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DETECTION, DIAGNOSIS AND MAPPING OF NATIVE AREAS INFESTED BY PHYTOPHTHORA SPECIES IN WESTERN AUSTRALIA. Giles E.S.J. Hardy1, Kevin Vale2 Emer O’Garra1 Nari Williams1 and Philip A.O’Brien1. Centre for Phytophthora Science and Management, School of Biological Sciences, Murdoch University, Western Australia 6159. Department of Environment and Conservation, Kensington, Western Australia. e-mail: g.hardy@murdoch.edu.au.

Phytophthora cinnamomi Rands is an exotic soilborne pathogen that is thought to have entered Australia with the early European settlers. It is now widespread across southern Australia, and along the east coast into the southeast. It causes the most serious epidemic impacts on plant communities across a range of ecosystems. These include those areas with a Mediterranean climate where mean annual rainfall exceeds 600 mm (south-western Australia, South Australia and southern Victoria); the temperate uniform, low elevated regions of Victoria and New South Wales; and in the winter-dominant rainfall areas in maritime climates of coastal and sub-montane Tasmania. For example, in the south-west botanical province of Western Australia approximately 2284 of the 5710 described plant species are susceptible to P. cinnamomi. In those areas where it causes epidemics it is considered a ‘biological bulldozer’ as it also impacts on many vertebrate and invertebrate fauna. Due to its threat to biodiversity and general ecosystem function it is considered by the Commonwealth Government’s Environmental Protection and Biodiversity Conservation Act 1999 as a ‘Key Threatening Process’ to Australia’s biodiversity.

Although Phytophthora cinnamomi is the predominant species, there are at least 32 other species of Phytophthora which have been identified throughout Australia, with 14 recorded in the wild. Of these, only P. cinnamomi, P. cryptogea and P. megasperma cause significant impact in natural ecosystems. This paper provides an overview of the detection, diagnosis and mapping of Phytophthora species in Western Australia.

There are a number of ways that Phytophthora can be managed in native ecosystems. These include (a) eradication by chemical treatments, (b) reducing its rate of spread through hygiene and quarantine, (c) reducing its activity either by biological control or by reducing its food base, (d) altering the physical conditions for the pathogen, and (e) use of chemicals like phosphite (neutralised phosphorous acid). However, before any of these can be implemented it is critical to know where the pathogen is present or absent in the landscape. In some plant communities its presence can be easily ascertained by the presence of dying and dead susceptible (or ‘indicator’) species. In other areas its presence can be extremely ‘cryptic’ and difficult to determine due to the absence of ‘indicator’ species. This can be further exacerbated by recent wildfires, drought, salinity and presence of other plant pathogens such as Armillaria species. Also in the post-epidemic period few susceptible species may be present. In many infested landscapes P. cinnamomi has been present for long periods of time and its autonomous movement or movement by anthropogenic means has resulted in a mosaic of disease and disease-free landscapes across millions of hectares. This increases the complexities associated with determining where the pathogen is present and accurately mapping it.

Accurate maps that precisely locate the boundaries between Phytophthora infested areas and those free of the pathogen are extremely valuable for land managers and agencies that are required to manage the pathogen through hygiene and quarantine restrictions. However, across a large landscape there are difficulties associated with effective sampling regimes, high costs of mapping and the fact that the presence of P. cinnamomi may be difficult to detect. Soil sampling for diagnostic purposes is also expensive.

Accurate mapping of the presence and absence of the pathogen using Geographical Information System (GIS) platforms overlayed with vegetation, soil, climate, and fire history maps to name a few also allow managers to make informed decisions. For example, determining which areas might be protectable from the autonomous and anthropogenic spread of the pathogen, and the threat posed on communities of rare and endangered flora and fauna all allow for informed decisions regarding the management of the pathogen to be made. In turn, this ensures that limited available funding is spent wisely in key and strategic areas with considerable emphasis placed on the accurate mapping of the pathogen.

In Western Australia, considerable resources are spent on ‘dieback interpreters’; those staff who are specifically trained in field based interpretation of dieback infestations. Dieback interpreters are specifically trained to have strong skills in the identification of plant species, knowledge of susceptible species, description and mapping of vegetation, soil and landform types across a range of ecosystems (forests, woodlands, heathlands), and the ability to read maps and aerial photographs. They are also expected to interrogate databases and interface the reporting of field based data with GIS platforms to generate or contribute to management plans. The information generated is the basis for the development of long-term management systems of hygienic access to areas of land identified as ‘protectable’ areas. Protectable areas are those that have been mapped free of disease symptoms and are amenable to management to restrict disease introduction in the future. High management priority may also be given to infested areas with special conservation values due to the presence of communities of rare/endangered flora or fauna that will benefit from treatment with phosphite, a chemical that induces defence responses in susceptible plant species (Hardy et al., 2001).

An important tool that is used to enhance the efficiency and accuracy of disease interpretation is 230mm-format large-scale aerial colour, shadowless photography (nominally 1:4,500 scale). Skills in the observation and interpretation of aerial photography and knowledge of disease patterns in different vegetation types are critical. If the time of photography captures reasonable disease expression, a trained interpreter can make accurate decisions about the disease status of an area. The area can then be visited to verify the observations and decisions made from the photographs.

Interpreters place their emphasis on determining those areas that are (a) infested with Phytophthora cinnamomi, (b) uninfested or free of P. cinnamomi, (c) uninterpretable or where the presence or absence of the pathogen cannot be ascertained, (d) unprotectable or areas that are either infested or are free of the pathogen but will become infested within a short time frame, and (e) protectable or free of P. cinnamomi and will remain so in the distant future. Once an area has been aerially mapped, the interpreter will visit the area and interpret it in a logical fashion using factors that
indicate whether the pathogen is present or absent, or whether the plant deaths observed on aerial photographs are due to other factors such as fire, drought, and other pathogens. Usually, emphasis is placed on identifying key species known to be susceptible to the pathogen. There are numerous ‘indicator’ species that are used to determine the presence of *P. cinnamomi*. The distribution of these will vary with vegetation types. Deaths associated with these are termed ‘indicator species death’ (ISDs), and are species which are reliably susceptible to *P. cinnamomi*. Indicator species deaths can be grouped into classes as follows (a) isolated ISDs, (b) scattered ISDs, (c) groups or clusters of ISDs, and (d) multiple ISDs. The larger the number and range of species dead in an area the greater the probability of *P. cinnamomi* being the cause of deaths. Where there are many susceptible plant species dying, there will frequently be a clearly defined ‘disease front’ delimited by a zone of healthy susceptible species alongside dead and dying susceptible species.

Once on site, the environmental conditions that either favour or disfavour the growth and spread of the pathogen need to be explored as well all aspects of the disease triangle to aid the interpretation process (Shearer and Tippett 1989). The photographs are interpreted and the interpreters mark the edge of the disease front with coloured tape. A buffer of between 10-20 m into the ‘uninfested’ side is taped. In the forest, if areas to be minered, logged or managed in any way that causes disturbance, these will be ‘blazed’. Blazing involves removing bark at head height on 3 sides of the tree and painting these yellow. The non-blazed side denotes the uninhibited area. Subsequent on-ground activities which involve earth moving, road building or mining ensures that machinery and vehicles do not move from infested areas across the ‘blazed’ line without adequate hygiene protocols such as vehicle wash down to ensure the pathogen is not spread. All of the information gathered in the field is transferred to aerial photographs and maps for eventual databasing on GIS platform. Normally, these ‘dieback’ maps have a three year life, before the area has to be remapped.

Diagnostic analysis of soil and plant material is used to confirm or clarify the interpretation of symptom expression in the field and is particularly important in the interpretation of cryptic sites in which there is little or no clear expression of the disease. Soil, root and stem samples are collected from under dying and dead plants and returned to the laboratory for confirmation of *Phytophthora* species in the laboratory. In the laboratory, the soil and plant samples are (a) baited (bioassays using susceptible plant material), (b) direct plating of plant material onto *Phytophthora* selective agar media, or (c) processed for molecular analyses. Currently in Australia, ‘baiting’ is the primary diagnostic method for *Phytophthora* in commercial laboratories. Baiting relies on the physical isolation of the pathogen from samples and as a result has many widely acknowledged limitations including: low sensitivity; labour intensive; and, high cost. Detection of *Phytophthora* species within the soil is complicated by several factors including the presence of different structures; oospores, chlamydospores, sporangia and zoospores, and differences in the ability of these to germinate or be recovered by standard assays.

The emergence of DNA based technology offers methods which overcome these inconsistencies by targeting the DNA of the pathogen and are independent of the structural composition of the population. This has led to the development of numerous PCR based protocols for the detection of *Phytophthora* species from soil (Ippolito et al. 2002). To date, the application of DNA based detection technology has been focussed on agricultural and horticultural applications in which the soil and plant species are relatively uniform and the population density of the pathogen more consistent due to the abundance of host plant species with consistent susceptibility to the pathogenic species. In contrast, infestations of native ecosystems by *P. cinnamomi* are characteristically heterogeneous with considerable variation in the host species, ecological substrate, hydrology and soil structure. Consequently the pathogen population is likely to be low and variable, and recoveries by both traditional and DNA based techniques are often inconsistent and patchy. This is further compounded by the variability in microhabitat within and between sites and the diversity of *Phytophthora* species present. Recent work in Western Australia comparing detection by baiting and PCR based protocols has shown good promise for the application of this technology to management of *Phytophthora* species in a limited number of native ecosystems. However, the key to ongoing effective application of DNA based diagnostics relies on critical assessment of detection across the range of habitats which are infested by the pathogen habitats.

Continued developments in DNA diagnostic technology such as the introduction of quantitative PCR (qPCR) coupled with improvements and simplification of DNA extraction technology have led to the widespread adoption of DNA diagnostic technologies in disease management (Wang et al. 2006). On site DNA detection has become a reality with simplification of the qPCR process (Tomlinson et al. 2005). Increased application of this technology to disease management will significantly enhance our understanding of the movement and viability of pathogens such as *P. cinnamomi* in soil.

Remote sensing tools such as hyperspectral and digital multi-spectral imagery are being explored as possible tools to accurately map the presence of the pathogen through disease expression as well as mapping rates of spread. In the future these and other such tools may well facilitate the processes described above.

In summary, the diagnosis, detection and mapping of *Phytophthora* species across complex and heterogeneous plant communities in natural ecosystems is complex, time consuming, costly and on-going. Expert knowledge is required about the biology and ecology of the pathogen, plant species susceptibility and environmental conditions that drive the disease process. Tools and methodologies including those at strategic (policy) and operational levels are now in place to effectively map the pathogen. However, as new technologies become available such as molecular tools, these can be incorporated into existing management structure to ensure ‘best practice’ is maintained.

**REFERENCES**


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O gênero Phytophthora, em soja, pode causar apodrecimento de plântulas em pré e pós-emergência, tombamento de plântulas ou podridão radicular e de haste em plantas adultas. O agente causal é o oomiceto *Phytophthora sojae* Kaufmann & Gerdemann (Kaufmann & Gerdemann,