

# *Phytophthora agathidicida*

## Overview

The genus *Agathis* (Araucariaceae) includes about 13 species of tropical to warm temperate trees found from Malesia, through Australia to New Zealand. Kauri (*Agathis australis*) is a dominant tree



Photo: S.E. Bellgard

Figure 1. Tāne Mahuta “Lord of the Forest” is a giant kauri tree (ca. 47 m high) in the Waipoua Forest of Northland Region, New Zealand. Its age is estimated to be 1,250 to 2,500 years.

## Morphology

Differential oogonium ornamentation, together with the size of the oospore, was first recognised to be a diagnostic characteristic by Beever et al. (2009) in their study of representative isolates of the members of Clade 5. The mean oospore widths for *P. cocois* and *P. heveae* were not significantly different from each other nor from the Clade 5 mean, but oospore width of *P. agathidicida* is significantly larger than all other species and *P. castaneae* is significantly smaller than all other species (Weir et al., 2015). Oogonium wall ornamentation of *P. agathidicida* is mildly stipulate. Oospores nearly fill the oogonia with a mean width of 27.7  $\mu\text{m}$ , and ranging between 19.8 –35  $\mu\text{m}$ . Antheridia are amphigynous, globose, and some have knots at the base (Fig. 2). *P. agathidicida* is homothallic.

in lowland forests of northern New Zealand (Fig. 1) (Steward and Beveridge, 2010). Giant individual trees can reach over 4.5 m in trunk diameter and exceed 1,000 years in age, and are cultural icons (Beever et al., 2009). In 1972 a *Phytophthora* was associated with dead and dying trees in a kauri forest stand on Great Barrier Island off the northern New Zealand coast (Gadgil, 1974). Symptoms included yellowing of foliage, canopy thinning and occasional tree death. The causative organism was identified as *P. heveae* by J. Stamps of the Commonwealth Mycological Institute (Gadgil, 1974). In 2006, *Phytophthora* ‘taxon *Agathis*’ was reported from kauri in a forest west of Auckland on regenerating and mature trees. The original identification of the causative organism as *P. heveae* was questioned, because the ITS–sequence of the newly isolated suspected pathogen was identical to *P. castaneae*. This raised the possibility that the kauri *Phytophthora* was a new species within Clade 5 (Blair et al., 2008). The ‘Kauri killing’ *Phytophthora* organism has now been formally described as *Phytophthora agathidicida* B.S. Weir, Beever, Pennycook & Bellgard (Weir et al., 2015).



M.A. Dick, Scion, Rotorua

15  $\mu\text{m}$

Figure 2. Light micrograph of *P. agathidicida* oospore (scale bar =15  $\mu\text{m}$ ).

Sporangia are globose to ovoid-ellipsoid, papillate, borne terminally on long, thin, branched sporangiophores and could be formed via internal proliferation (Fig. 3). Sporangia are non-caducous (although some isolates have a somewhat defined septum near the base of the sporangium, see Fig. 3, left). Sporangia have a mean width of  $28.4\ \mu\text{m}$ , ranging between  $(12.4\text{--}50)\ \mu\text{m}$ , and a mean length of  $39.6\ \mu\text{m}$ , ranging between  $14.9\text{--}75\ \mu\text{m}$ .



Figure 3. Left: globose to ovoid-ellipsoid, papillate sporangium. Right: differentiation of the cytoplasm within papillate sporangia into zoospores. Specimen was stained with acid fuchsin (scale bar =  $15\ \mu\text{m}$ ).

### Genetics:

DNA sequences of eight genes (i.e. RPL10, COX1, ENL, HSP90, ITS, ND1, TIGA, and YPT1) were obtained from 29 isolates recovered from throughout the range of kauri, and concatenated to form a supermatrix alignment of 7527 bp. A Bayesian inference phylogenetic analysis of this dataset is presented (Fig. 4). This tree is annotated with the species boundaries of the taxa that we accept.

We identified and described two new species in Clade 5: *P. agathidicida* associated with a root and collar rot of kauri, and *P. cocois* associated with a pod rot of coconut. Our proximal hypothesis remains that *P. agathidicida* is an exotic incursion to New Zealand. The “founder effect” observed in the *P. agathidicida*-branch of the phylogenetic tree of Clade 5 is a reflection of the loss of genetic variation that occurred when the “new” population was established in New Zealand by a very small number of individuals from a larger population from a still unknown origin.

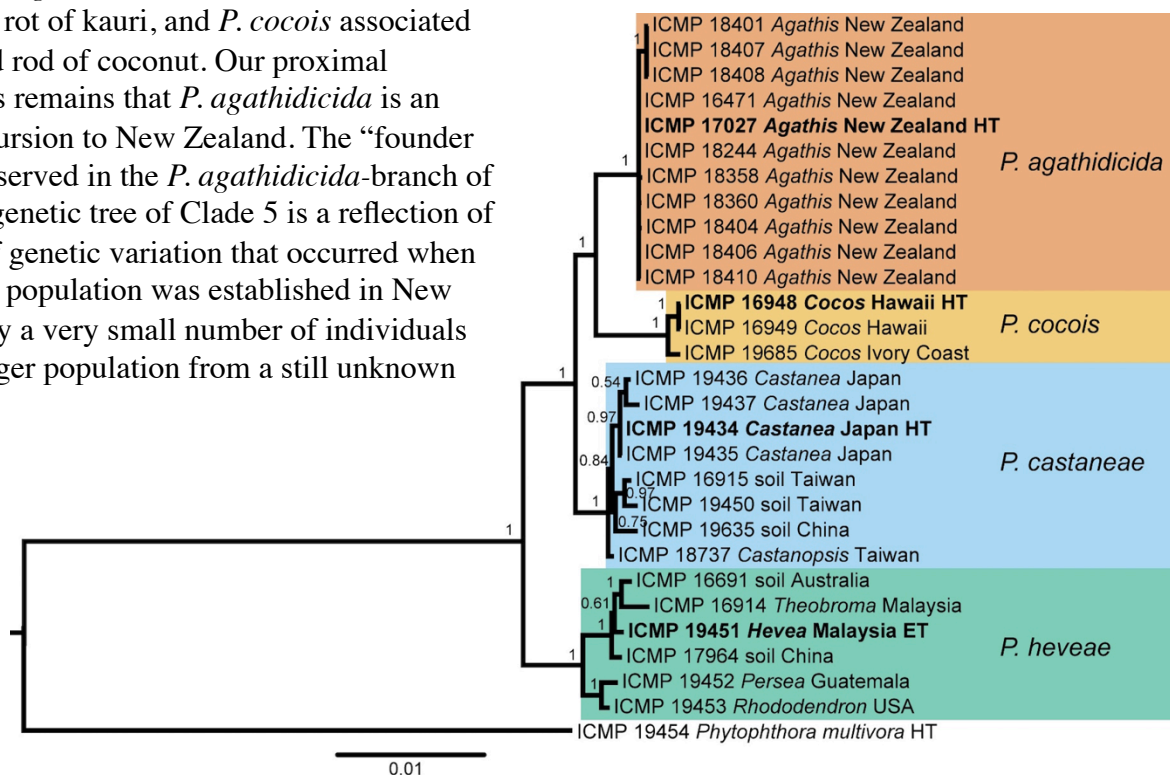


Figure 4. Phylogenetic tree of 28 isolates in the *Phytophthora* Clade 5 group. Bayesian posterior probability values  $\geq 0.5$  are shown above nodes. Culture accession numbers are listed along with host plant genus and country of origin. Ex-type cultures are emphasised in bold font (HT = ex-holotype; ET= ex-epitype). Species delimitations are indicated with coloured boxes, and *P. multivora* (Clade 2) was used as an outgroup. The scale bar indicates the number of expected changes per site (Weir et al., 2015).

### Growth in culture:

Vegetative hyphae are simple, with slight swellings, and lacking chlamydospores in culture. Colony morphology after 7 days was very uniform across the isolates tested on most of the media examined. Colonies are loosely aerial. On 5% clarified V8-juice agar, there is a weakly stellate radial pattern (Fig. 5).

Minimum growth temperature is 6°C; maximum 25°C; optimum 21.5°C. *P. agathidicida* also has a relatively faster growth rate than other members of Clade 5, especially at temperatures ranging from 10-20°C (Weir et al., 2015).

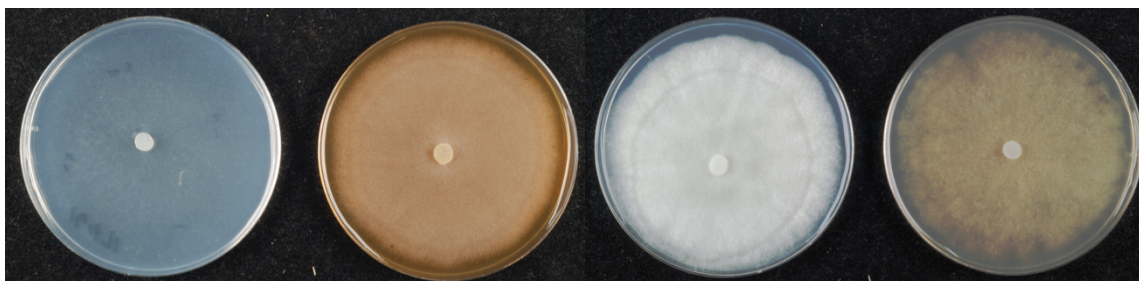


Figure 5. Diffuse, non-patterned colony morphology of ICMP 16471 (the original “Gadgil isolate”) after 10-days incubation at 20°C in the dark. Left to right: CMA, MEA, PDA, Clarified V8 juice agar.

### Distinguishing characteristics for identification

The most reliable morphological character to differentiate *P. agathidicida* from the other members of Clade 5 is gametangial morphology, in particular the degree of oogonium wall ornamentation, which ranges from completely smooth in *P. heveae*, to coarsely bullate in *P. castaneae* (Fig. 6). Oospore width, together with surface morphology (i.e. degree of wall ornamentation), have been used historically in synoptic and dichotomous keys of *Phytophthora*, to separate these species (e.g. Ho, 1981; Stamps et al., 1990). Antheridial morphology is also important, particularly with the often reflexed antheridia of *P. cocois* being a unique characteristic of the “coconut” *Phytophthora*.

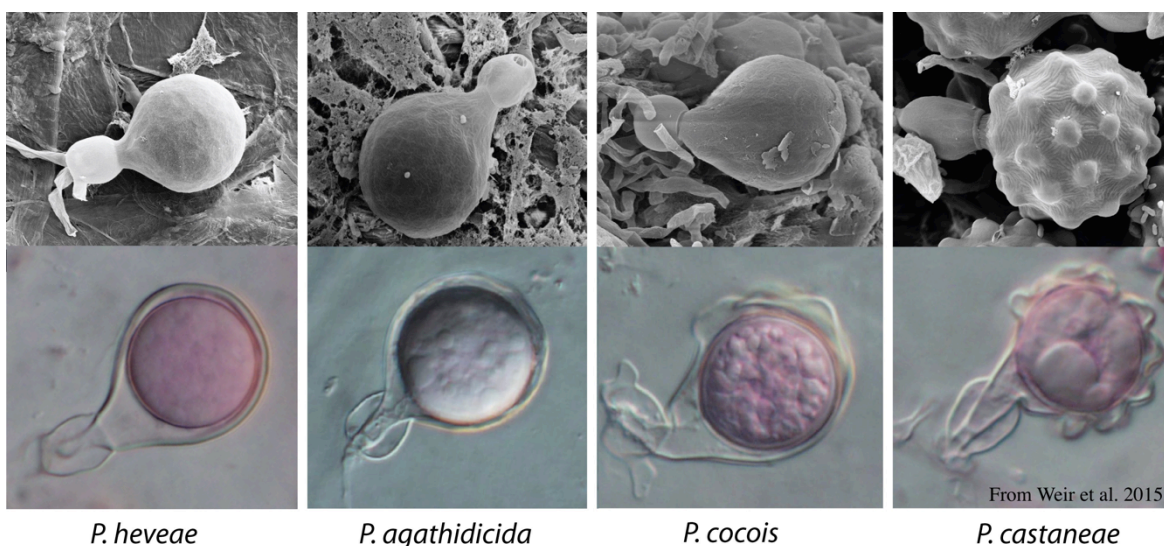


Figure 6. Comparative gametangial morphology of *Phytophthora* Clade 5 species, with SEM (top) and light microscopy (bottom). *P. heveae* has smooth-walled oogonia with funnel-shaped, amphigynous antheridia. *P. agathidicida* has mildly stipulate oogonia with globose amphigynous antheridia. *P. cocois* has mildly bullate oogonia with reflexed amphigynous antheridia. *P. castaneae* has coarsely bullate oogonia with rugose protuberances and narrow amphigynous antheridia (Weir et al., 2015).

## Disease History

New Zealand kauri, *Agathis australis*, is an iconic, ancient southern conifer belonging to the family Araucariaceae. To Māori, the indigenous people of New Zealand, kauri holds a very significant place in their creation mythology, with iconic trees having their own names, for example Tane Mahuta “the God of the Forest”. With approximately 1% left in old-growth, kauri has an International Union for Conservation of Nature listing as “Conservation Dependent”. Kauri is now threatened by the introduced soilborne pathogen, *Phytophthora agathidicida* – a newly described pathogen in Clade 5. Other hosts are not known, and the origin of the pathogen has not been determined. Kauri dieback was first reported in 1972 on Aotea (Great Barrier Island), associated with regenerating “ricker” kauri. It was not recognized on the mainland until 2006. Since 2009, via delimitation surveys instigated by a multi-agency, government response (so-called “Joint Agency Response “JAR”), it has been identified that the pathogen is distributed throughout the natural range of kauri except Little Barrier Island and the Hunua Ranges in the Auckland Region (Waipara et al., 2013; Scott and Williams, 2014). Kauri represents a climax, keystone taxon, and the premature loss of this species may cause a change in the composition of kauri-dominated forests, to forests dominated by podocarps (Beever et al. 2009).

## Impacts in the forest

The symptoms of kauri dieback are recognized as crown decline (Fig. 7), and resin production (“gummosis”) at the collar and lower trunk region (Fig. 8). However, this represents the chronic phase of the disease, with initial fine-root infections occurring many years before the onset of above-ground disease symptoms; the disease trajectory is purportedly dependent upon environmental factors, tree age, and inoculum density. Trees of all ages are killed in both natural forest remnants and plantations established in the 1950’s.



Figure 7. Crown decline of mature kauri, with branchlets with few or no leaves.



Fig. 8. Advancing triangular lesion extending up the trunk of an 80 cm DBH kauri tree in the Huia Dam Site along Twin Peaks Track, Waitakere Regional Park.

*P. agathidicida* parasitizes the secondary (cork) cambium, growing to the root crown and up the lower stem, forming lesions which are evident through the exudation of resin (Fig. 9). A demarcation between brownish, infected phloem below and white healthy tissue above (Fig. 10a,10b) is visible when the outer bark is removed.

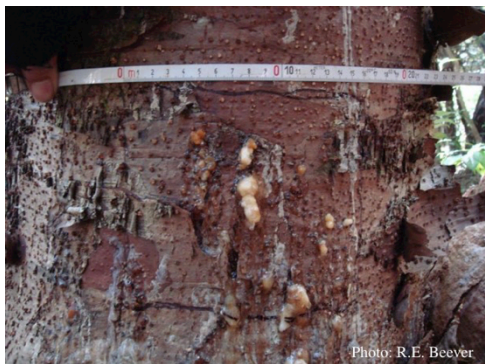


Figure 9. Close up of gum oozing out of lower trunk lesions of a young kauri tree at Maungaroa Ridge, Piha region of Waitakere Regional Park.



Figure 10a. Gum oozing out of longitudinal lesion.



Figure 10b. Excavated lesion, with outer bark removed showing extent of disease front.

Field diagnosis in kauri is based on the crown decline symptoms, gummosis at the collar, presence of necrotic fine roots, and potentially, a strong demarcation between necrotic and healthy tissue, advancing from the large leader roots to the root crown in dying trees. The pathogen can be isolated from recently killed tissues by removing the outer bark, and plating the cork cambium to *Phytophthora* selective media. Commercial ELISA kits have not proven to be reliable, possibly due to the resin-laden plant material. Soil bioassays utilizing a drying and wetting phase, followed by baiting with cedar needles and germinated lupine radicles, is routinely used. A real time PCR-based assay has been developed for soil and plant material (Than et al., 2013), as well as a species-specific, fluorescent in-situ hybridization assay to assist with visualizing the infection process *in planta*.

The New Zealand central government has initiated and coordinates a joint agency response to kauri dieback. “Keep Kauri Standing” is the program to stop the further spread of the pathogen, contain the pathogen in infested areas, and if necessary, restrict access to certain diseased forest areas. All forest management and concessionaire activities in kauri forests are evaluated for their potential effects on the kauri resource, and activities are modified or appropriate mitigating measures are undertaken as necessary (Fig. 11). Principal actions include trail use restrictions such as wet season or permanent closure, use of elevated boardwalks, and sanitation through washing of boots (Fig. 12).

Phosphite trunk injections are being trialed as a remedial therapy, following successful applications in Australia for the management of *Phytophthora cinnamomi* (Horner et al., 2013). Resistance to *P. agathidicida* in the kauri population has not as yet been ascertained. The Healthy Trees Healthy Future program (led by Scion, New Zealand’s federal forest research institute and funded by the Ministry of Business, Innovation and Employment and JAR) is currently funding the testing of kauri for resistance to *P. agathidicida*. It is hoped that some differential host responses may provide the basis for the identification of trees with useful resistance, which will be used as part of a selected breeding program.



Figure 11. Jogger running over a plastic-reinforced, foam-mat containing a 2% solution of Trigen<sup>TM</sup> Advance (quaternary ammonium compound) as part of a cross-country event, in the Waitakere Regional Park.



Figure 12. Use of hypochlorite solution applied through a “livestock drench-gun”, integrated with a soil grate to allow potentially contaminated soil to be collected.

### Forest and Wildland Hosts and Symptoms:

*Phytophthora agathidicida* can infect plants over a broad temperature range, with the pathogen recovered from soil (via soil bioassay) throughout the year. It is carried in mud, soil and infected root-debris and potentially washes downslope in water. In recreational parks, transport has been primarily along trails, while downslope movement may occur in streams and potentially through overland flow during periods of heavy winter rains.

Oospores are formed after three months in deliberately inoculated seedlings of kauri (Fig. 13), and *P. agathidicida* can be recovered after at least 9 years from soil stored at 10°C. It is hypothesized that resting spores, in root debris, are transported in mud on footwear during wet weather. This has spurred instigation of vertebrate culling programs (e.g. feral pigs, *Sus scrofa*), because they are considered a potential vector, as they are fond of foraging under kauri trees for giant kauri snails and earthworms (Krull et al., 2012).

The consequences of kauri dieback are visually dramatic, however, the ecological consequences are not well studied, with significant, plot-level studies only commencing in 2011. Standing dead trees are quickly attacked by pin-hole borers, leaving a bleached “stag head”. Kauri is no longer logged and so trees have protected status. The utilization of recently killed trees for cultural purposes remains unresolved, due to the timber of the lower bole being a potential pathway for inoculum dissemination. Distinctive plant associations with up to 36 species, including many rare endemic epiphytic species, reside in or on kauri. The pathogen removes one of the canopy dominants to be potentially replaced by other canopy members of the closely-related Podocarpaceae family, e.g. tanekaha (*Phyllocladus trichomanoides*) and kahikatea (*Dacrycarpus dacrydioides*).

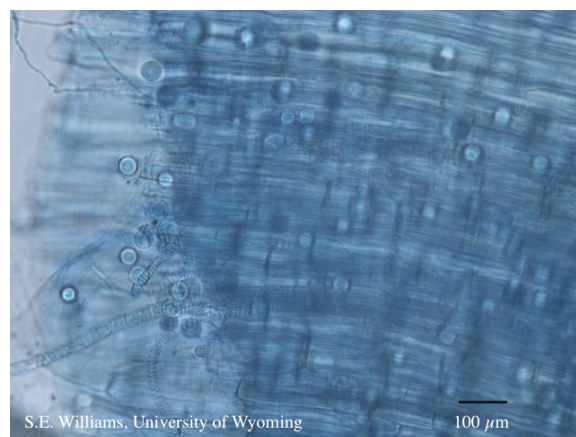


Figure 13. Oospores in the roots of kauri seedlings inoculated with *P. agathidicida*. The root has been cleared with potassium hydroxide and bleached with peroxide before being stained with trypan blue (scale bar = 100  $\mu$ m).

*P. agathidicida* is known only from kauri (*Agathis australis*) trees and associated soil in northern (<38°S lat.) New Zealand's mixed kauri-podocarp forest. Locations include, in the Northland Region: Trounson Park, Raetea, Waipoua Forest; in the Coromandel Region: Great Barrier Island (Kaiarara), Whangapoua; in the Auckland Region: Pakiri, Titirangi, Waitakere Ranges, Cascades Regional Park, Piha and in the Whangarei Region: Glenbervie, and Russell Forest.

Host Latin Name	Host Common Name	Symptoms	Habitat	Region
<i>Agathis australis</i>	New Zealand kauri	Root and collar rot	Forest and plantation	North Island of New Zealand

## Management and education resources

The Kauri Dieback Response: <http://www.kauridieback.co.nz/>

Kauri 2000: <http://www.kauri2000.co.nz/>

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