BOTANIC GARDENS OF SYDNEY

Cunninghamia

Date of Publication:
December 2023

A journal of plant ecology for eastern Australia

ISSN 0727- 9620 (print) • ISSN 2200 - 405X (Online)

New records of plant pathogen *Phytophthora* species in Kosciuszko National Park, southeastern Australia

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Abstract: *Phytophthora* species in Australia are microscopic soil- and water-borne pathogens, which have caused great economic and environmental damage. Recent surveys of Kosciuszko National Park have detected 15 *Phytophthora* species through baiting and a further 10 species from high throughput DNA sequencing. Some of these species may be native pathogens. The hosts in the park of only two of the 15 baited species are known: *Pimelea bracteata* is a host of *Phytophthora gregata* and *Nematolepis ovatifolia* is a host of *Phytophthora cambivora*. Of the species recorded, *Phytophthora cinnamomi, Phytophthora gregata* and *Phytophthora multivora* are likely to be the most destructive of environmental values.

Very little of the park is currently suitable for *Phytophthora cinnamomi* but the area of suitable habitat is likely to increase in the coming decades as mean temperature increases; a few subalpine plant species are already known to be badly affected by it following infection. Preventing the dispersal of *Phytophthora* species in Kosciuszko National Park will be difficult because of the unfettered movement of vehicles, mountain bikes, horse riders and walkers through much of the park, and the increasing numbers of large feral animals present. The identification of protectable at-risk plant populations will be a priority for land managers in the future.

Cunninghamia (2023) 23: 067–075
doi: 10.7751/cunninghamia.2023.007

Introduction

The Phylum Oomycota is a group of micro-organisms collectively called oomycetes and commonly known as water moulds. Many oomycetes are pathogenic and are responsible for common plant diseases such as damping off (*Pythium* species), downy mildew (e.g. *Peronospora* species), and dieback, a term encompassing a range of symptoms caused by *Phytophthora* species. *Phytophthora* species are amongst the world's most destructive plant pathogens, causing catastrophic economic damage (e.g. *P. infestans* to potatoes, contributing to the Irish famine of the 19th Century) and environmental degradation (e.g. *P. cinnamomi* in heathlands and forests of southern Australia and *P. ramorum* in oak forests of North America) (Kroon et al. 2012). All species of *Phytophthora* recorded in Australia are soil- or water-borne, colonising roots and lower stems.

Burgess et al. (2021) compiled a list of 99 *Phytophthora* species in Australia from almost 8000 records, an increase from the 27 species known in the year 2000, following extensive surveys and improvements in molecular diagnostic techniques. Some of the species recorded in Australia are likely to be native, although the exact number is unknown because surveys have not been as extensive overseas (i.e. the global distribution is often unknown and trade networks have undoubtedly dispersed geographically isolated species).

Kosciuszko National Park (KNP) is the largest reserve for nature conservation in New South Wales (NSW) and is characterised by its steep environmental gradients, which produce a great diversity of habitats (from rain-shadow *Callitris* woodlands, to rainforest and alpine communities); the park supports almost one quarter of the New South Wales (NSW) flora and many endemics (Doherty et al. 2015). Until 2014, to our knowledge, there had been no surveys for *Phytophthora* species in the park. Before that time, the subject of most pathogen surveys in Australia was *P. cinnamomi*, and cold, mountainous areas were thought to be unsuitable for its activity (e.g. Podger et al. 1990). Since then, surveys associated with the Mountain Invasion Research Network (MIREN; Burgess et al. 2017, Khaliq et al. 2021) and those responding to observations of poor plant health have added greatly to knowledge about *Phytophthora* diversity in KNP and high mountain areas of Australia in general.

Phytophthora species may be detected in soils and plant material in two ways. The first involves baiting of soil and plants in a wet slurry using attractants, such as agar or susceptible plant material (e.g. lupins), to attract motile *Phytophthora* zoospores. If *Phytophthora* species are taken up by the baits, they are then cultured and identified by morphology and / or DNA sequencing. Gene sequence databases may be used to identify entities to species level. At times, a species name cannot be given to the detected entity either because it has not been detected before (and may be a new species) or the gene sequence was inadequate for matching. Importantly, species identified in this way were alive when sampled, either as vegetative material or spores (short-lived zoospores or resting spores known as a chlamydospores). High Throughput Sequencing (HTS) may also be used to identify *Phytophthora* species in soil and plant samples. This locates fragments of DNA and matches them against known sequences in the database. This technique may identify both live organisms and fragments of DNA from organisms that were once present but have not persisted at a site.

In this paper, we review the results of surveys for *Phytophthora* in KNP since 2015, some of which are unpublished, and present the results of a repeat survey in 2022 of sites sampled in 2017. We then focus on the current and future modelled occurrence of *P. cinnamomi* in the park, and provide management recommendations for the protection of flora at risk from *Phytophthora* pathogens in KNP.

Previous studies of *Phytophthora* presence in Kosciuszko National Park

The Australian Alps Liaison Committee funded a survey for *Phytophthora* in early 2015 along the Alpine Walking Track in KNP and beyond. Within KNP, 47 soil samples were collected and baited at the Royal Botanic Gardens Sydney. No species of *Phytophthora* were detected in the samples, although two species of the related genus *Pythium* (*Pythium macrosporum* and *Pythium undulatum*) were detected (McDougall and Wright, unpublished data).

A study of *Phytophthora* diversity in Australia (Burgess et al. 2017) tested 17 soils from MIREN plots beside Kosciuszko and Tooma Roads and a selection of other roadsides in KNP for *Phytophthora* presence. Twenty species, species aggregates or taxa of uncertain status were detected using HTS. These species may not persist in the locations where they were detected in the park, and most probably do not. For instance, *Phytophthora cinnamomi* was detected by Burgess et al. (2017) at several sites in the alpine area as high as 2125 m above sea level. However, in a subsequent study, *Phytophthora cinnamomi* was not detected at the same sites using baiting (see Khaliq et al. 2021).

Green (2016) detected *Phytophthora cambivora*, using baiting, in the roots of dying *Nematolepis ovatifolia* shrubs in the alpine zone of KNP near Charlottes Pass.

McDougall et al. (2018) detected *Phytophthora gregata* through baiting in dying *Pimelea bracteata* at two sites in northern KNP. Unlike many *Phytophthora* infections, the pathogen caused distinct lesions in plant collars (in the vicinity of the soil surface) rather than in roots.

Khaliq et al. (2019) isolated six *Phytophthora* species from soil samples using baiting along road and track edges in KNP, including *Phytophthora oreophila*, a newly described species currently only known from the Type location near Merritts Creek in the alpine zone. *Phytophthora cryptogea* was isolated from the summit of Mount Kosciuszko and *Phytophthora gregata* in the vicinity of Perisher ski resort at 1750 m above sea level.

Khaliq et al. (2021) sampled MIREN plots on three roads in KNP in 2017: Kosciuszko Road, Snowy Mountains Highway, and Tooma Road. Two samples were taken at each site:

one on the roadside in highly disturbed vegetation and one 50 – 100 m from the road in natural vegetation. *Phytophthora* detection was done using both baiting and HTS techniques. Nine species were detected using baiting and 16 species were detected using HTS; two species were detected by both baiting and HTS.

Methods for the 2022 survey

A survey in January 2022 sampled soil from the same natural vegetation plots as Khaliq et al. (2021) but only baiting was used for *Phytophthora* detection. Rhizosphere soil samples including fine roots were collected in each plot. Samples were collected from five different places (approximately 10–15 m apart) and placed in a single ziplock bag for each site (up to about 400 g in total). The trowel used for sampling was disinfected using 70% methylated spirits after each sample.

Methods used for *Phytophthora* species detection and identification in 2017 and 2022 were similar in that they used baits to attract any *Phytophthora* species present, cultured the pathogens on selective agar, identified species based on morphological traits and confirmed the identifications by sequencing rDNA. However, in 2017, soil samples were baited at Murdoch University (Western Australia) with juvenile leaves of *Rhododendron* spp., *Quercus ilex*, *Q. suber*, *Pimelea ferruginea*, *Poplar* sp., *Scholtzia involucrata*, and *Hedera helix* (see Khaliq et al. 2021 for a detailed description of the method) while in 2022 they were baited at the Royal Botanic Gardens Sydney using *Lupinus angustifolius* (see McDougall et al. 2018 for a detailed description of the method). While the methods differed between years, the outcomes should similar, given that soils were collected from the same sites.

Results

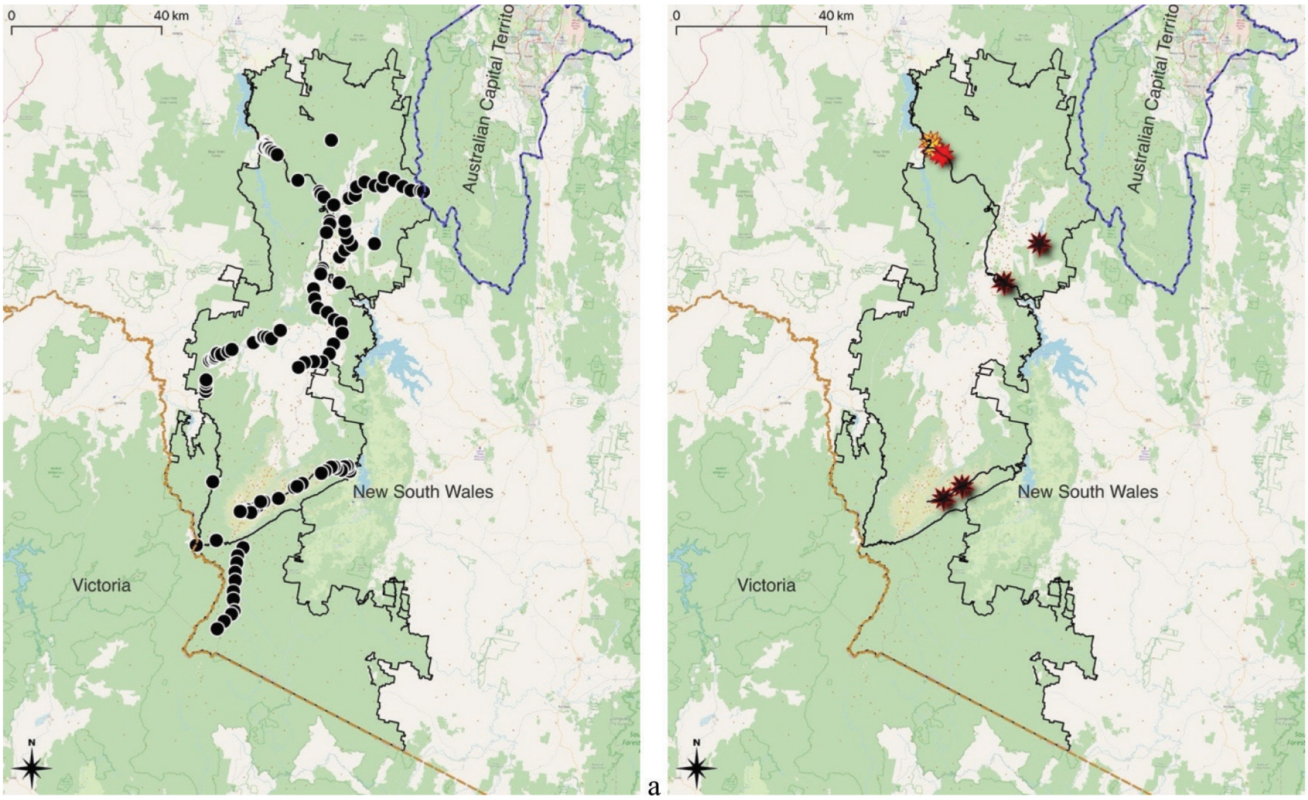
From all surveys, 15 *Phytophthora* species have been detected in Kosciuszko National Park using baiting techniques – these are species that were alive at the time of sampling. A further 10 species have only been detected using HTS and these may not persist in the park (Table 1). For all *Phytophthora* species except *Phytophthora cambivora* and *Phytophthora gregata*, hosts within KNP are unknown and for many *Phytophthora* species, knowledge about hosts extends only to genera present in the park, based on susceptibility testing of species elsewhere (and mostly Western Australia). The optimum temperatures for growth of the species detected in KNP (as per Abad et al. 2023) are between 20 and 30°C but all are capable of growth at 10°C and some as low as 2°C (Table 1).

Table 1. List of *Phytophthora* species detected in Kosciuszko National Park through baiting and High Throughput Sequencing (HTS). Data sources for baiting and HTS detections: 1 - Green (2016); 2 - Burgess et al. (2017); 3 – McDougall et al. (2018); 4 - Khaliq et al. (2019); 5 - Khaliq et al. (2021); 6 – this study. Names not accepted by Burgess et al. (2021) are not included. Minimum – optimum – maximum temperatures for growth are from https://idtools.org/phytophthora/ (Abad et al. 2023). Hosts are listed for each *Phytophthora* species as genera occurring in KNP (based on detections in species not occurring in KNP; McDougall and Liew (unpublished data)), and as species occurring in KNP (in bold; based on glasshouse susceptibility testing by Rigg et al. (2018) and Wan et al. (2020)).

***Phytophthora* Species**	**Baiting**	**HTS**	**Growth temperatures (°C)**	**Australian plant hosts**
Baiting				
P. cactorum	4,5,6	2	4 – 24 - 30	*Banksia, Calytrix, Crowea, Grevillea, Hakea, Pimelea*
P. cambivora	1		2 – 23 - 32	***Epacris petrophila, Grevillea victoriae, Nematolepis ovatifolia, Oxylobium ellipticum, Phebalium squamulosum, Prostanthera cuneata***
P. chlamydospora	5		? – 26.5 – 36.5	None known
P. cinnamomi	6	2	9 – 27 - 30	***Epacris petrophila, Grevillea victoriae* subsp. *nivalis*, *Nematolepis ovatifolium, Oxylobium ellipticum, Phebalium squamulosum, Podocarpus lawrencei, Prostanthera cuneata***; very large host range, see McDougall (2016)
P. cryptogea	4	2	3 – 24 - 30	*Allocasuarina, Banksia, Bauera, Crowea, Eucalyptus, Grevillea, Hakea, Leucopogon, Lomandra, Patersonia, Pimelea, Podocarpus, Xanthorrhoea*
P. elongata	5,6	2,5	5 – 25 – 32.5	*Banksia, Eucalyptus, Hakea, Leucopogon, Patersonia, Persoonia, Styphelia, Xanthorrhoea*
P. fallax	5	2	2 – 25 - 30	None known
P. gonapodyides	4		3 – 25.5 - 30	None known
P. gondwanensis	5		5 – 27.5 - 32	None known
P. gregata	3,4,5,6	2	7.5 – 25 – 32.5	***Pimelea bracteata***, *Banksia, Boronia, Correa, Eucalyptus, Pultenaea, Xanthorrhoea*
P. megasperma	6		3 – 18 - 27	*Allocasuarina, Banksia, Boronia, Hakea, Xanthorrhoea*

***Phytophthora* Species**	**Baiting**	**HTS**	**Growth temperatures (^{o}C)**	**Australian plant hosts**
P. multivora	5	2	5 – 25 – 32.5	*Acacia, Allocasuarina, Banksia, Elaeocarpus, Eucalyptus, Grevillea, Hakea, Leucopogon, Mirbelia, Patersonia, Persoonia, Podocarpus, Styphelia, Xanthorrhoea*
P. oreophila	4		4 – 20 – 32.5	None known
P. pseudocryptogea	4,5,6	5	3 – 25 - 35	*Acacia, Banksia, Xanthorrhoea*
P. thermophila	5	2	10 – 33 - 35	*Banksia, Eucalyptus, Persoonia, Xanthorrhoea*
P. acerina		2	6 – 25 - 32	None known
P. arenaria		2,5	10 – 25 – 32.5	*Banksia, Eucalyptus, Hakea, Xanthorrhoea*
P. sp. AUS9B		5	Not known	None known
P. bilorbang		2	4 – 25 – 32.5	None known
P. citricola		2	3 – 24 - 30	None known
P. litoralis		2	10 – 30 - 35	*Banksia, Callitris, Eucalyptus, Xanthorrhoea*
P. moojotj		2	10 – 27 – 32.5	*Banksia, Eucalyptus*
P. niederhauserii		2	10 – 30 - 37	*Banksia, Eucalyptus*
P. nicotianae		2	9 – 24 - 33	*Banksia, Bauera, Billardiera, Boronia, Callistemon, Correa, Crowea, Eucalyptus, Eutaxia, Grevillea, Hakea, Kunzea, Melaleuca, Pimelea, Westringia, Xanthorrhoea*
P. versiformis		5	4 – 20 - 28	None known

Many detections of *Phytophthora* species from baiting were on the low elevation sections of the three roads from which most samples came: Snowy Mountains Highway (in the vicinity of Talbingo Mountain), Kosciuszko Road and Tooma Road (Fig. 1). The exceptions to this are *Phytophthora gregata*, which was detected at elevations between 1300 and 1750 m on the eastern side of the park, *Phytophthora cambivora*, *Phytophthora gonapodyides*, and *Phytophthora oreophila*, which are only known above 1660 m near Kosciuszko Road, *Phytophthora cryptogea*, found on the summit of Mount Kosciuszko (but recorded at lower elevations close to KNP in Bago State Forest and Namadgi National Park), and *Phytophthora cactorum*, found in subalpine grassland near the Snowy Mountains Highway turnoff to Long Plain.



a b

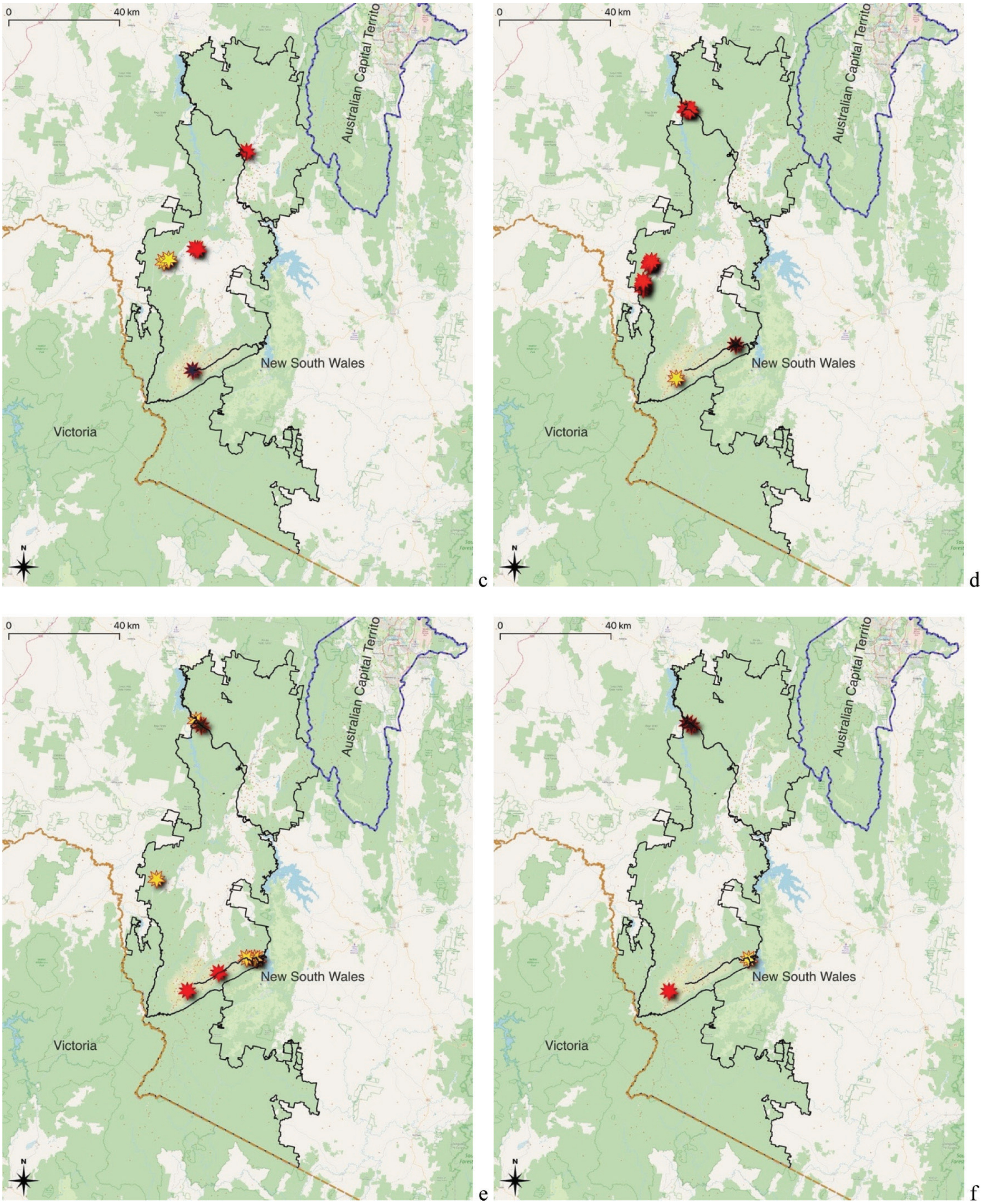


Figure 1. Distribution of *Phytophthora* species detected by baiting in Kosciuszko National Park (black outline) **a.** *Phytophthora* sample sites (black dots); **b.** red = *P. cinnamomi*, black = *P. gregata*, yellow = *P. multivora* (potentially the most destructive *Phytophthora* species in KNP); **c.** red = *P. cactorum*, black = *P. cambivora*, yellow = *P. chlamydospora*; **d.** red = *P. elongata*, black = *P. megasperma*, yellow = *P. oreophila*; **e.** red = *P. gonapodyides,* black = *P. gondwanense*, yellow = *P. pseudocryptogea*; **f.** red = *P. cryptogea*, black = *P. fallax*, yellow = *P. thermophila*.

The single record of *Phytophthora cinnamomi* in KNP was from forest beside the Snowy Mountains Highway near the Black Perry Lookout where the modelled *Phytophthora cinnamomi* habitat suitability is low (0.01 on a scale from 0 to 1; McDougall and Liew 2020) and the modelled mean annual temperature is 10°C. The minimum reported temperature for growth of *P. cinnamomi* is 9°C (Abad et al. 2023). While mean annual air temperature will not capture the annual extremes of temperature, some of which might be unfavourable or lethal to *Phytophthora cinnamomi*, temperature fluctuations in the soil rhizosphere where the pathogen occurs will not be as great as in the air above. The mean annual air temperature is perhaps therefore a reasonable predictor of growth and survival. This suggests that the site near Black Perry Lookout is marginal for *Phytophthora cinnamomi*. If this is the case, only the western edge of KNP and the lower Snowy River valley are currently marginally suitable habitat for *Phytophthora cinnamomi* (Fig. 2a). By 2070, using the CSIRO-Mk3-6-0 climate model (Gordon et al. 2002) and depending on the modelled climate scenario, more of the park will become suitable; under rcp 4.5 (an intermediate climate change scenario), much of the west and north of the park and all of the lower Snowy River area will be suitable (Fig. 2b), while under a rcp 8.5 scenario (a worst case on current projections), all but higher elevations between the Main Range and the Jagungal area will be suitable (Fig. 2c). Rainfall is unlikely to limit growth and survival of *P. cinnamomi* anywhere in the park, now or in the future.

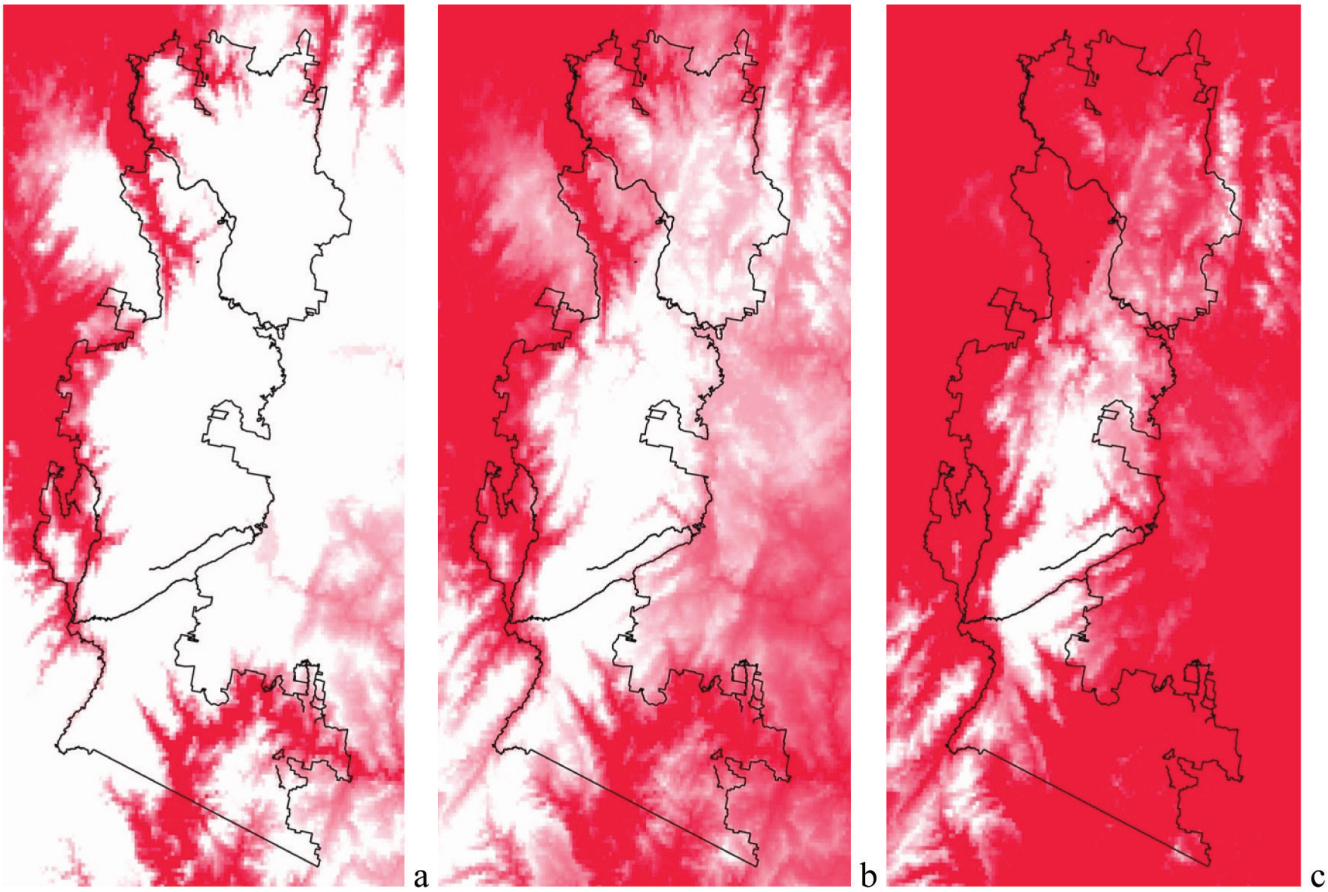


Figure 2. The red shaded area in each panel represents the 10°C isotherm, the mean annual temperature of the site of the only *Phytophthora cinnamomi* record in Kosciuszko National Park (with white areas having a mean annual temperature less than 10°C) – the park boundary is a continuous black line; **a.** current climate, **b.** projected climate in 2070 using the CSIRO-Mk3-6-0 climate model for the rcp 4.5 scenario, **c.** projected climate in 2070 using the CSIRO-Mk3-6-0 climate model for the rcp 8.5 scenario.

Repeat plots

In 2017, *Phytophthora* species were detected through baiting by Khaliq (2021) at 13 sites in natural vegetation (23% of sites sampled) while in 2022 we detected *Phytophthora* species at only 4 sites (7% of sites sampled; Table 2). Six species were identified in samples in 2017, *Phytophthora elongata* being the most frequently detected (7 sites). Four species were detected in 2022, at least two of which were not identified in 2017 – it is likely that *Phytophthora* cf. *cryptogea* detected in 2022 corresponds with *Phytophthora pseudocryptogea* found nearby in 2017. Curiously, none of the species detected in 2022 corresponded with species detected at the same sites in 2017. The detection of *Phytophthora cinnamomi* in 2022 is the first record of this species by baiting in Kosciuszko National Park. Although the soil sample was taken in forest at least 50 m from the road edge, there was considerable disturbance at the road edge from reconstruction of a barrier destroyed by the 2019 / 20 bushfire.

Table 2. *Phytophthora* species recorded in surveys of MIREN natural vegetation plots in January 2017 and January 2022 through baiting of soils.

Site	Road	Elevation (m)	2017	2022
AK2	Kosciuszko	1050	*P. pseudocryptogea*	
AK4	Kosciuszko	1170		*P. cf. cryptogea*
AK8	Kosciuszko	1405		*P. megasperma*
AS6	Snowy Mts Hwy	680	*P. elongata, P. fallax*	
AS8	Snowy Mts Hwy	790	*P. elongata*	
AS9	Snowy Mts Hwy	860	*P. fallax*	
AS10	Snowy Mts Hwy	900	*P. gondwanense*	
AS12	Snowy Mts Hwy	1030		*P. cinnamomi*
AS17	Snowy Mts Hwy	1320	*P. cactorum*	
AT2	Tooma	500	*P. elongata*	
AT3	Tooma	565	*P. elongata*	
AT4	Tooma	640	*P. elongata*	
AT8	Tooma	860	*P. elongata*	
AT9	Tooma	920		*P. elongata*
AT10	Tooma	980	*P. elongata*	
AT12	Tooma	1100	*P. chlamydospora*	
AT13	Tooma	1140	*P. chlamydospora*	

Discussion

The *Phytophthora* species detected in KNP that are most destructive in native vegetation in Australia are *Phytophthora cinnamomi*, *Phytophthora gregata* and *Phytophthora multivora*. *Phytophthora cinnamomi* has a very large host range (Cahill et al. 2008) and infection of native plants by this pathogen is a key threatening process under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999 and NSW *Biodiversity Conservation Act 2016*; over 100 plant species and about 30 plant communities are regarded as directly threatened by it. *Phytophthora multivora* has been implicated in the death of the dominant forest tree species *Eucalyptus gomphocephala* in Western Australia (Scott et al. 2012), *Wollemia nobilis* in New South Wales (Puno et al. 2015) and *Araucaria* species in Queensland (Shuey et al. 2019). *Phytophthora gregata* is an emerging threat and appears to be prevalent in wetlands and riparian areas, especially in cool climates. It has been commonly detected in the stem collars of dying *Pimelea bracteata* in KNP (McDougall et al. 2018) and is suspected of also infecting and causing death to *Pimelea pauciflora* there. The rapid decline in the *Pimelea bracteata* population warranted its listing as critically endangered on the *Biodiversity Conservation Act 2016*.

Although 15 *Phytophthora* species have now been recorded from baiting in KNP and all are likely to be pathogenic, the current hosts of only two species are known. *Phytophthora cambivora* has been isolated from the roots of symptomatic plants of the alpine shrub *Nematolepis ovatifolia* but unusual climatic conditions are thought to be the primary cause of poor plant health (Green 2016). *Phytophthora gregata* can be very destructive in populations of *Pimelea bracteata* in KNP; however, this shrub has re-emerged since the 2019 / 20 fires despite the continued presence of the pathogen, suggesting that it may be less susceptible when young. The true impact of *Phytophthora* in KNP may be much greater than currently recognised because there has been almost no testing for *Phytophthora* presence in the roots of plants displaying poor health symptoms (and this can be very difficult for large trees).

Some of the *Phytophthora* species detected in KNP are likely to be native to Australia. Indeed, one species, *Phytophthora oreophila* has only been recorded in KNP and may be endemic to the park. Of the other 14 *Phytophthora* species detected from soil baiting in KNP, Burgess et al. (2016) list six as being native to Australia and one species (*Phytophthora cryptogea*) as being of uncertain status. One of the putative native species found in KNP, *Phytophthora gregata*, has more recently been found in several other countries, suggesting it is either introduced to Australia or cosmopolitan. Regardless of origin, some *Phytophthora* species are likely to require management in KNP.

The vegetation at the sites where *Phytophthora cinnamomi* and *Phytophthora multivora* were detected in soil appeared asymptomatic at the time of collection. While these pathogens have a very large host range and are associated with poor vegetation health in many parts of Australia, they may also persist in the vegetation without causing extensive deaths. For instance, common plant species such as *Themeda triandra* and *Rytidosperma* species can become infected by *Phytophthora cinnamomi* but they rarely show symptoms (e.g. Phillips and Weste 1984). This may, however, not always be the case, especially as temperatures rise and more of the park becomes suitable to *Phytophthora* species. Rigg et al. (2018), for instance, found in glasshouse experiments that some subalpine species of KNP (*Epacris petrophila, Grevillea victoriae* subsp. *nivalis, Nematolepis ovatifolium, Oxylobium* *ellipticum, Phebalium squamulosum, Podocarpus lawrencei* and *Prostanthera cuneata*) are susceptible to *Phytophthora cinnamomi* infection. In addition, there is evidence that *Phytophthora cinnamomi* can adapt to lower temperatures than are regarded as being ideal for its growth and reproduction; in the laboratory it can be trained to produce zoospores and sporangia at 7.5°C (Khaliq 2019) – i.e., lower than the 9°C minimum temperature for growth documented for this species (Abad et al. 2023). *Phytophthora cinnamomi* will probably coexist with highly susceptible plant species in the coming decades and population declines in some species can be expected.

Phytophthora cinnamomi cannot be eradicated once introduced because it persists in asymptomatic plants after highly susceptible hosts are killed, and there are few options for control (Cahill et al. 2008). The same is likely to be true for other *Phytophthora* species. Given the amount of disturbance in KNP from visitor use (vehicles, walkers, mountain bikes, horse trail rides etc.), construction associated with the Hydro Scheme and ski industry, and widespread large introduced herbivores, preventing further dispersal is likely to be impossible. Despite that, it is worth attempting good hygiene, at least for management and construction vehicles accessing remote areas. Identifying which species need protection and which can be protected using hygiene and quarantine are the first steps in minimising the detrimental impact of *Phytophthora* species in KNP.

The low number of sites where *Phytophthora* species were detected in 2022 precludes any assessment of the effect of fire on these pathogens. Fire can have both positive and negative effects on *Phytophthora cinnamomi* (see McDougall and Liew 2020) but even the hottest fire is unlikely to remove it from soils. The greatest risk with fire is the increase in disturbance and soil movement typically occurring through the construction of firebreaks.

The changes in *Phytophthora* species composition at the plot level we detected between 2017 and 2022 are substantial and highlight the difficulty of monitoring these soil pathogens. However, sampling roots and soils for *Phytophthora* presence at sites with poor plant health is essential for tracking the movement of these and other pathogens in the park. The results of surveys since 2014 provide good baseline data for future assessments of *Phytophthora* distribution.

Acknowledgements

We are most grateful to Ed Liew, Matt Laurence, Allison Mertin and Clara Pang from Royal Botanic Gardens Sydney for their work in baiting soils collected in 2022 and identifying the *Phytophthora* species present. Funding support for resampling and analysing the soils in 2022 was part of the Australian Government's Wildlife and Habitat Bushfire Recovery investment.

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Manuscript accepted 1 November 2023