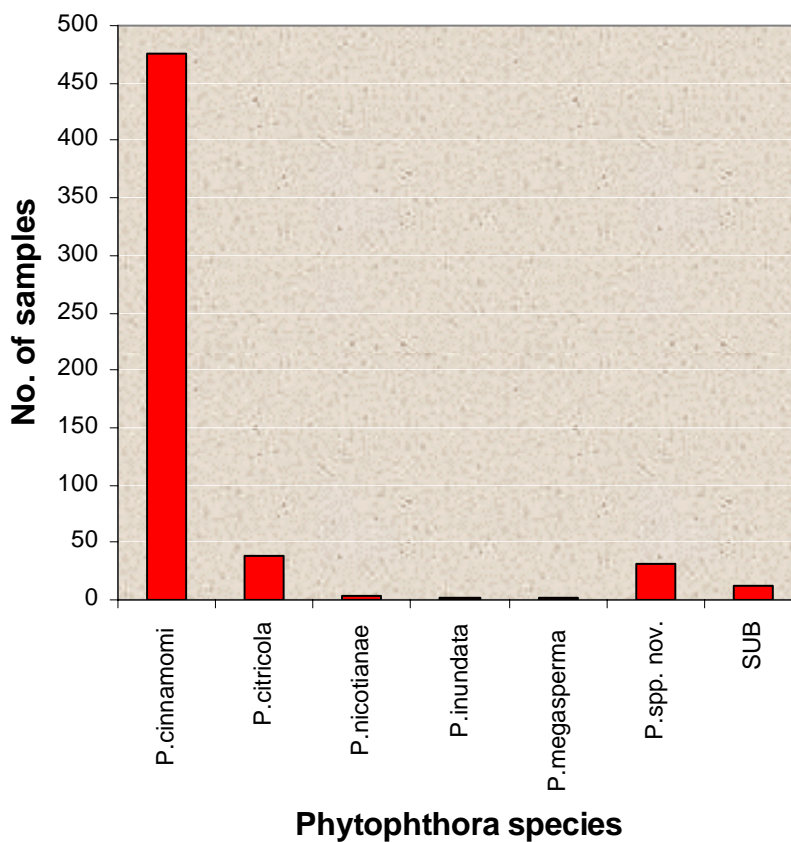
**Table 3** and **Figure 3**. Numbers of samples from which *Phytophthora* species were isolated by the VHS in 2006-2007.

<i>Phytophthora</i> species	No. of samples with positive <i>Phytophthora</i> recovery	
	Primary result	Second species <sup>1</sup>
<i>P. cinnamomi</i>	476	
' <i>P. citricola</i> '( <i>P.sp. 4</i> )	35	4
<i>P. nicotianae</i>	4	
<i>P. inundata</i>	2	
<i>P. megasperma</i>	1	
<i>P. spp. nov.</i> <sup>2</sup>	31	
SUB <sup>3</sup>	12	1
<b>TOTAL</b>	<b>561</b>	<b>5</b>

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

<sup>2</sup>*P. spp. nov.* – these isolates represent undescribed new *Phytophthora* taxa (see Section 3.1), excluding *P. sp. 4* '*P. citricola*'.

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



### 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.

DNA sequencing and data analysis: 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).

We reported in the *VHS Annual Report 2005-06* that:

- DNA sequencing by CPSM had confirmed at least three “new” *Phytophthora* taxa among unidentified VHS isolates recently obtained from the south-west of WA.
- Two of these are undescribed new species (designated “**P.sp. 1**” and “**P.sp. 2**”), and the third was identified as *Phytophthora inundata* (a new record for WA).
- All three had been found associated with dying plants in native forest or heath-land.

Very significant progress has been made in the last year. We have now received ITS rDNA sequence data for a total of over 160 recent and historical isolates of *Phytophthora*, mostly obtained from natural ecosystems in WA. **Table 3a** shows the associations of the ‘new’ *Phytophthora* taxa with dying native plant species that have been recorded so far. 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 - 10**”). See the **phylogenetic tree (Attachment)**; explanatory notes are given in **Section 9** (p. 11).
- All ten undescribed *Phytophthora* species have been found associated with dying plants in native forest or heath-land in WA (**Table 3a**).
- A new record (for WA) of one other known *Phytophthora* species (*P. gonapodyides*) has been confirmed.

**Table 3a.** Undescribed *Phytophthora* taxa (P. spp. 1 – 10) and new species records from Western Australia to 30<sup>th</sup> June 2007, with associated dying native plant species.

<i>Phytophthora</i> sp.	Dying native plant species
P. sp. 1	<i>Banksia menziesii</i> , <i>B. littoralis</i> , <i>B. attenuata</i>
P. sp. 2	<i>Eucalyptus marginata</i> , <i>B. grandis</i> , <i>Patersonia xanthina</i>
P. sp. 3 / 8	<i>E. marginata</i> , <i>B. attenuata</i> , <i>B. grandis</i>
P. sp. 4 ‘ <i>P. citricola</i> ’	<i>E. marginata</i> , <i>B. attenuata</i> , <i>B. grandis</i> , <i>B. littoralis</i> , <i>B. menziesii</i> , <i>B. prionotes</i> , <i>Xanthorrhoea gracilis</i> , <i>Podocarpus drouyniana</i> , <i>Patersonia</i> sp.
P. sp. 5	<i>B. grandis</i> , <i>X. gracilis</i> , <i>Patersonia</i> sp., <i>Crowea angustifolia</i> , <i>Isopogon buxifolius</i>
P. sp. 6	<i>Grevillea mcutcheonii</i> , <i>X. preissii</i>
P. sp. 7	<i>X. preissii</i>
P. sp. 9	<i>B. attenuata</i> , <i>Dryandra cirsioides</i> , <i>Isopogon</i> sp.
P. sp. 10	<i>B. prionotes</i>
<b><i>P. inundata</i><sup>1</sup></b>	<i>B. littoralis</i> , <i>X. preissii</i> , <i>Adenanthos cuneata</i>

<sup>1</sup>*P. inundata* – This species was formally named only recently (Brasier *et al.* 2003). It was first reported in Australia in 2006, from Victoria (Cunnington *et al.* 2006), where it was not associated with plant disease although isolated from soil in horticultural sites. However, in Europe and South America, *P. inundata* is a recognised pathogen of woody trees and shrubs, including *Olea*, *Prunus*, *Salix* and *Vitis*. Some of these known host species are grown commercially in WA.

### **3.2 Management implications – the need for further investigation**

Many of the ten undescribed new *Phytophthora* taxa (reported in **Section 3.1** and **Table 3a**) 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*). 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* are all potential pathogens**, as has already been indicated by their associations with dying native flora (**Table 3a**). It must be remembered that no *Phytophthora* has yet been documented as a “benign” organism – all are pathogens. 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 *P. sp. 4* ‘*P. citricola*’), but not all, are associated mostly with low-impact dieback sites. It is important at this stage that they should not 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 native flora.

**The undescribed *Phytophthoras* should therefore now all be regarded as a threat, and managed accordingly, until it is proven otherwise.** Consistent 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 that are now starting (**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.**



#### 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 22,543 samples have been processed for *Phytophthora* detection (to 30<sup>th</sup> June 2007) (**Table 4** and **Figure 4**).

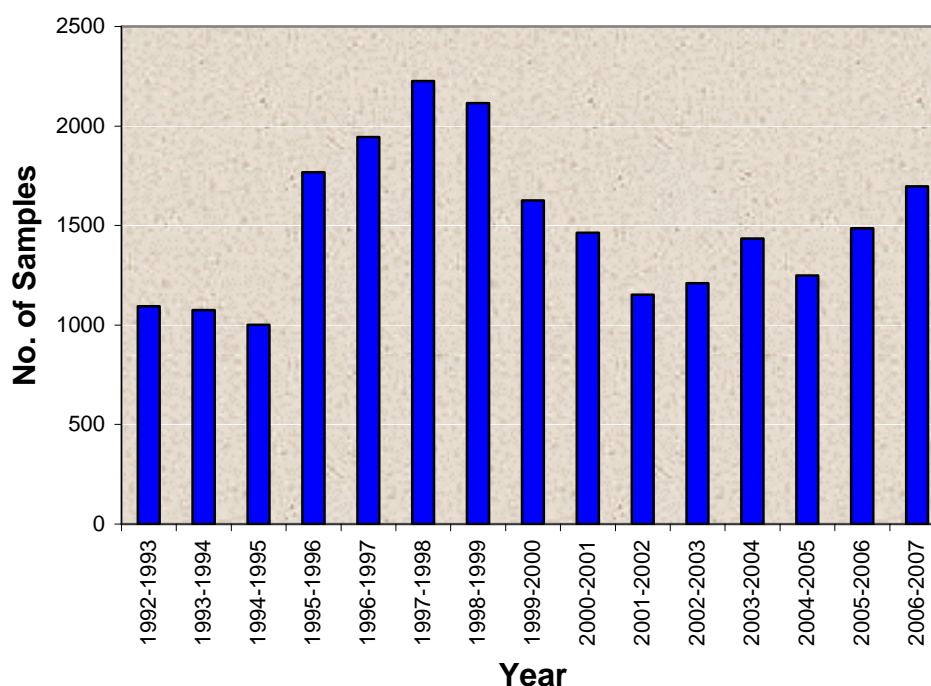
Prior to 1992, the routine sample processing was carried out at Dwellingup Research Centre. All identification of *Phytophthora* species isolated from samples processed between 1985 and 1992 was completed by Mike Stukely at Kensington. Records from these earlier years, for which map references are available, have been included in the **VHS database** which now has a total of 29,293 records.

The VHS was managed between 1992 and 2001 by Francis Tay, and from 2001 by Mike Stukely. Technical support (1 FTE) is provided on a time-share basis by Janet Webster and Juanita Ciampini.

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 unidentified new and historical *Phytophthora* isolates, which has led to the discovery of the ten new, undescribed taxa and also new records for WA of two known species (**Section 3**). Student projects investigating the new *Phytophthoras* have 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 2006-07.

Year	No. of Samples	Year	No. of Samples
1992-1993	1095	2000-2001	1463
1993-1994	1075	2001-2002	1153
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	<b>TOTAL</b>	<b>22,543</b>



## 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 and FPC personnel and sections.

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 (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 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 all *Phytophthora* 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.
- b. Honours project – **Biology and pathology of a *Phytophthora megasperma*-like pathogen** – seeking a student and expected to start in 2007-08 at Murdoch University.

[c. Honours project – **Biology and pathology of a *Phytophthora citricola*-like pathogen**. A student at Murdoch University (Melissa Bexley) commenced this project in 2006 to examine and describe the “P.sp.2” cultures and investigate their pathogenicity. Unfortunately the project was not completed, and key parts have now been included in (a) above.]

## 7. Publications and Presentations

- Research paper on the undescribed *Phytophthora*, “P.sp.2”, published in *Australasian Plant Disease Notes* 2: 49-51. Stukely *et al.* (2007): **A new homothallic *Phytophthora* from the jarrah forest in Western Australia.**
- Research paper on a new *Phytophthora* record for WA, submitted to *Australasian Plant Pathology*. Stukely *et al.*: ***Phytophthora inundata* from native vegetation in Western Australia.**
- Oral Presentation to Dieback Information Group (DIG) meeting, 21 July 2006 (also a presentation to DIG on 13 July 2007). Stukely: **New *Phytophthora* species in WA.**
- Poster prepared for 4<sup>th</sup> IUFRO Meeting on Phytophthoras in Forests and Natural Ecosystems, Monterey, California, USA, 26-31 August 2007. Stukely *et al.* (2007): **Molecular testing uncovers new *Phytophthora* taxa from natural ecosystems in Western Australia.**

## **8. References**

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- Cunnington JH, Jones RH, de Alwis S, Minchinton EJ (2006). Two new *Phytophthora* records for Australia. *Australasian Plant Pathology* **35**: 383-384.
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- D'Souza NK, Webster JL, Tay FCS (1997). *Phytophthora boehmeriae* isolated for the first time in Western Australia. *Australasian Plant Pathology* **26**: 204.
- Maseko B, Burgess TI, Coutinho TA, Wingfield MJ (2007). Two new *Phytophthora* species from South African *Eucalyptus* plantations. *Mycological Research* (accepted for publication).
- Stukely MJC, Shearer BL, Tay FCS, Hart RM, Hart RP (1997). *Phytophthora* species in natural vegetation in Western Australia. In: 'Programme and Summaries', 11<sup>th</sup> Biennial Conference, Australasian Plant Pathology Society, Perth, Western Australia, p.199. (Poster).

## **9. Attachments, notes and acknowledgments**

### **1. Map showing results of *Phytophthora* sample testing by DEC Vegetation Health Service, 2006-2007.**

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

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

### **2. Phylogenetic tree (based on ITS rDNA sequences) showing a limited selection of the known species of *Phytophthora*, and ten undescribed WA taxa (indicative only).**

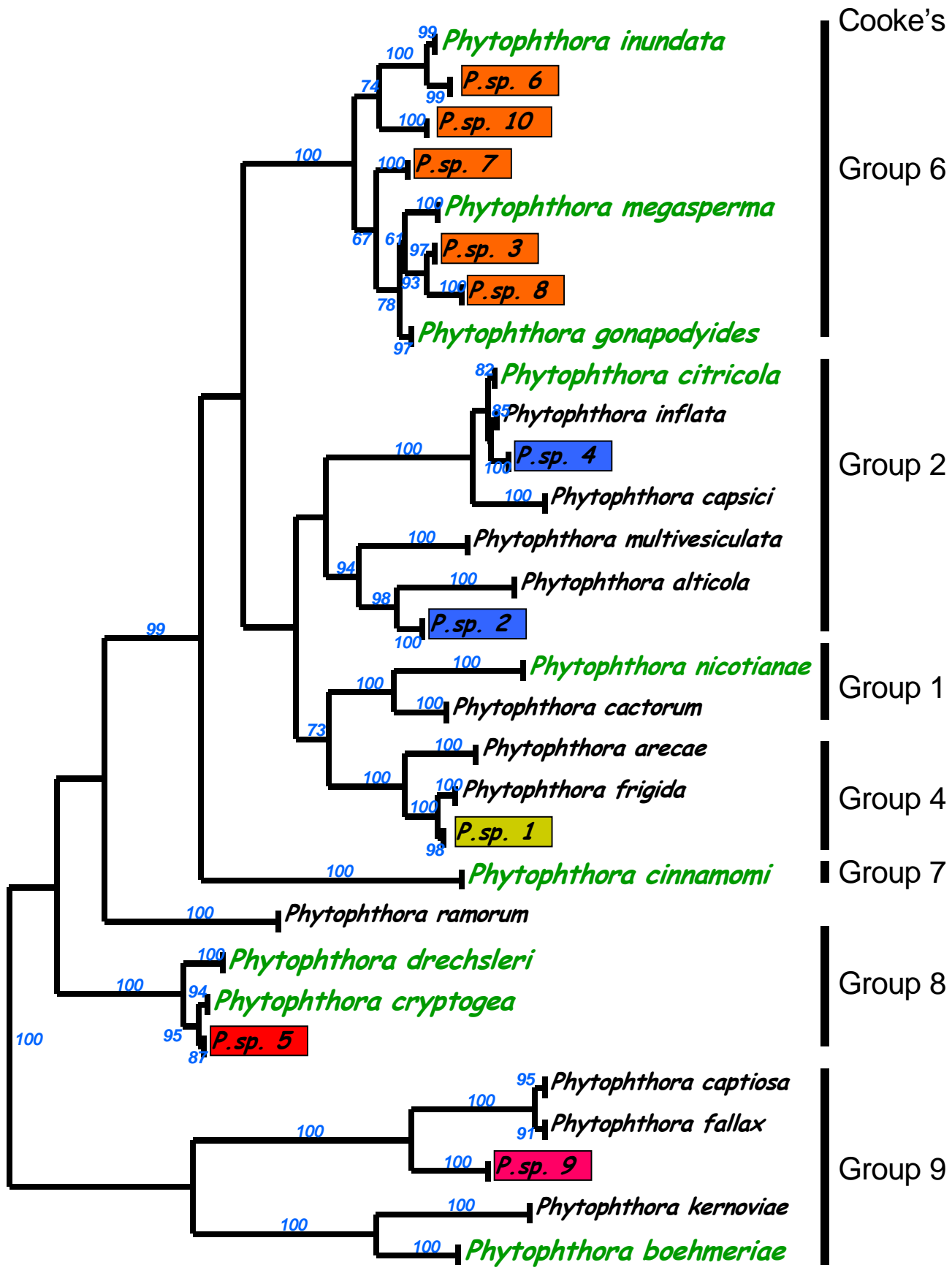
The ten undescribed taxa isolated from WA samples (“**P. spp. 1-10**”) are colour-coded according to the groupings proposed by Cooke *et al.* (2000), and Cooke's Groups 1, 2, 4, and 6-9 are shown at the right.

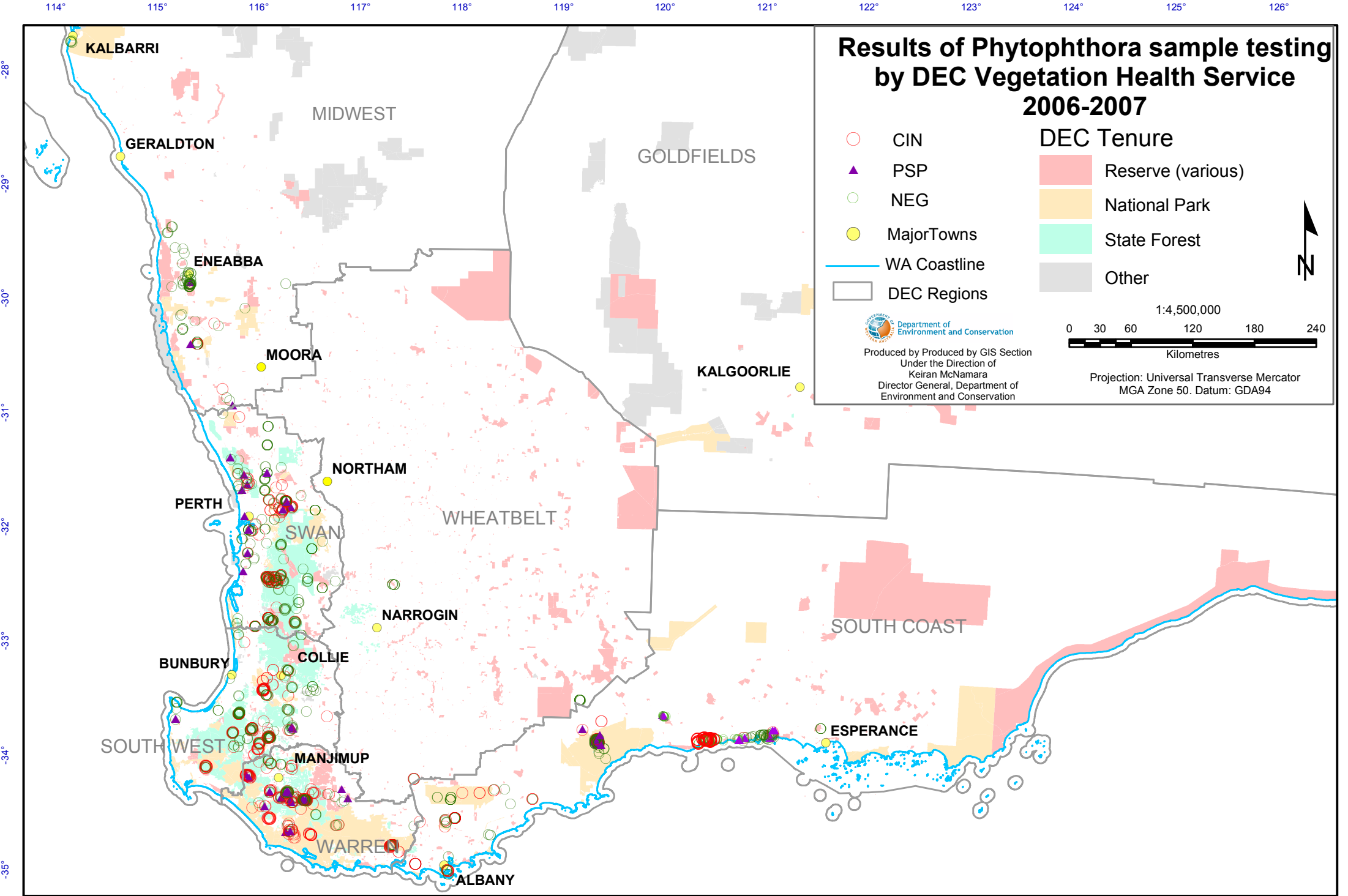
*Phytophthora* species shown in green text are those known previously to be present in WA natural ecosystems, based on the traditional morphological identification of pure cultures (Stukely *et al.* 1997; D'Souza *et al.* 1997).

Other species shown include 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.


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

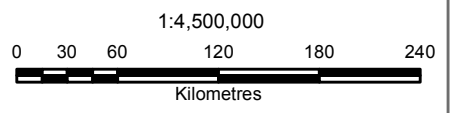




# Results of Phytophthora sample testing by DEC Vegetation Health Service 2006-2007

- CIN
  - ▲ PSP
  - NEG
  - Major Towns
  - WA Coastline
  - DEC Regions
- 
- Reserve (various)
  - National Park
  - State Forest
  - Other


 Department of Environment and Conservation  
 Produced by Produced by GIS Section  
 Under the Direction of  
 Keiran McNamara  
 Director General, Department of Environment and Conservation



Projection: Universal Transverse Mercator  
 MGA Zone 50. Datum: GDA94



114° 115°  
Graticule shown at 1 degree intervals

116° 117° 118° 119° 120° 121° 122° 123° 124° 125° 126° 127°  
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