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Science Division

Phytophthora Dieback – detecting the pathogen

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Background

Phytophthora dieback was listed as a Key Threatening Process under the Australian Government's *Environment Protection and Biodiversity Conservation Act, 1999*. In the South-west Botanical Province of Western Australia, it has been estimated that 2 284 species of the 5 710 described native plant species are susceptible to the soil-borne dieback pathogen, *Phytophthora cinnamomi*.

Extensive vegetation health survey in the WA jarrah forest, using shadowless colour aerial photography to map the extent of *Phytophthora* dieback disease, has been in continuous operation since 1978. Validation of this mapping involves the routine testing of soil and root samples – collected primarily from beneath dying, *Phytophthora*-sensitive native plants known as "indicator species" – for the presence of the pathogen. Samples from other native ecosystems including forests and heathlands, as well as from parks, gardens, nurseries and plantations, are also tested.

A *Phytophthora* dieback "graveyard" in the Northern Jarrah Forest.
Photo: Bryan Shearer

The Vegetation Health Service

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. The VHS laboratory was established in 1992 at the Department of Environment and Conservation's (DEC's) Kensington Research Centre; earlier, sample processing had been carried out at Dwellingup.

Samples received by the VHS include a mixture of soil and plant-root material, which is baited for *Phytophthora* using the *Eucalyptus sieberi* cotyledon baiting method. The baits are plated to a selective agar medium for incubation, and any possible *Phytophthora* colonies that emerge are then isolated to pure culture. Roots can also be surface-sterilised and direct-plated to the selective agar for *Phytophthora* isolation.

All cultures are identified to species in the VHS by microscopic examination and measurement of their morphological characters. DNA sequencing is also used for selected isolates at the Centre for *Phytophthora* Science and Management (CPSM) at Murdoch University.

Findings

In the 2007-2008 financial year, a record total of 2 353 samples were tested by the VHS for *Phytophthora*. They were collected by DEC staff based at offices from Geraldton to Esperance, as well as by other government departments and local government bodies, environmental consultants, researchers and students, conservation groups, mining companies and private individuals. *Phytophthora* species were isolated from 650 samples; 573 of these were *P. cinnamomi*.

Samples of soil and plant roots being baited for *Phytophthora* in plastic trays in the VHS Bait Room. The *E. sieberi* cotyledons floating in the right tray have lost the purple pigmentation on their lower surface after infection by *Phytophthora*

For all samples, results (both *Phytophthora*-positive and negative) and information including map references are added to the VHS database; this contains over 32 500 records going back to the early 1980s, and is made available to researchers and managers as required. Representative *Phytophthora* cultures are added to the VHS Culture Collection which contains over 1 500 different live cultures. As most of these were collected in WA native vegetation, this is a unique and very valuable research resource.

Since 1980, six other *Phytophthora* "morpho-species" have been identified in samples from natural ecosystems in WA – *P. citricola*, *P. cryptogea*, *P. drechsleri*, *P. megasperma*, *P. nicotianae* and *P. boehmeriae*. Species other than *P. cinnamomi* regularly account for 10-15% of all positive *Phytophthora* results. Occasionally, more than one *Phytophthora* species is found in a sample.

DNA sequencing for *Phytophthora* species identification has been available through the CPSM since 2005. Over 230 new and historical WA isolates from the collection had been tested to 2008, resulting in first records for WA of five additional Phytophthoras known from elsewhere: *P. inundata*, *P. asparagi*, *P*. taxon pgChlamydo, *P*. taxon personii, and *P*. taxon niederhauserii. It has also led to the discovery of nine potentially new and undescribed *Phytophthora* taxa, designated "*P*.spp.1–11", from WA native vegetation. The formal description of one of these, *P. multivora* ("*P*.sp.4"), has been published in 2009; this species was previously mis-identified as *P. citricola* based on its morphology. [Indeed, none of the WA isolates tested so far has a DNA sequence matching that of the "true" *P. citricola*.]. Several of the new taxa are associated with deaths of plants from multiple families. In addition, five *Phytophthora* isolates with unique ITS-DNA sequences have been found.

Management Implications

P. cinnamomi is already widespread in WA's south-west, especially where annual rainfall exceeds 600mm, but every effort must be made to protect non-infested sites that are considered "protectable", especially where endangered species of flora or fauna, or threatened ecological communities, are present. Effective and accurate monitoring of the presence and spread of *P. cinnamomi* is therefore fundamental to its management, and to the broader decision-making of land managers. The *Phytophthora* detection work of the VHS underpins our knowledge of the pathogen's distribution, and land managers are encouraged to make full use of these services.

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.

Negative test results do not prove the absence of *Phytophthora*, and should be treated with caution.

Further investigation of the new *Phytophthoras* is under way to determine their pathogenicity, distribution and other key characteristics, and the level of threat they pose to biodiversity. It is not known if they are endemic to WA or introduced. Most are known to have been associated with plant deaths in natural ecosystems, so it will be prudent to regard all *Phytophthoras* as a threat until it is proven otherwise, and to manage them accordingly.

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