

**Department of Environment and Conservation**

**Vegetation Health Service**

**Annual Report 2008-2009**

***Phytophthora* Detection**

**Mike Stukely, Janet Webster and Juanita Ciampini**

Vegetation Health Service, Science Division, Kensington  
Telephone: 9334 0317

**6 October 2009**

# Vegetation Health Service – Annual Report 2008-2009

## *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, the Forest Products Commission (FPC), NRM groups, and for Natural Heritage Trust projects. No other *Phytophthora* testing service is available on this scale in WA.

**Processing of samples:** 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 Centre for *Phytophthora* Science and Management (CPSM) at 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, associated plant species, land tenure, etc. The database now contains 33,376 records (at 30<sup>th</sup> June 2009), 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 1,208 cultures. Cultures from the Collection are made available to researchers in DEC and the CPSM, 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 2008-2009 financial year, the VHS received 1,784 samples for testing for the presence of *Phytophthora* (**Table 1** and **Figure 1**). This was below the previous year's record total of 2,353, which was boosted significantly by the BCI project (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**.

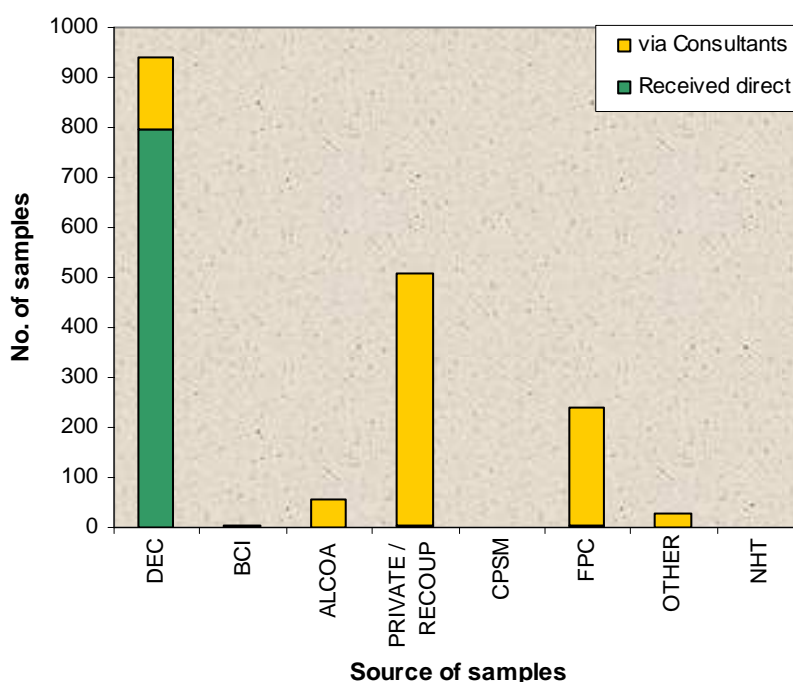
**CPSM** – samples associated with projects carried out at the **Centre for *Phytophthora* Science and Management** at Murdoch University.

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 again 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, Dieback Treatment Services, NPC Consulting, Moore Mapping, RPS Environmental, Coffey Environments, Ficifolia Consulting and Enviroworks Consulting – supplied 972 samples from various sources including DEC land and Alcoa (**Table 1** and **Figure 1**). In addition to Alcoa World Alumina Australia, mining companies sending samples direct to the VHS included Iluka Resources Ltd, and Wesfarmers Premier Coal; samples were also received direct from Southern Gateway Alliance in association with the Perth-Bunbury Highway project. No samples were received from NHT projects in 2008-09.

VHS facilities have again been made available for the processing of samples for **other DEC research projects** associated with the BCI (Dr Chris Dunne).

**Table 1 and Figure 1.** Numbers of samples received from major sources, including consultants, in 2008-2009. Numbers of samples received from the same sources in 2007-2008 are given in the Table for comparison.

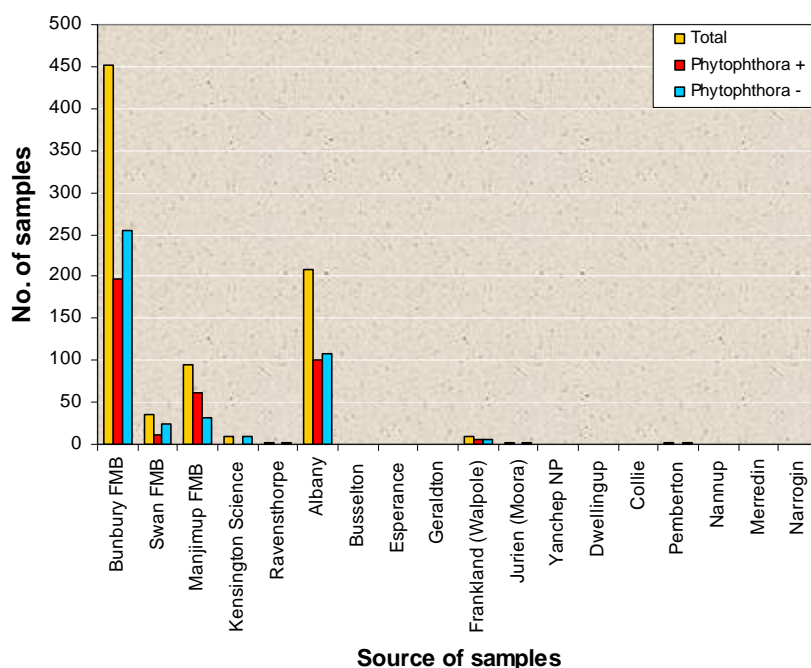
Source	No. of samples			
	Received direct	Received via Consultants	Total 2008-2009	Total 2007-2008
DEC	798	143	941	998
BCI	6	0	6	633
ALCOA	0	58	58	59
PRIVATE / RECOUP	5	505	510	515
CPSM	0	0	0	29
FPC	3	236	239	85
OTHER	0	30	30	34
NHT	0	0	0	0
<b>TOTAL</b>	<b>812</b>	<b>972</b>	<b>1784</b>	<b>2353</b>



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 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 2008-2009, and numbers giving positive or negative recoveries of *Phytophthora*. Total numbers of samples received from the same sources in 2007-2008 are given in the Table for comparison. DEC samples received from consultants are not included.

DEC Office	No. of Samples			
	Total received 2008-2009	Ph. Positive 2008-2009	Ph. Negative 2008-2009	Total received 2007-2008
Bunbury FMB	451	197	254	386
Swan FMB	35	11	24	143
Manjimup FMB	94	62	32	233
Kensington Science	10	0	10	38
Ravensthorpe	2	0	2	30
Albany	208	101	107	62
Busselton	0	0	0	8
Esperance	0	0	0	27
Geraldton	0	0	0	14
Frankland (Walpole)	10	5	5	3
Jurien (Moorra)	1	0	1	9
Yanchep NP	0	0	0	0
Dwellingup	0	0	0	49
Collie	0	0	0	2
Pemberton	2	0	2	1
Nannup	0	0	0	7
Merredin	0	0	0	0
Narrogin	0	0	0	0
<b>TOTAL</b>	<b>813</b>	<b>376</b>	<b>437</b>	<b>1012</b>



### **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 believed 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.1–12**", from dying native vegetation in WA (see **Section 3.1**) (Stukely *et al.*, 2007c; Stukely *et al.*, 2008; Burgess *et al.*, 2009). The first of the new species ('Psp4') was formally described as *Phytophthora multivora* sp. nov. (Scott *et al.*, 2009).

During 2008-2009, *Phytophthora* was isolated from 711 of a total of 1,784 samples processed (compared with 650 of 2,353 in 2007-2008) (**Table 3, Figure 3**). *Phytophthora cinnamomi* was, as usual, the species most frequently isolated (648 samples). *P. cryptogea* was isolated from seven samples, *P. inundata* from one, and *P. nicotianae* from nine.

The newly described species *P. multivora*, closely related to *P. citricola* (and morphologically very similar to it), was isolated from 15 samples and identified by DNA sequencing. An additional 18 isolates, identified on morphology only, are likely also to be *P. multivora* (see **Section 3.1**). Nine samples gave isolates that were shown by DNA sequencing to be members of other new (as yet undescribed) *Phytophthora* taxa (see **Section 3.1**): **Psp 1** (four isolates), **Psp2** (three), **Psp9** (one), and **Psp11** (one).

One sample yielded both *P. cinnamomi* and *P. inundata*, and one contained both *P. cinnamomi* and *P. multivora*. Another six *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 1,208 cultures stored in the VHS Collection. In addition, the VHS holds *Phytophthora* cultures from the following earlier collections that were stored and numbered by researchers and consultants: MJS series (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 the CPSM, 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 2008-2009. Corresponding numbers of samples for 2007-2008 are given in the Table for comparison.

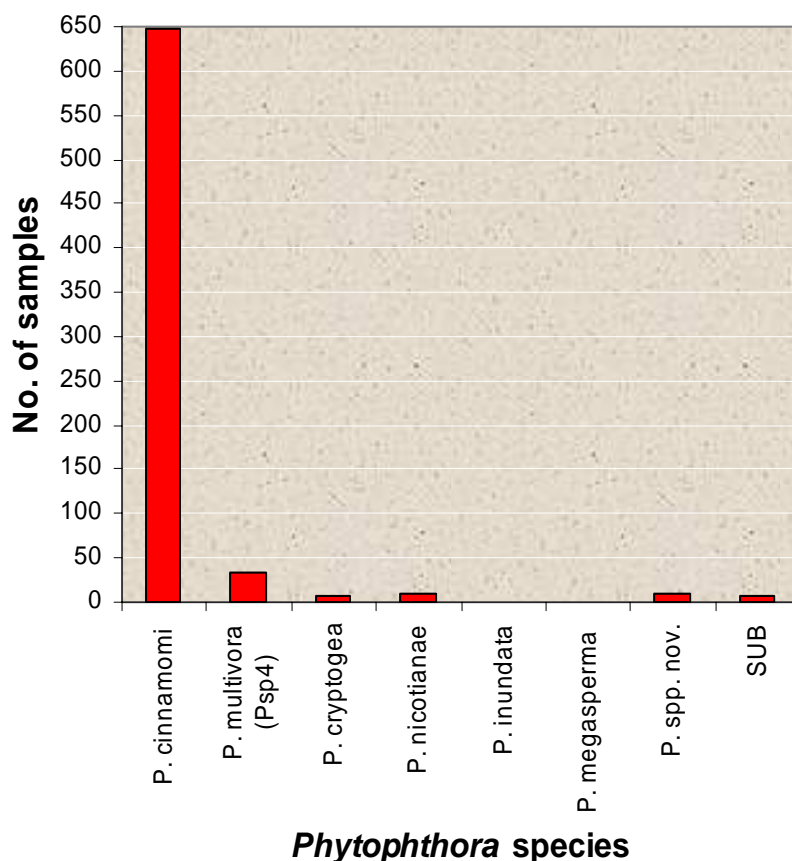
<i>Phytophthora</i> species	No. of samples with positive <i>Phytophthora</i> recovery 2008-2009		No. of samples with positive <i>Phytophthora</i> recovery 2007-2008	
	Primary result	Second species <sup>1</sup>	Primary result	Second species <sup>1</sup>
<i>P. cinnamomi</i>	648		573	
<i>P. multivora</i> <sup>2</sup> ('Psp4')	32	1	46	2
<i>P. cryptogea</i>	7		8	
<i>P. nicotianae</i>	9		3	
<i>P. inundata</i>	0	1	5	
<i>P. megasperma</i>	0		0	
<i>P. spp. nov.</i> <sup>3</sup>	9		13	
SUB <sup>4</sup>	6		2	
<b>TOTAL</b>	<b>711</b>	<b>2</b>	<b>650</b>	<b>2</b>

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

<sup>2</sup>*P. multivora*, described in 2009, was formerly identified as *P. citricola*.

<sup>3</sup>*P. spp. nov.* – these isolates represent undescribed new *Phytophthora* taxa 'P.spp.1-12' (see Section 3.1), excluding *P. multivora* ('Psp4').

<sup>4</sup>SUB – these *Phytophthora* cultures are still in the process of being 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**).

Very significant progress has been made in the last year. We have now received ITS rDNA sequence data for a total of over 260 recent and historical isolates of *Phytophthora*, most of which were obtained from samples collected in 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, our findings to date on the new *Phytophthora* taxa are:

- 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 - 12**”).
- 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).
- The first of the new WA species, *P. multivora* sp. nov. [‘Psp4’], was described in 2009.
- In addition to the above ten new species (for each of which there are multiple isolates), there are several **unique *Phytophthora* isolates** from natural ecosystems in WA.
- There is strong new evidence (2009) that some of the unique WA *Phytophthora* isolates are **hybrids**. Further investigation of these is now required.
- Only **two WA isolates** 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 *P. multivora* and some ‘Psp2’ (the latter has a distinct morphology).
- **None** of the isolates identified earlier as *P. drechsleri* (on their morphology) had a 100% match to sequences for *P. drechsleri* on GenBank. These isolates all represent new taxa.
- Many of the ten new taxa, and all of the 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 ‘niederhauserii’ 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 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’.
- WA isolates previously identified as *P. gonapodyides* align with the undescribed *P.* taxon ‘PgChlamydo’.
- The large group of isolates designated earlier as Psp5 are now included as *P. cryptogea*.

### **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 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 *Phytophthora* taxa are **indigenous or introduced**.
- New evidence (2009) shows that several unique *Phytophthora* **hybrids** are present.

Some of the new *Phytophthoras* (eg *P. multivora* [‘Psp4’], formerly identified as ‘*P. citricola*’), but not all, are widespread and are associated mostly with low-impact dieback sites. **However, it is important at this stage that they should not all be treated as indigenous (and hence “not threatening”), until they have been 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 WA’s 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* and hybrids 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. Clearly, this issue now needs to be addressed by land managers with a well-considered, adaptive management approach.

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, and restricting operations to “dry soil” conditions) may not always be appropriate, nor directly applicable without modification, for managing the ‘new’ *Phytophthora* species.

The newly discovered *Phytophthora* **hybrids** and their origins are being investigated further. Their presence reinforces the importance and urgency of preventing the spread of *Phytophthoras* wherever possible. **The opportunities for new hybrids to form must be minimised.**

**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, DDS), a total of 26,695 samples have been processed for *Phytophthora* detection (to 30<sup>th</sup> June 2009) (**Table 4** and **Figure 4**). The 2008-2009 total (1,784) was below the previous year's record total (2,353), which was boosted significantly by the BCI project. Nevertheless, the 2008-2009 total was the second-highest achieved in the last 10 years.

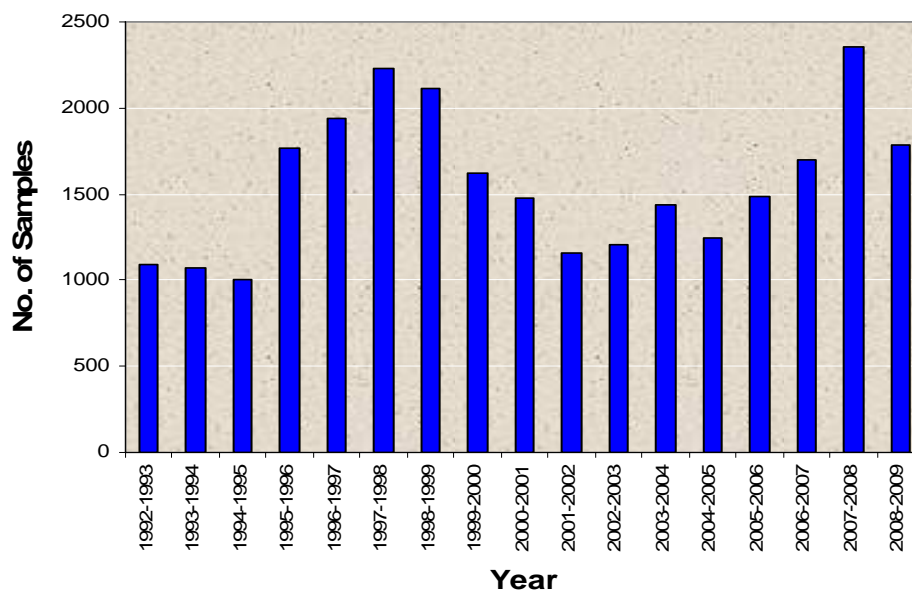
Prior to 1992, the routine sample processing was carried out at Dwellingup Research Centre. Between 1984 and 1992 this was done by Janet Webster. All identification of *Phytophthora* species isolated from these samples between 1985 and 1992 was completed by Mike Stukely at Kensington. Records from these earlier years, for which map references are available, have been included in the **VHS database** which now contains a total of 33,376 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; additional casual assistance is utilised as required. 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 and hybrids, and of new records for WA of several known species and undescribed species that are known from elsewhere (**Section 3**). A student project investigating the new *Phytophthoras* is in progress at Murdoch University under joint supervision by Prof. Hardy, Dr T Burgess and M Stukely (**Section 6**).

**Table 4 and Figure 4.** Numbers of samples processed by the VHS for *Phytophthora* detection from 1992-93 to 2008-09.

Year	No. of Samples	Year	No. of Samples
1992-1993	1095	2001-2002	1154
1993-1994	1075	2002-2003	1210
1994-1995	1001	2003-2004	1435
1995-1996	1767	2004-2005	1250
1996-1997	1944	2005-2006	1486
1997-1998	2227	2006-2007	1696
1998-1999	2115	2007-2008	2353
1999-2000	1626	2008-2009	1784
2000-2001	1477	<b>TOTAL</b>	<b>26,695</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 personnel and sections. No other service that is 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 and essential 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 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.

## **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, 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.** 4<sup>th</sup> IUFRO Meeting on Phytophthoras in Forests and Natural Ecosystems, Monterey, California, USA, 26-31 August 2007.
- DEC Internal 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.** 3<sup>rd</sup> 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 published in international journal. Burgess TI, Webster JL, Ciampini JA, White D, Hardy GESTJ, Stukely MJC (2009): **Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia using molecular techniques.** *Plant Disease* **93**:215-223.
- Paper published in international journal. Scott PM, Burgess TI, Barber PA, Shearer BL, Stukely MJC, Hardy GESTJ, Jung T (2009): ***Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia.** *Persoonia* **22**:1-13.
- DEC Science Division Information Sheet #8/2009. Stukely M (2009): ***Phytophthora* Dieback – detecting the pathogen.**

## **8. Other References Cited**

- CALM (2002). *Phytophthora cinnamomi* and disease caused by it. Volume I – Management Guidelines.
- 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.
- Dick MA, Dobbie K, Cooke DEL, Brasier CM (2006). *Phytophthora captiosa* sp. nov. and *P. fallax* sp. nov. causing crown dieback of *Eucalyptus* in New Zealand. *Mycological Research* **110**: 393-404.
- 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* **111**:1321-1338.
- 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, 2008-2009.**

*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).**

**Tree 1:** 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 believed 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 international **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.

**Tree 2:** 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). Five **unique** WA isolates are shown in purple boxes – new evidence (2009) shows that four of these (and several others) are hybrids.

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

# Results of *Phytophthora* sample testing by DEC Vegetation Health Service 2008-2009

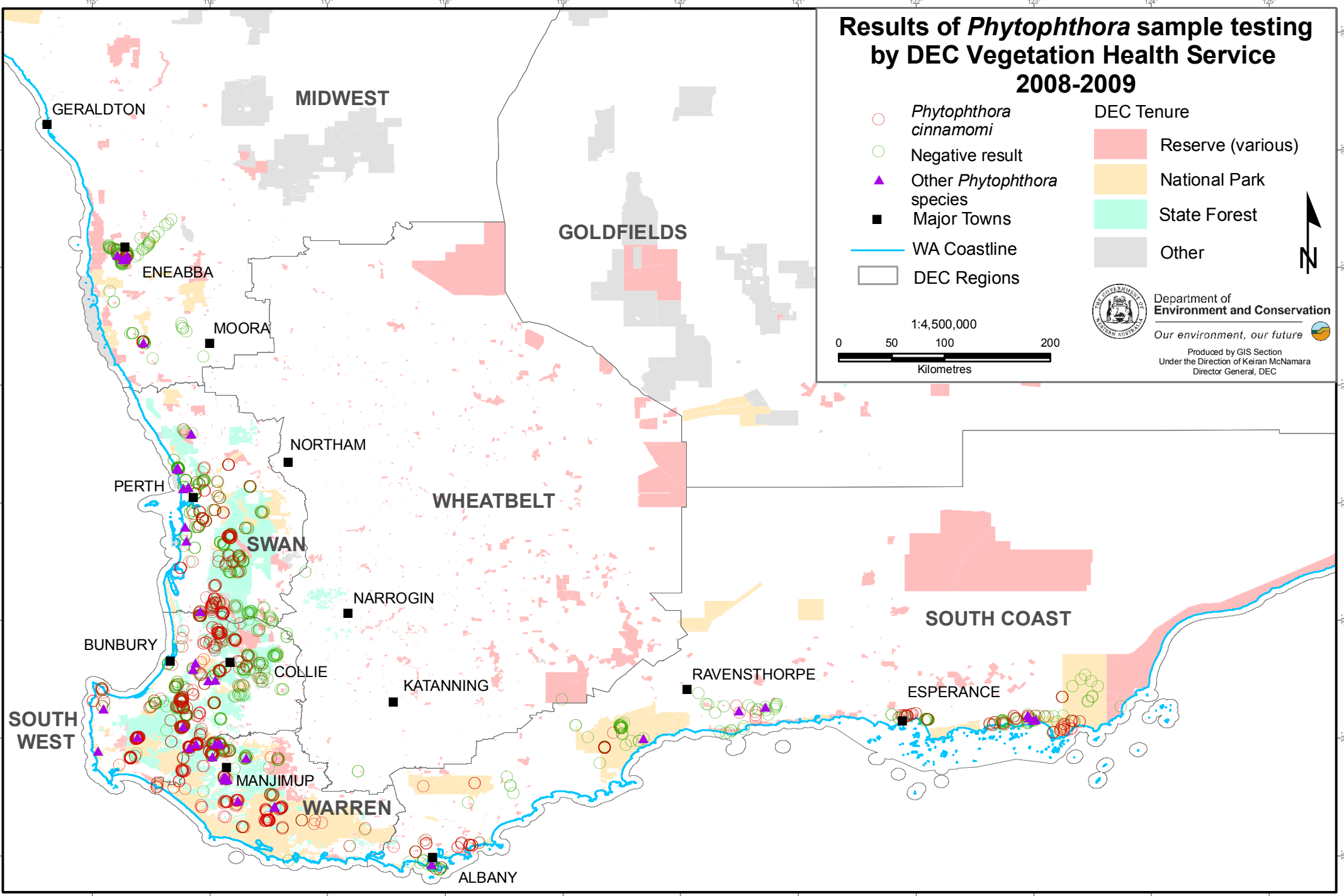
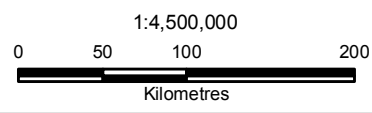
- *Phytophthora cinnamomi*
  - Negative result
  - ▲ Other *Phytophthora* species
  - Major Towns
  - WA Coastline
  - DEC Regions
- DEC Tenure
  - Reserve (various)
  - National Park
  - State Forest
  - Other



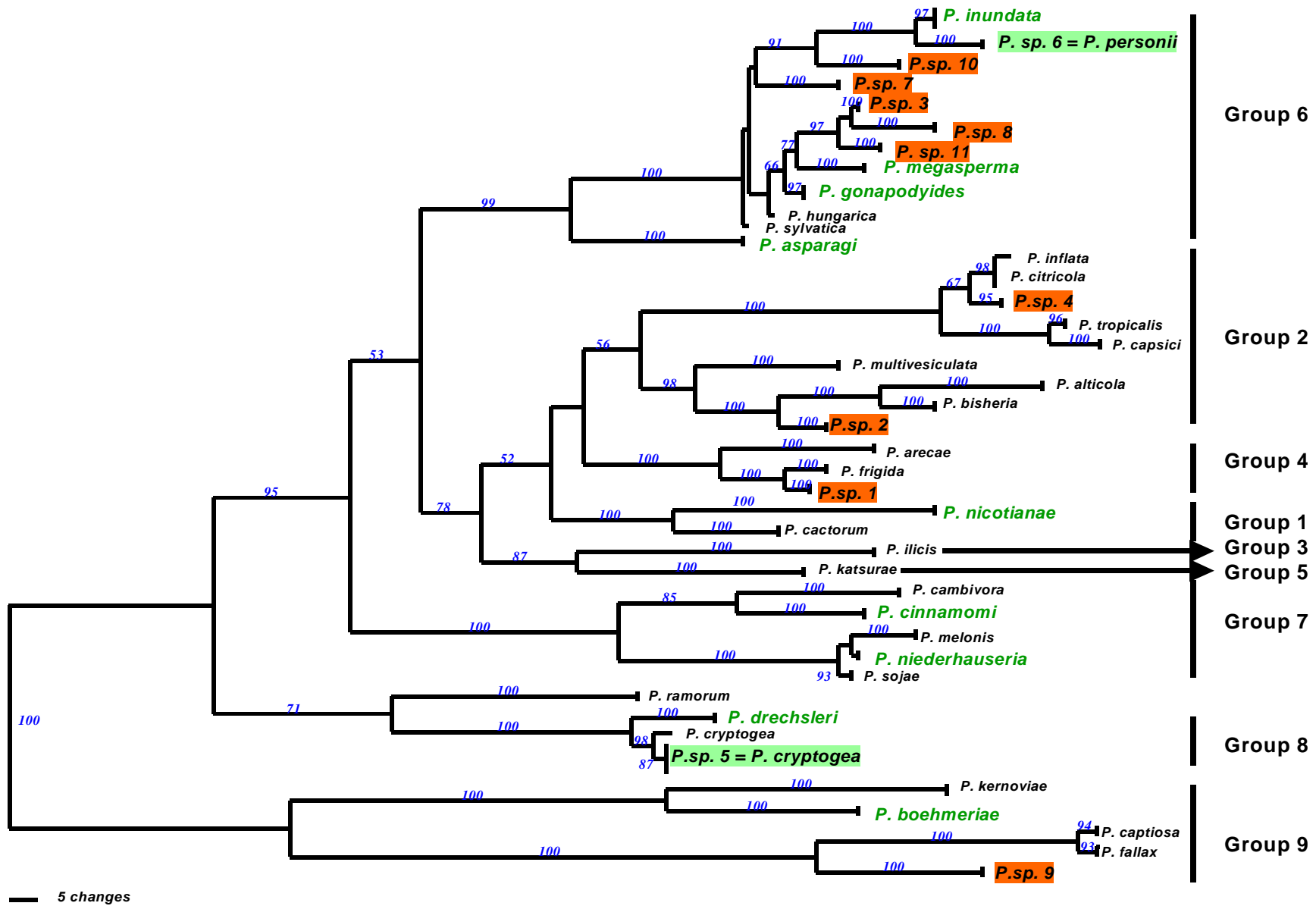
Department of  
**Environment and Conservation**

*Our environment, our future*

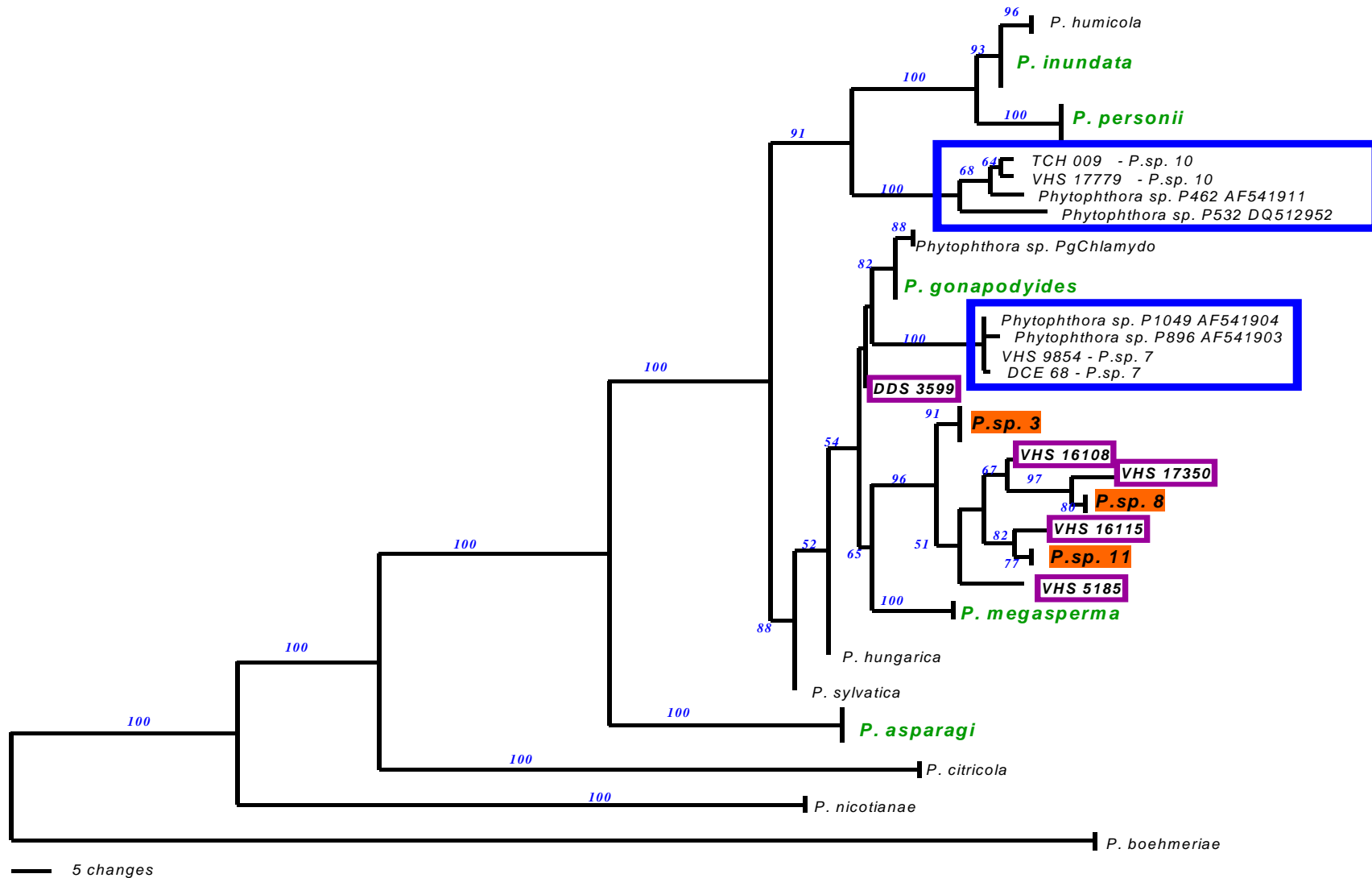
Produced by GIS Section  
Under the Direction of Keiran McNamara  
Director General, DEC



Graticule shown at 1 degree intervals



Phylogenetic Tree 1 – *Phytophthora* Groups 1-9 (Cooke): New taxa 'P. spp. 1-11' are highlighted red.



**Phylogenetic Tree 2 – *Phytophthora* Group 6 (Cooke): New taxa ‘P. spp. 10, 7, 3, 8, 11’; and five unique isolates (see purple boxes). VHS Annual Report 2008-2009.**