***Phytophthora* in Kirstenbosch National Botanical Gardens**

Trudy Paap 25/02/2020

The oomycete genus *Phytophthora* is more closely related to brown algae and diatoms than true fungi and has been placed in a separate kingdom, the Stramenopiles. The genus contains many important plant pathogens, and they have a propensity to spread rapidly throughout the world. A survey of *Phytophthora* species present in Kirstenbosch National Botanical Gardens was undertaken to obtain baseline diversity data, and to inform management options for plant disease issues present in the gardens.

Soil sampling was carried out in various sections of the gardens, with the most intensive sampling focused on the Protea beds. Twenty-one Protea beds were sampled, *Phytophthora* was recovered from 17 of the beds. *Phytophthora multivora* was the most commonly isolated species, present in 15 of the beds. *Phytophthora cinnamomi* was also regularly recovered, with its presence confirmed in seven beds. *Phytophthora asperagi* and *P. capensis* were each recovered from one bed. Bed U10, a bed not receiving irrigation but in the vicinity of the Protea beds, was sampled to determine *Phytophthora* presence. Both *P. cinnamomi* and *P. multivora* were recovered from this bed.

In addition to the sampling of the Protea beds, samples were collected from other sections of the garden, generally from rhizosphere soils under declining trees. Five of these samples were positive for *Phytophthora. Phytophthora multivora* was recovered from under declining *Ficus macrophylla* and *Cinnamomum camphorum, P. cinnamomi* was recovered from declining *Quercus suber,* the provisionally described *P.* sp. emzanzi was isolated from declining *Podocarpus elongata,* and an isolate of *P. elongata* was recovered from under declining *Aloe arborescens.*

From the nursery area, two potting media samples were tested, with *P. multivora* detected in the General Mix, and *P. chlamydospora* isolated from the Fynbos mix. Lastly, a symptomatic *Leucadendron saligna* seedling (potted, Nursery N82) was sampled, *P. cinnamomi* and *P. multivora* were both recovered.

Details of sampling locations, associated hosts and sampling dates are provided in the accompanying spreadsheet. In total, seven species of *Phytophthora* were detected in the garden. A brief description of each is provided here.

Phytophthora multivora, the most frequently isolated species from the gardens, was first described from declining *Eucalyptus* and *Banksia* in Western Australia (Scott et al. 2009). It is known to have a global distribution, and is emerging as a significant pathogen with a wide host range of woody plants. In South Africa it has frequently been found in asymptomatic natural vegetation (Oh et al. 2013). Observations made during the current study indicate that this species does not behave as a pathogen towards indigenous plants, however, it may play a role as a fine root pathogen of exotic tree species present in the garden. As such it is likely a contributing factor in complex tree declines, for example, it may be a contributing factor to the *Ficus macrophylla* decline observed in the gardens.

Phytophthora capensis and *P.* sp. emzansi are both closely related to *P. multivora,* with all three species residing in the *Phytophthora* Subclade 2c. *Phytophthora capensis* was first detected in South Africa from stream water, *Olea capensis* and *Curtisia dentata* (Oudemans et al. 1994). Until recently it was not known to occur outside of South Africa, however Jung et al. (2017; 2020) isolated this species from streams in Taiwan and Vietnam. Likewise, *P.* sp. emzansi was first identified in South Africa, from commercially cultivated *Agathosma* (Bezuidenhout et al. 2010). This species has also been detected from natural forests in South Africa (Oh et al. 2013). It has been suggested that the three closely

related species (*P. capensis, P.* sp. emzansi and *P. multivora*) are all of South African origin (Oh et al. 2013), however Jung et al (2020) has hypothesised that *Phytophthora* clade 2 is native to Indochina. Further work is needed to resolve the question of origin.

Phytophthora cinnamomi was the second most frequently isolated species from Kirstenbosch. It is known to be a very damaging and important invasive pathogen, globally. There are over 5000 known susceptible hosts, including many highly susceptible Proteaceae in southwest Western Australia (Shearer et al. 2007). *Phytophthora cinnamomi* was frequently isolated from Proteaceae showing symptoms of dieback, root and collar rot. Specifically, there is a high rate of mortality of the silver trees (*Leucadendron argenteum*) in the garden, *P. cinnamomi* was readily isolated from roots and collar lesions of silver trees (Figure 1). This species was also recovered from roots and rhizosphere soil of *Q. suber.* The involvement of *P. cinnamomi* in the decline and mortality of *Q. suber* has been demonstrated in their natural range (southwest Europe, northwest Africa) (Brasier et al. 1993).

Fig. 1 (a) Recently dead *Leucadendron argenteum* (b) with bark removed exposing collar lesion

Phytophthora chlamydospora has been recovered from stream water and soils in many parts of the world, including South Africa (Oh et al. 2013). It has been shown to be a pathogen of horticultural hosts (causing root and crown rot of walnut, almond and cherry trees), as well as causing foliar lesions and shoot dieback on ornamental species in nurseries. While it has occasionally been recovered from root rot and cankers of trees in forest situations, these infections may be largely opportunistic, and its status as a true pathogen in forests is yet to be established (Hanson et al. 2018).

Phytophthora asperagi was first described from diseased asparagus plants, causing spear and root rot, in Michigan (United States). This species has been isolated from *Agave* and *Aloe* with leaf rot symptoms in the Royal Botanic Gardens, Melbourne Australia (Cunnington 2005). Of note is that

some taxonomic schemes recognise the Asparagaceae, Agavaceae and Asphodelaceae (which includes *Aloe*), all as members of the Asparagales. This is the first report of this species in South Africa, the potential for this species to impact South African *Aloes* should be examined.

Phytophthora elongata has been isolated in Western Australia from natural forest and heathlands since the early 1980s, it has been associated with dead and dying plants of several species present in these ecosystems. It's pathogenicity to *Eucalyptus* and *Banksia* species has been demonstrated (Rea et al. 2010). More recently it has been recovered from symptomatic nursery plants in Maryland (United States) (Bienapfl & Balci 2014). This finding is the first report for South Africa, further work is required to determine the origin of this species, and whether it has the potential to be pathogenic to indigenous South African plant species.

There are currently many gaps in our knowledge of origin of the various species (and their status as alien or native), we also have little understanding of the role many of the species present in the gardens have in causing plant disease or mortality, particularly to indigenous South African plants. Pathogenicity trials are required to provide substantiated data to answer these questions. Of the species found, *P. cinnamomi* is by far the most well researched. A strong body of work exists on the impact of *P. cinnamomi* in Australia, particularly the Proteaceae dominated natural vegetation of southwest Western Australia. Previous studies in South Africa have identified *P. cinnamomi* as the cause of Protea root rot and silver tree decline (van Wyk 1973; von Broembsen 1984). It is evident that *P. cinnamomi* is causing mortality of Proteaceae (particularly silver trees) in Kirstenbosch gardens.

With regards to management, care should be taken when moving between beds. Boots, tools and machinery should be cleaned after use to avoid movement of soil between different areas of the garden.

The systemic fungicide phosphorous acid (HPO_3^-) or phosphite (Phi) is the main form of chemical control of *Phytophthora* diseases in natural and managed systems worldwide. Phosphite induces a defence response in *Phytophthora*-challenged plants and has been shown to increase the resistance of susceptible plant species to infection by *P. cinnamomi.* It has been used extensively for the last two decades in the southwest floristic region of Western Australia, to protect highly threatened flora and ecological communities impacted by *P. cinnamomi.* Barrett & Rathbone (2018) assessed the impact of *P. cinnamomi* and phosphite application on species assemblages, richness, abundance and vegetation structure in this region, at sites with 7-16 years of treatment history. They fpound that in the absence of treatment, *P. cinnamomi* had a profound impact on species assemblages, richness, abundance and vegetation structure. There was no evidence of adverse effects of phosphite treatment on phosphorus-sensitive species, even after fire. Treatment with phosphite enhanced the survival of key susceptible species and mitigated disease mediated changes in vegetation structure. The application of phosphite to protect Proteaceae in Kirstenbosch gardens will likely be a valuable management tool, and trails should be established to determine its efficacy in this setting.

References

Barrett, S., & Rathbone, D. (2018). Long-term phosphite application maintains species assemblages, richness and structure of plant communities invaded by *Phytophthora cinnamomi*. *Austral Ecology* **43**, 360-374.

Bezuidenhout, C. M., Denman, S., Kirk, S. A., Botha, W. J., Mostert, L., & McLeod, A. (2010). *Phytophthora* taxa associated with cultivated *Agathosma*, with emphasis on the *P. citricola* complex and *P. capensis* sp. nov. *Persoonia: Molecular Phylogeny and Evolution of Fungi* **25**, 32-49.

Bienapfl, J. C., & Balci, Y. (2014). Movement of Phytophthora spp. in Maryland's nursery trade. *Plant Disease* **98**, 134-144.

Brasier, C. M., Robredo, F., & Ferraz, J. F. P. (1993). Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology* **42**, 140-145.

Cunnington, J. H., De Alwis, S., Pascoe, I. G., & Symes, P. (2005). The 'asparagus' *Phytophthora* infecting members of the Agavaceae at the Royal Botanic Gardens, Melbourne. *Australasian Plant Pathology* **34**, 413-414.

Hansen, E., Reeser, P., and Sutton, W., 2018. *Phytophthora chlamydospora. Forest Phytophthoras* **8.**

Oh, E., Gryzenhout, M., Wingfield, B. D., Wingfield, M. J., & Burgess, T. I. (2013). Surveys of soil and water reveal a goldmine of Phytophthora diversity in South African natural ecosystems. *IMA Fungus* **4**, 123-131.

Oudemans P, Forster H, Coffey MD. 1994. Evidence for distinct isozyme subgroups within *Phytophthora citricola* and close relationships with *P. capsica* and *P. citrophthora. Mycological Research* **98,** 189–199.

Rea, A., Jung, T., Burgess, T. I., Stukely, M. J. C., and Hardy, G. E. S. (2010). *Phytophthora elongata* sp. nov. a novel pathogen from the *Eucalyptus marginata* forest of Western Australia. *Australasian Plant Pathology* **39**, 477–491.

Scott, P. M., Burgess, T. I., Barber, P. A., Shearer, B. L., Stukely, M. J. C., Hardy, G. S. J., & 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.

Shearer, B. L., Crane, C. E., Barrett, S., and Cochrane, A. (2007). *Phytophthora cinnamomi* invasion, a major threatening process to conservation of flora diversity in the South-west Botanical Province of Western Australia. *Australian Journal of Botany* **55**, 225–238.

van Wyk, P. S. (1973). Root and crown rot of silver trees. *Journal of South African Botany* **39.** 255-260.

von Broembsen, S. (1984). Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. *Phytophylactica* **16**, 221-226.

Yang, X., Tyler, B. M., & Hong, C. (2017). An expanded phylogeny for the genus *Phytophthora*. *IMA Fungus* **8**, 355-384.