Department of Environment and Conservation

Vegetation Health Service

Annual Report 2011-2012

Phytophthora Detection

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1. Introduction

The DEC **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. VHS staff salaries are funded by DEC's Sustainable Forest Management Division (90%) and Nature Conservation Division (10%). The VHS is run by Science Division staff at the Kensington Research Centre. Its services are offered free of charge to all DEC sections and personnel, and to non-profit community groups sponsored by DEC such as SCRIPT and the NRM groups. The service is also available to external clients at a standard fee of $77 per sample, with discounted rates applying to samples collected for Alcoa World Alumina Australia, the Forest Products Commission (FPC), and Caring for our Country (formerly NHT) 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 examined microscopically in the VHS and most are identified to species here from their **morphological characters**. **DNA sequencing** is used for selected isolates through the service provided by the Centre for *Phytophthora* Science and Management (CPSM) at Murdoch University, enabling these isolates also to be identified to species.

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 39,092 records (at 30th June 2012). 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). This now contains 1,138 cultures, plus earlier collections. 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, as well as the public, with other plant disease problems in forests, plantations, parks and reserves, and nurseries.

2. Annual summary – samples processed

During the 2011-2012 financial year, the VHS received 2,198 samples for testing for the presence of *Phytophthora* (**Table 1** and **Figure 1**). This was well above the previous year's total of 1,936 and is the third-highest yearly total processed by the VHS (see **Table 4** and **Figure 4**). The sources of the 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.

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 (private consultants, land managers or owners).

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

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

CC – samples associated with projects funded by **Caring for our Country** (formerly **Natural Heritage Trust, NHT)**.

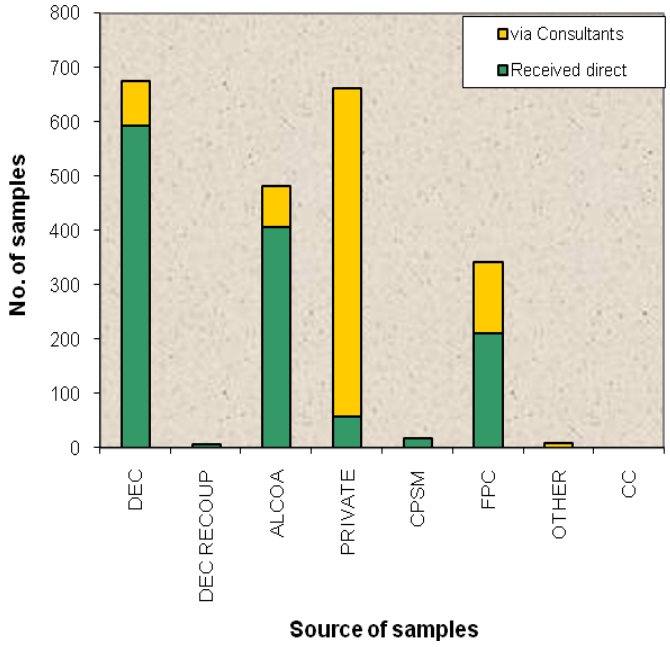
CPSM – samples associated with projects carried out at the **Centre for *Phytophthora* Science and Management** at Murdoch University.

The number of samples collected by, or for, DEC (675) was significantly lower this year (total 947 in 2010-2011). 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. Samples are also received from various DEC District offices (**Table 2** and **Figure 2**). More samples were received from FPC this year (total 343). Private Environmental Consultants – Glevan Consulting, Dieback Treatment Services, NPC Consulting, Moore Mapping, NRG Consulting, RPS Environmental, Natural Area Consulting, Great Southern Biologic, Bark Environmental Consulting, and Ekologica – supplied 899 samples (1,008 in 2010-2011) from various sources including from DEC land and Alcoa (**Table 1** and **Figure 1**). Alcoa World Alumina Australia was the only mining company sending samples direct to the VHS again this year, and these included a major sampling program from Dieback-Free Pits. No samples were received from Caring for our Country (formerly NHT) projects in 2011-12. Other sources included NRM groups, government authorities, and shires.

VHS facilities have again been made available for the processing of samples for **other DEC research projects** (Dr Chris Dunne, Colin Crane).

Table 1 and Figure 1. Numbers of samples received by the VHS from major sources, including consultants, in 2011-2012. Numbers of samples received from the same sources in 2010-2011 are shown in the Table for comparison. Abbreviations of sources are as listed above.

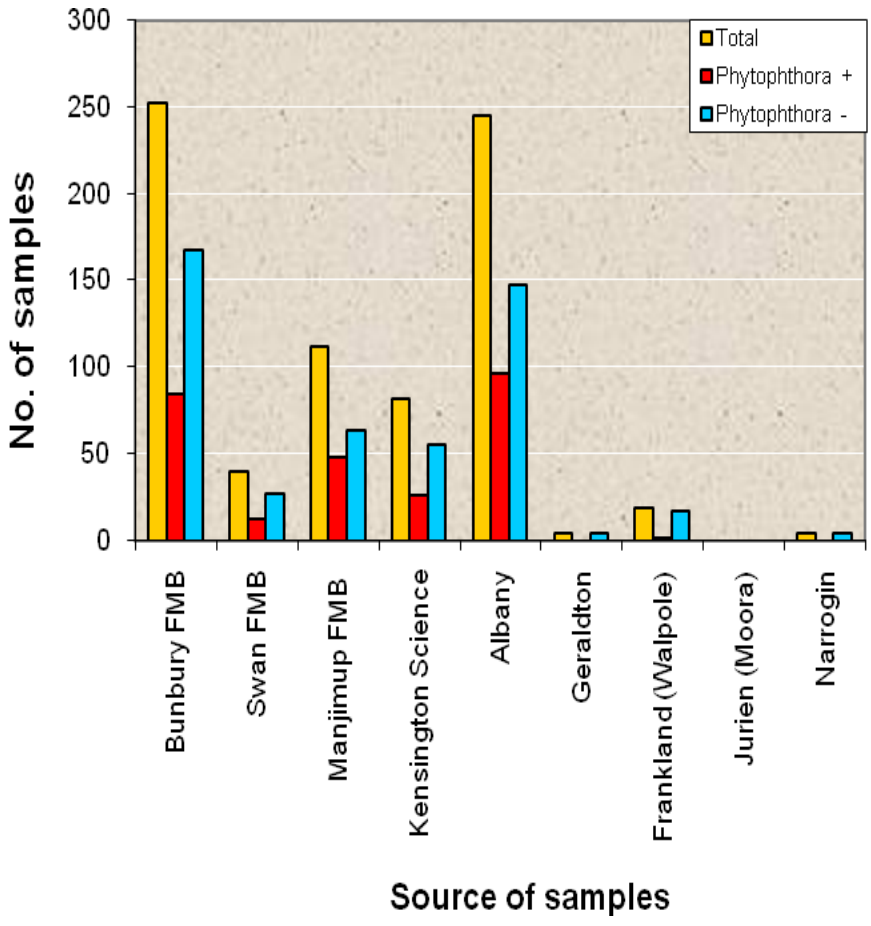
Source (Job Type, as per Sample Information Sheet)	No. of samples			
	Received direct	Received via Consultants	Total 2011-2012	Total 2010-2011
DEC	594	81	675	947
DEC RECOUP	8	0	8	(Incl. with Private)
ALCOA	408	74	482	84
PRIVATE	59	603	662	646
CPSM	18	0	18	0
FPC	211	132	343	204
OTHER	1	9	10	55
CC	0	0	0	
TOTAL	**1299**	**899**	**2198**	**1936**



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

Table 2 and Figure 2. Numbers of samples received by the VHS from DEC offices in 2011-2012, and numbers giving positive or negative recoveries of *Phytophthora*. Total numbers of samples received from the same sources in 2010-2011 are shown in the Table for comparison. DEC samples received from consultants are not included.

DEC Office	No. of Samples			
	Total received 2011-2012	*Ph*. Positive 2011-2012	*Ph*. Negative 2011-2012	Total received 2010-2011
Bunbury FMB	253	85	168	227
Swan FMB	40	13	27	213
Manjimup FMB	112	48	64	115
Kensington Science	82	26	56	142
Albany	245	97	148	294
Geraldton	5	0	5	0
Frankland (Walpole)	19	2	17	0
Jurien (Moora)	0	0	0	2
Narrogin	5	0	5	0
TOTAL	**761**	**271**	**490**	**993**



3. *Phytophthora* species in WA natural ecosystems

Based upon the traditional methods of identification of *Phytophthora* isolates by microscopic examination of their morphological characters in pure culture, the following six morpho-species (in addition to *P. cinnamomi*) had previously been documented in association with dying plants 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). Representative isolates of the WA species had been sent to international authorities such as CABI (UK) and CBS (The Netherlands) for verification, and their identities confirmed from their morphology.

In addition to the above morpho-species, ***P. inundata*** had been identified in WA by DNA sequencing prior to June 2007 (Stukely *et al*., 2007b). Testing of new and historical isolates by this technique since 2005 has led to the discovery of more than fifteen **new and previously undescribed *Phytophthora* species** in WA natural ecosystems. These were initially designated "**P.sp.1, 2, …"**, and were mostly associated with dying vegetation (see **Section 3.1**) (see Stukely *et al*., 2007c; Stukely *et al*., 2008; Burgess *et al*., 2009). Descriptions of nine species (***Phytophthora multivora***, ***P. elongata, P. thermophila, P. gibbosa, P. gregata, P. litoralis, P. arenaria, P. constricta*** **and** ***P. fluvialis***) had been published to June 2011, and ***P. amnicola*** now brings the total to ten. References are given in **Section 7** (p. 12). Pathogenicity has so far been tested and confirmed on native plants for *P. multivora*, *P. elongata*, *P. arenaria* and *P. constricta*, and further testing of new species is to be carried out. Several additional new WA taxa await formal description. New records for WA of several other *Phytophthora* taxa known from elsewhere have also been discovered, and these are listed in **Section 3.1** (p.7).

During 2011-2012, *Phytophthora* was isolated from 757 of a total of 2,198 samples processed (compared with 590 of 1,936 in 2010-2011) (**Table 3, Figure 3**). ***Phytophthora cinnamomi*** was, as usual, the species most frequently isolated (from 625 samples, compared with 544 in 2010-2011). Significant numbers of isolates were recovered for ***P. multivora*** (44), ***P. elongata*** (27) and ***P. nicotianae*** (11), with other named species being found less frequently. Thirty-three samples gave isolates that were shown by DNA sequencing to be members of other new (as yet undescribed) *Phytophthora* taxa (see **Section 3.1**). Most of these (23) were ***P.* aff. *arenaria***, from an infestation in a wheat-belt nursery supplying oil mallee seedlings to DEC, and further investigations on this are proceeding. Others included ***P.* aff. *humicola***, ***P.* aff. *elongata***, ***P.* aff. *rosacearum***, ***P.* aff. *captiosa***, ***P.* taxon stagnum**, and some other unique isolates.

One sample yielded both *P. cinnamomi* and *P. multivora*, one *P. cinnamomi* and *P. nicotianae*, and one *P. inundata* and an undescribed *P.* species.

The locations where DEC's and other samples were collected are shown on the attached **Map**, with results categorised to distinguish a). *P. cinnamomi*; b). Other *Phytophthora* spp. (including undescribed *Phytophthora* taxa); and c). Negative.

Representative pure living cultures of all *Phytophthora* species isolated, and 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. From 2010, Hemp seed is progressively being introduced as the standard storage medium. There are now 1,138 live cultures stored in the main VHS Collection. In addition, the VHS holds *Phytophthora* cultures from the following earlier collections that were stored and numbered by researchers and consultants: the MJS series (M Stukely), DCE (E Davison), TCH (T Hill) and HSA (Hart, Simpson and Associates). Various backup cultures are also held. 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, whose maintenance receives a high priority.

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

A review of our present knowledge of these Phytophthoras found in WA natural ecosystems, and an outline of some implications and recommendations for management, are given below.

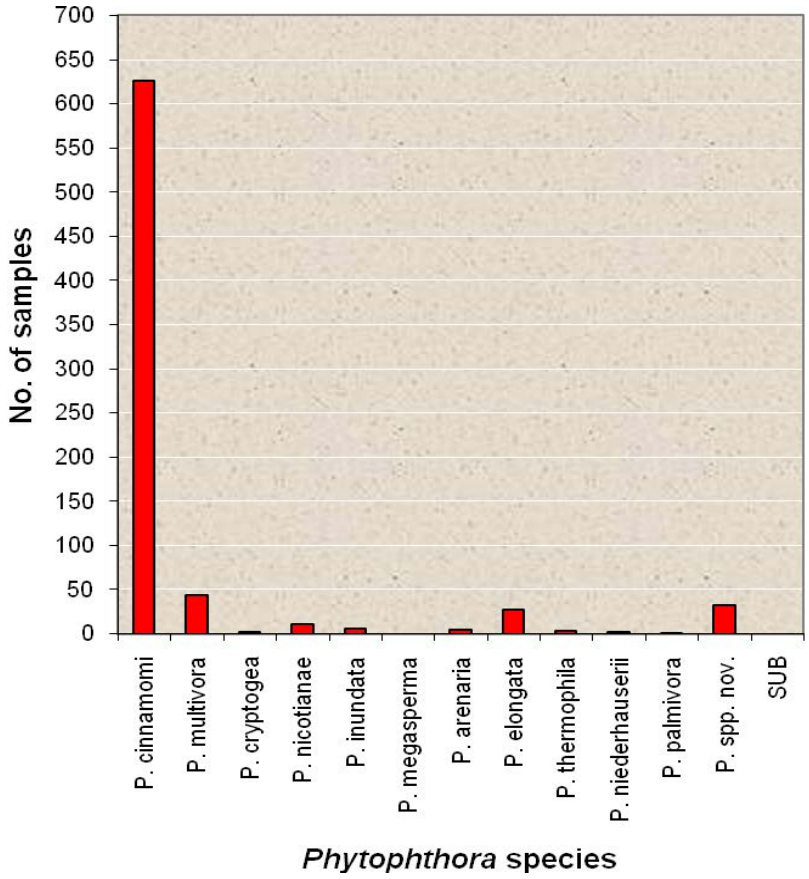
Table 3 and Figure 3. Numbers of samples from which *Phytophthora* species were isolated by the VHS in 2011-2012. Corresponding numbers of samples for 2010-2011 are shown in the Table for comparison.

***Phytophthora* species**	**No. of samples with positive *Phytophthora* recovery 2011-2012**		**No. of samples with positive *Phytophthora* recovery 2010-2011**	
	Primary result	**Second species[1]**	**Primary result**	**Second species[1]**
P. cinnamomi	625	0	544	
P. multivora	43	1	12	
P. cryptogea	2	0	19	1
P. nicotianae	10	1	4	
P. inundata	6	0	4	
P. megasperma	0	0	0	
P. arenaria	5	0		
P. elongata	27	0		
P. thermophila	4	0		
P. niederhauserii	2	0		
P. palmivora	1	0		
***P.* spp. nov.[2]**	**32**	**1**	2	
SUB[3]	**0**	**0**	5	
TOTAL	**757**	**3**	**590**	

[1]Second species – Three samples yielded more than one *Phytophthora* species as shown. Their primary results were *P. cinnamomi*, *P. cinnamomi*, and *P. inundata* (respectively).

[2]*P.* spp. nov. – these isolates represent undescribed new *Phytophthora* taxa, including hybrids (see Section 3.1).

[3]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 in the last decade has caused something of a revolution in *Phytophthora* taxonomy, with well over 50 new species having been discovered, worldwide, since 2000. To put this into perspective, a total of just 55 *Phytophthora* species had been formally described up to 1999. 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. In addition, DNA sequencing has confirmed that the *P. cinnamomi* isolates have been correctly identified from their morphology.

> Overview of DNA sequencing and data analysis: DNA was extracted from pure cultures of *Phytophthora* grown on half-strength potato dextrose 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 then constructed (see Attachment).

Very significant progress has been made again in the last year. We have now received ITS rDNA sequence data for a total of 725 recent and historical isolates of *Phytophthora* (to 30 June 2012), 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 database of DNA sequences that was created in 1988 and is in the public domain.]

In brief, our findings to date on the new *Phytophthora* taxa are:

- There are now known to be **more than fifteen previously undescribed new species of *Phytophthora* present in natural ecosystems in WA**.
- 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. 13).
- **Ten** of the new WA species have now been formally described: ***P. multivora, P. elongata, P. thermophila, P. gibbosa, P. gregata, P. litoralis, P. arenaria, P. constricta, P. fluvialis*** and ***P. amnicola.*** Several additional new WA taxa await formal description (see **phylogenetic tree)**. [Another new species, ***P. bilorbang***, associated with declining Blackberry in southern WA, was described by Murdoch University researchers in 2012 (Aghighi *et al*. 2012). This species has not yet been found among the isolates in the VHS collection.]
- **Pathogenicity** has been tested and confirmed on selected WA native plant hosts for ***P. multivora, P. elongata, P. arenaria*** and ***P. constricta*** (Murdoch University projects).
- Two of these species, *P. arenaria* and *P. constricta*, are believed to be endemic in WA.
- There have been **nine** new records for WA of *Phytophthora* taxa that are known elsewhere: ***P. inundata, P. niederhauserii, P. asparagi, P. palmivora***, ***P. rosacearum***, ***P.* taxon personii, *P.* taxon PgChlamydo** and ***P. lacustris*.**
- In addition to the above new species and records there are several unique isolates, as well as some ***Phytophthora*** **hybrid** isolates from natural ecosystems in WA **(Burgess *et al.* 2010)**. Larger numbers of hybrids have been isolated from WA waterways in the "Fishing for *Phytophthora*" project at CPSM (and also in South Africa). Further investigation of these *Phytophthora* hybrids and their origins is in progress. The number and diversity of the *Phytophthora* hybrids found in WA and South Africa represents a unique situation worldwide, to date.
- The previously recorded presence in WA natural ecosystems of **four** *Phytophthora* species other than *P. cinnamomi* (***P. cryptogea, P. nicotianae, P. megasperma, P. boehmeriae***) has been confirmed by DNA sequencing of stored isolates.
- However, two species previously believed to be present based on morphology (***P. citricola*** and ***P. drechsleri***) are in fact **not present** among the isolates tested to date.

- Still only **two WA isolates** of the many identified earlier as ***P. megasperma*** (based on their morphology) have had a 100% match to DNA sequences for *P. megasperma* on GenBank. The other isolates represent several diverse new taxa.
- Of the ten newly-described *Phytophthora* species, all but one (*P. fluvialis*) have been found **associated with dying indicator plants** in WA native forest or heath-land.
- The extent of the **host ranges** of the new *Phytophthora* taxa is unknown. Several of them are associated with deaths of native WA plants representing multiple families. The number of plant species associated with the new Phytophthoras is still increasing rapidly, as new isolates are found.
- Some *Phytophthora* taxa appear to have limited **distribution**, while others like *P. multivora* are widespread with an overall range in WA similar to that of *P. cinnamomi*. However, some Phytophthoras may be active in a broader range of site conditions than those favouring *P. cinnamomi* (e.g. *P. multivora* in calcareous soils, affecting tuart).
- Many (but not all) of the ten newly-described *Phytophthora* species have so far been recorded **only in WA**. However, *P. multivora*, for example, has been found also in South Africa where it is asymptomatic in natural ecosystems, and recently from a number of European and Asian countries, North America and New Zealand where it has been almost exclusively associated with horticultural or nursery plants.

3.2 Management implications and recommendations

Many of the previously undescribed, new *Phytophthora* taxa (**Section 3.1**) are genetically quite distinct from the Phytophthoras known earlier 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 that they may pose different potential and immediate levels of threat to biodiversity. This must now be investigated. However, it appears that they are not particularly new introductions to WA, with some isolates of most species having been obtained from samples collected in WA in the 1980s and 1990s, as well as more recently.

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

The previously undescribed Phytophthoras, as well as the known species newly recorded in WA, 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 completely "benign" organism – all are pathogens, with host specificities varying from a single plant species (eg *P. sojae*) to well over 2,000 species (*P. cinnamomi* in WA alone). Detailed investigations must therefore be conducted on each new *Phytophthora* species to determine its host range, pathogenicity, distribution, ease of spread, preferred environmental conditions, survival structures and mechanisms, and other characteristics such as its response to the inhibitor, phosphite. Pathogenicity has so far been tested and confirmed on selected WA native plant hosts for *P. multivora, P. elongata, P. arenaria* and *P. constricta*.

- Some (but not all) of the new WA *Phytophthora* taxa are likely to be **indigenous**.
- Several *Phytophthora* **hybrids** are also present in WA natural ecosystems.

Some of the new Phytophthoras (eg *P. multivora*, formerly identified as the morpho-species '*P. citricola'*), <u>but not all</u>, are widespread and are often associated with low-impact dieback sites. **However, it is important at this stage that they should <u>not</u> all be assumed to be "non-threatening", due to their often relatively low observed impact levels, until they have been investigated further.** This applies also to the apparently indigenous species.

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 has been known since the 1980s that, for example, the "*P. megasperma"* morpho-species causes most damage following heavy 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 Precautionary Principle should now be applied to the management of Phytophthoras in natural ecosystems generally: the new *Phytophthora* species and hybrids should all be regarded as a threat to biodiversity, and managed accordingly, until it is unequivocally proven otherwise. Consistent, pro-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.

Demarcation of the actual areas infested by particular species other than *P. cinnamomi* in the field, while desirable, will be difficult and unreliable unless there is high disease impact. Disease fronts may not be clearly defined, and often only sparse point distribution data are available from positive sample test results. Sometimes, multiple *Phytophthora* species may be present. It will still be important, as a priority, to identify and manage areas infested with *P. cinnamomi* so as to minimise the risk of introducing *P. cinnamomi* to new areas (including, for example, avoiding its transfer to adjacent areas that may be infested with other *Phytophthora* species but not yet with *P. cinnamomi*). Minimising the spread of **any** *Phytophthora* species to areas where they are not yet present will of course be important, but there are clear limitations to the practicality of this where several *Phytophthora* species may occur in close proximity. Resources must continue to be allocated so as to protect priority areas, based on risk assessment.

It is a matter of concern that *Phytophthora* was isolated from seedlings in one **nursery** (*P*. aff. *arenaria*), and from the floor in another (*P. cryptogea* and *P*. aff. *humicola*), in 2012. These nurseries are located in the wheat-belt and supply seedlings to DEC and other agencies for nature conservation and commercially-oriented revegetation projects. The seedlings are then widely distributed in the wheat-belt and other areas. While lower rainfall areas are generally thought not to be conducive to *Phytophthora*, this does preclude the existence of localised and seasonal wetter conditions that are highly conducive to the pathogen, where it could establish. For example, a new *P cinnamomi* infestation was found in Narrogin in 2012. It is therefore important to maintain vigilance and hygiene in lower rainfall areas and to minimise potential spread of *Phytophthora* to and within these areas as well as the wetter regions. Staff engaging nurseries need to ensure that the nurseries are accredited and/or appropriately tested for *Phytophthora*. I particularly acknowledge DEC staff, including Peter White and John Bartle, for the sampling carried out in and around nurseries in 2012 and for discussions on these issues.

A key point is that all of the new *Phytophthora* taxa discovered in WA so far are **soil-borne**, so the current, proven approaches and procedures being used to manage *P. cinnamomi* are generally appropriate at this stage for managing all of them. Some species, however, are more easily able to form thick-walled resting oospores than *P. cinnamomi*, and this will assist them to survive adverse conditions such as drought more effectively. *P*. aff. *arenaria*, isolated in 2012 from a wheat-belt nursery, is one such homothallic species.

Research projects (**Section 6**) are providing 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 additional specific or more intensive management strategies involving intervention are appropriate or necessary.

However, it is important to note that the controls applied currently for *Phytophthora cinnamomi* (such as phosphite application, forest hygiene, and restricting operations to "dry soil" conditions) may not always be appropriate, nor directly applicable without modification, for managing some potential 'new' *Phytophthora* species, eg those now causing severe tree disease overseas but not yet present here (*P. ramorum* and *P. kernoviae*). These two species produce **air-borne** spores and represent major **biosecurity risks** for Australia.

In relation to biosecurity, it should also be noted that four other recently-described *Phytophthora* species have been reported as causing disease in plantation eucalypts overseas: *P. captiosa* and *P. fallax* in New Zealand (Dick *et al*. 2006) and *P. alticola* and *P. frigida* in South Africa (Maseko *et al*. 2007). Of these four species only *P. fallax* has been found in Australia

(Victoria), but not in association with diseased plants. It is noteworthy that a single isolation of a new species related to *P. captiosa* (***P.* aff. *captiosa***) was made in the Jarrah forest in 2011-2012.

The newly discovered WA ***Phytophthora* hybrids** and their origins and pathogenicity are being investigated further through CPSM and in collaboration with South African researchers. Their presence reinforces the importance and urgency of preventing or minimising the spread of all Phytophthoras wherever possible. The formation of hybrids is believed to be a continuing process. **The opportunities for new *Phytophthora* hybrids to form, through the contact and interaction of compatible Phytophthoras, must therefore also be minimised**.

In general, a pro-active response with intervention at the earliest possible opportunity, to identify, 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 directly benefit the commercial agricultural and horticultural sectors. 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 32,410 samples have been processed for *Phytophthora* detection (to 30th June 2012) (**Table 4** and **Figure 4**). The 2011-2012 total (2198) was well above the previous year's total (1,936), and is the third-highest achieved in the VHS's 20 years of operation.

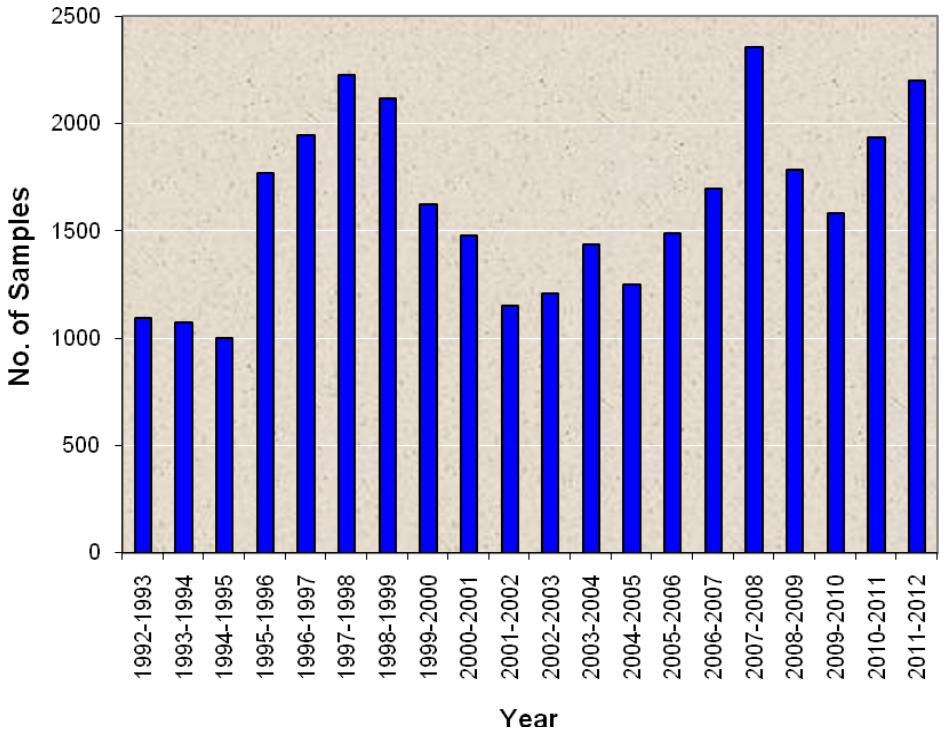
Background: Prior to 1992, the routine sample processing was carried out at Dwellingup Research Centre. Between 1984 and 1992 this was done by Janet Webster. All identification of *Phytophthora* species isolated from these samples between 1985 and 1992 was completed by Mike Stukely at Kensington. Records from these earlier years, for which map references are available, have been included in the **VHS database** which now contains a total of 39,092 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, with additional casual assistance as required. The long-term continuous involvement by these 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, previously undescribed species, the new records for WA of several species and undescribed taxa that are known from elsewhere, and the hybrids (**Section 3**). A second student project investigating several more of the new Phytophthoras has commenced at Murdoch University under joint supervision by Prof. Hardy, Dr Treena Burgess and M Stukely (**Section 6**).

Table 4 and Figure 4. Numbers of samples processed by the VHS for *Phytophthora* detection from 1992-1993 to 2011-2012.

Year	No. of Samples	Year	No. of Samples
1992-1993	1095	2002-2003	1210
1993-1994	1075	2003-2004	1435
1994-1995	1001	2004-2005	1250
1995-1996	1767	2005-2006	1486
1996-1997	1944	2006-2007	1696
1997-1998	2227	2007-2008	2353
1998-1999	2115	2008-2009	1784
1999-2000	1626	2009-2010	1581
2000-2001	1477	2010-2011	1936
2001-2002	1154	2011-2012	2198
		TOTAL	**32,410**



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, and DEC-sponsored community groups. 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 non-infested 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 the re-assessment and re-testing** for a given area of land will depend upon several factors, including:

- 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</u> *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), Murdoch University – **Classical and molecular taxonomy and pathogenicity testing of *Phytophthora* species** – completed in 2011-2012.

b. PhD project (Agnes Simamora), Murdoch University – **Multiple new *Phytophthora* species from Western Australia: taxonomy, pathogenicity, and disease control** – commenced Nov. 2011.

c. CPSM project – Investigation of *Phytophthora* hybrids from WA natural ecosystems.

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 Presentations to WA Dieback Information Group (**DIG**) meetings: 4 July 2008, 22 July 2011. 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.
- 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**. 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 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**. DEC Science Division.
- Papers (2) presented at international conference. A paper on the new ***Phytophthora* taxa *P.*sp.2, *P.*sp.1 and *P.*sp.9**, and a second paper on **the occurrence in WA of unique *Phytophthora* hybrids**, were presented (by Dr T Burgess, CPSM) at the **5th IUFRO Conference, "*Phytophthora* in Forests and Natural Ecosystems"**, Rotorua, New Zealand, 7-12 March 2010.
- Paper published in international journal. Rea AJ, Jung T, Burgess TI, Stukely MJC, Hardy GE St J (2010) ***Phytophthora elongata* sp. nov. a novel pathogen from the *Eucalyptus marginata* forest of Western Australia.** *Australasian Plant Pathology* **39,** 477-491.
- Paper published in international journal – descriptions of *P. thermophila, P. gibbosa, P. gregata, P. litoralis*. Jung T, Stukely MJC, Hardy GE St J, White D, Paap T, Dunstan WA, Burgess TI (2011) **Multiple new *Phytophthora* species from ITS clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications.** *Persoonia* **26,** 13-39.
- Paper published in international journal – descriptions of *P. arenaria* and *P. constricta*. Rea AJ, Burgess TI, Hardy GE St J, Stukely MJC, Jung T (2011) **Two novel and potentially endemic species of *Phytophthora* associated with episodic dieback of Kwongan vegetation in the south-west of Western Australia.** *Plant Pathology* 60:1055-1068.
- Conference paper. Burgess T, Stukely M, Jung T, White D, Huberli D, Hardy G (2011) **Molecular characterisation of a *Phytophthora* hybrid swarm in native ecosystems and waterways in Western Australia.** Australasian Plant Pathology Society – 18th Biennial Conference, Darwin, NT, 26-29 April 2011.
- Paper published in international online journal. Jung T, Burgess TI, Hüberli D, Hardy GEStJ, Stukely M (2011) ***Phytophthora fluvialis* T. Jung & T.I. Burgess, *sp. nov*.** *Persoonia* **26**:146-147.
- Paper presented at international conference. Jung T, Hardy GEStJ, Stukely MJC, Rea AJ, Scott P, White D, Paap T, Dunstan WA, Burgess TI (2011) **Multiple new *Phytophthora* species associated with natural ecosystems in Australia: evolutionary and ecological implications.** Cooperation in Science and Technology (COST), Action FP0801 Established and Emerging *Phytophthora*: Increasing Threats to Woodland and Forest Ecosystems in Europe – Working Group 4: Management and Control of *Phytophthora* diseases. Meeting 21-22 November 2011, Budapest, Hungary.
- Paper published in international online journal. Burgess TI, Huberli D, Hardy GEStJ, Stukely MJC, Jung T (2012) ***Phytophthora amnicola* T.I. Burgess & T. Jung, *sp. nov*.** *Persoonia* **28**:140-141.
- Invited Paper published in special "Plant Pathogen" issue of national journal. Stukely MJC (2012) **New Phytophthoras in Western Australia's natural ecosystems.** *Microbiology Australia* **33**:31-33.

8. Other References Cited

Aghighi S, Hardy GEStJ, Scott JK, Burgess TI (2012) ***Phytophthora bilorbang*** **sp. nov., a new species associated with the decline of *Rubus anglocandicans* (European blackberry) in Western Australia.** *European Journal of Plant Pathology* (doi:10.1007/s10658-012-0006-5)

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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.

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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.

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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. (Poster).

9. Attachments, Notes and Acknowledgments

1. **Maps (2):**

   - **Results of *Phytophthora* sample testing by DEC Vegetation Health Service, 2011-2012.**

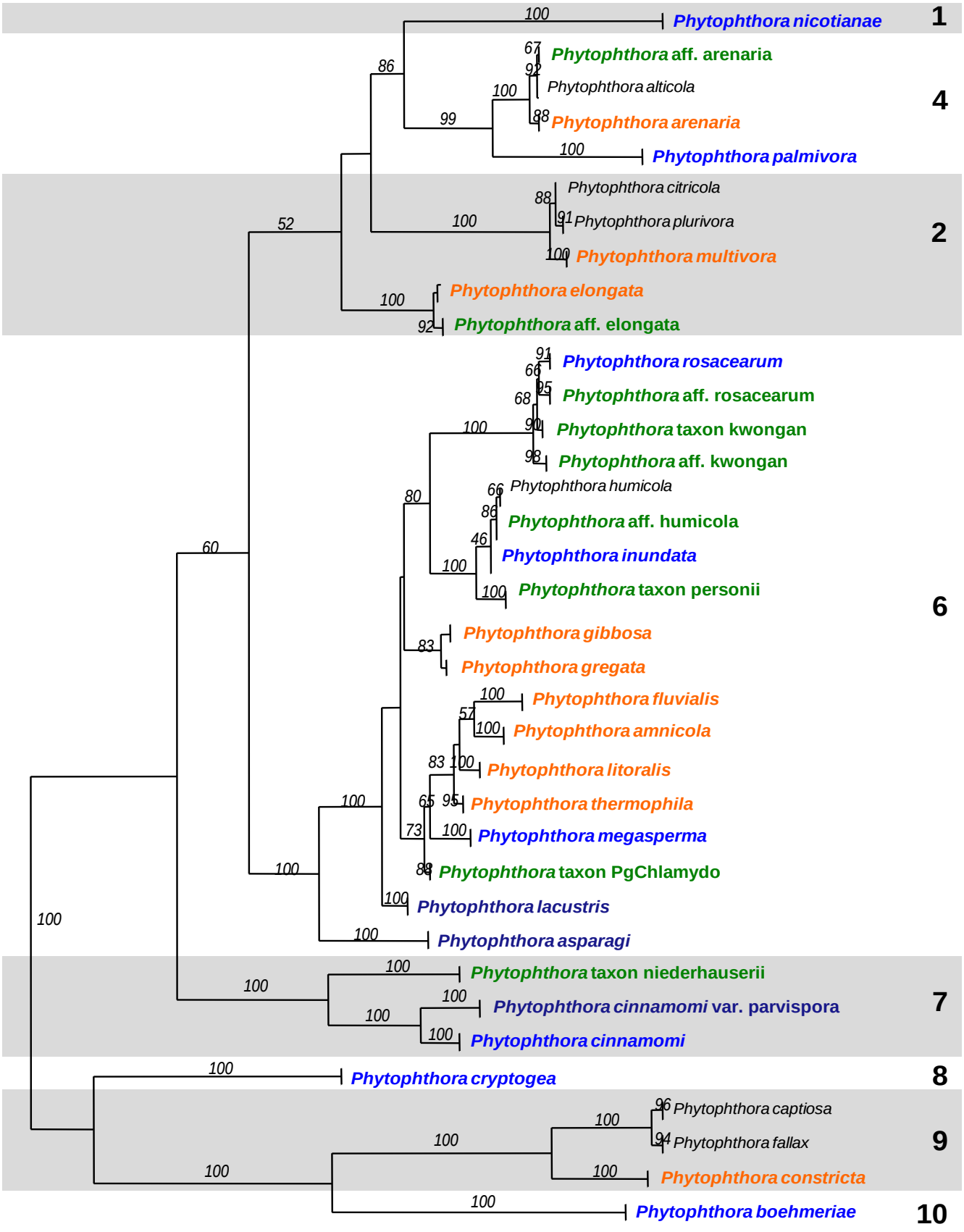
     Phytophthora cinnamomi (CIN), Other *Phytophthora* species (PSP), and Negative (NEG) recoveries are shown separately.

   - **Results of *Phytophthora* sample testing by DEC Vegetation Health Service, Years 1982-2012, *P. cinnamomi*.**

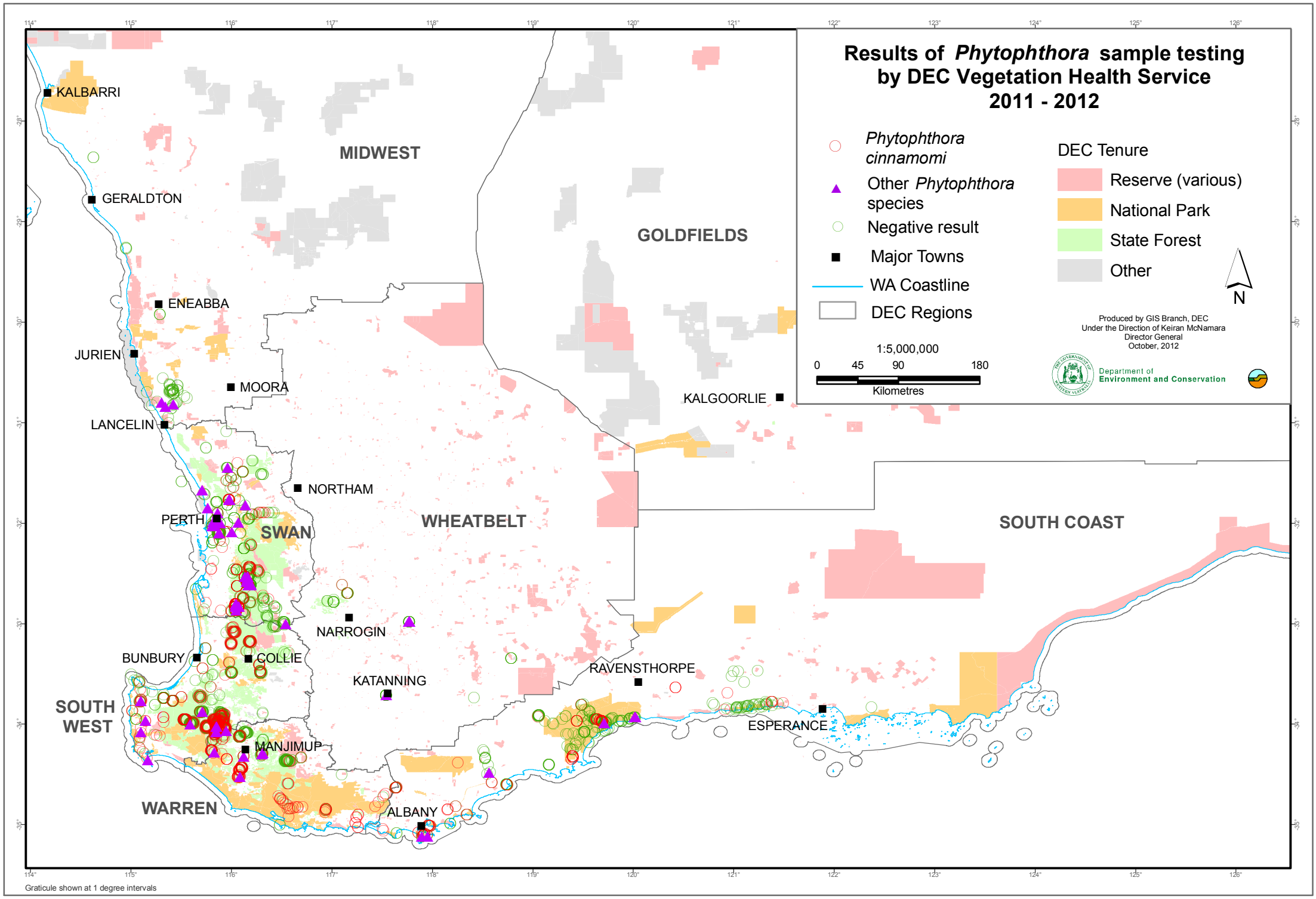
   Acknowledgment: ***The maps were produced by Holly Smith (DEC Geographic Information Services).***

2. **Phylogenetic tree (based on ITS rDNA sequences) showing the newly-described *Phytophthora* species and new records from Western Australian natural ecosystems, to 2012.** Numbers above branches represent bootstrap support values. The Clades delineated by the shaded blocks, with their numbers (1–10) shown at the right, are after Cooke *et al.* (2000). Species found in WA and known from elsewhere, and recognised undescribed taxa, are shown in blue and green print respectively; newly described species are shown in orange. Other species in black print are shown for reference.

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



Phylogenetic Tree – Genus *Phytophthora*, showing selected species from Clades 1-10 (after Cooke *et al.* 2000). Newly described WA species (orange font); species found in WA and known from elsewhere (blue); and recognised undescribed taxa found in WA (green). *(Courtesy TI Burgess)*



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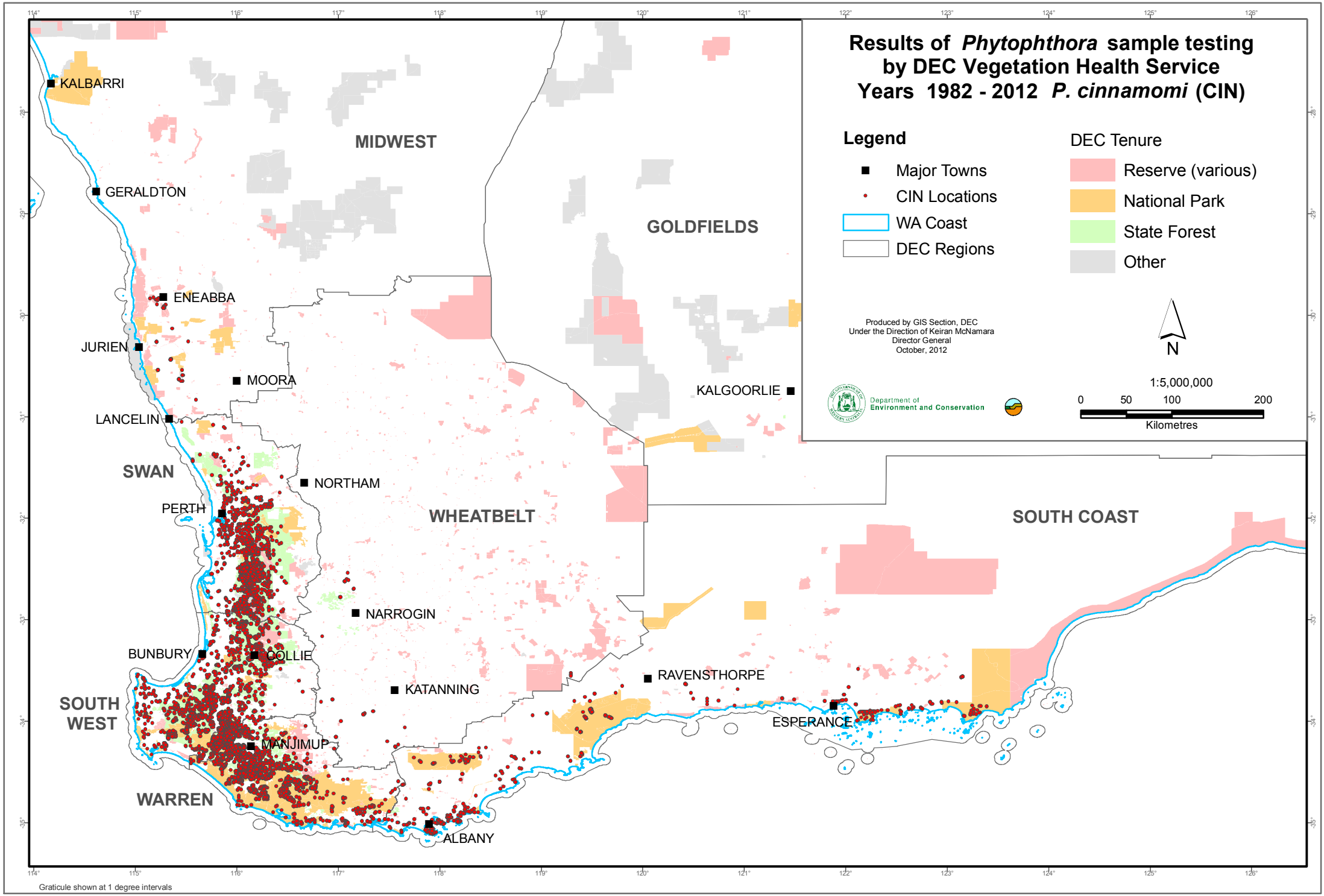
Results of *Phytophthora* sample testing
by DEC Vegetation Health Service
Years 1982 - 2012 *P. cinnamomi* (CIN)
Legend
Major Towns
CIN Locations
WA Coast
DEC Regions
DEC Tenure
Reserve (various)
National Park
State Forest
Other
Produced by GIS Section, DEC
Under the Direction of Keiran McNamara
Director General
October, 2012
Department of Environment and Conservation
N
1:5,000,000
0 50 100 200
Kilometres
KALBARRI
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ENEABBA
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MOORA
LANCELIN
KALGOORLIE
NORTHAM
PERTH
NARROGIN
BUNBURY
COLLIE
KATANNING
RAVENSTHORPE
ESPERANCE
MANJIMUP
ALBANY
MIDWEST
GOLDFIELDS
SWAN
WHEATBELT
SOUTH COAST
SOUTH WEST
WARREN
Graticule shown at 1 degree intervals
Job Ref: G:\Projects\08122C04_Phytophthora_Mapping\2011\MXD\ Phytophthora_AllYears.mxd