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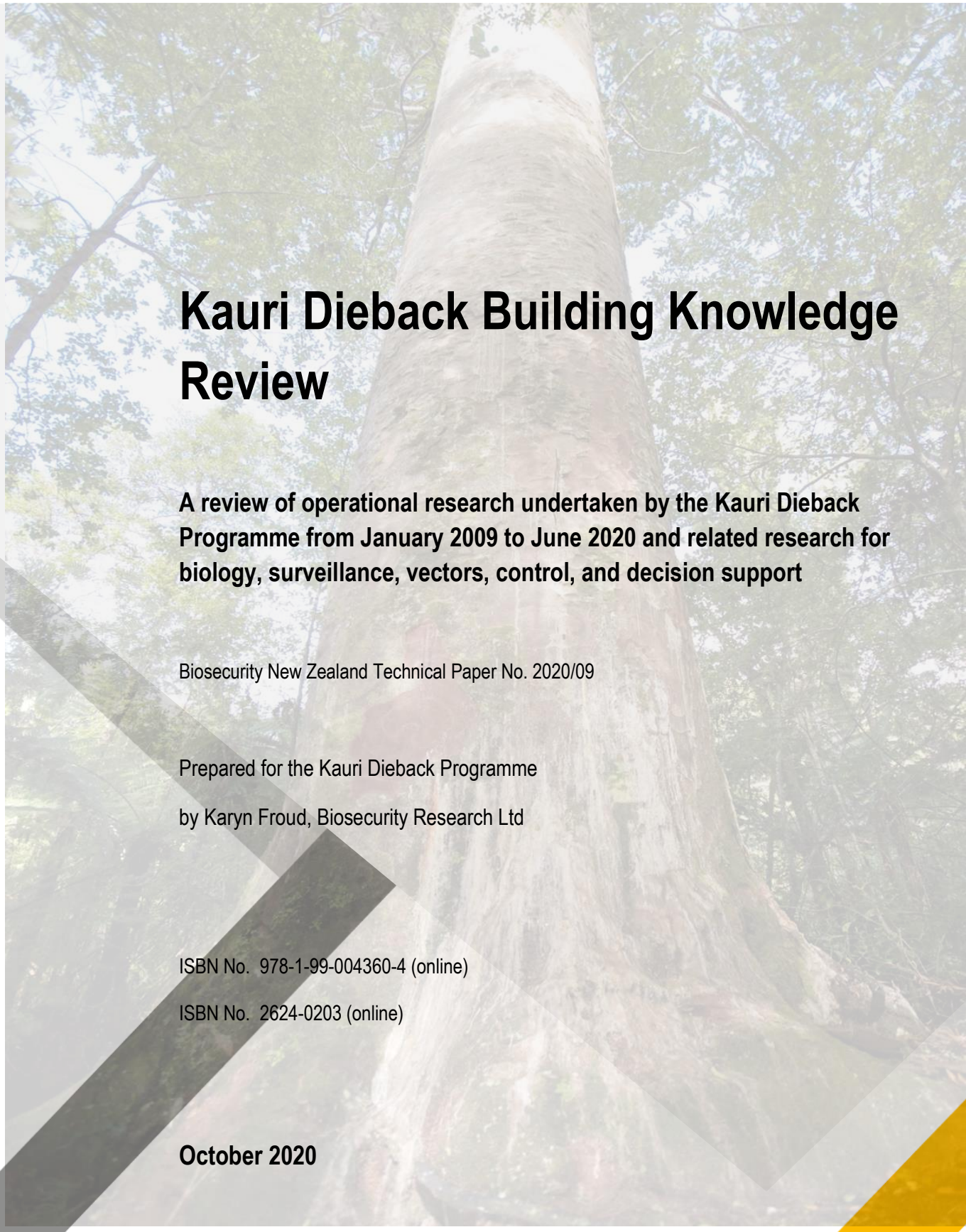

Kauri Dieback Building Knowledge Review

A review of operational research undertaken by the Kauri Dieback Programme from January 2009 to June 2020 and related research for biology, surveillance, vectors, control, and decision support

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This work was commissioned by the Ministry for Primary Industries to produce a review of 10 years of operational research, in the context of other research, to describe: what we have done (current state), what we have learnt, what are the gaps and barriers and future lines of enquiry.

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1 KAURI DIEBACK PROGRAMME REVIEW OBJECTIVES

This document aims to:

1. review Kauri Dieback Programme- (KDP-) funded research in the following areas:
    a. understanding the disease – origin, taxonomy, biology and impacts;
    b. surveillance, detection, diagnostics and pathways
    c. controlling the disease – phosphite, oospore control; biological control; genetic resistance and tolerance; hygiene;
    d. decision support – kauri mapping, best practice guidelines, prioritisation framework;
2. build a complete picture of the current state of KDP-funded research, which will let us identify the operational areas that have been addressed and those areas have not;
3. identify the barriers, considerations and technical uncertainties in undertaking the research and to transfer knowledge gained to a pragmatic operational tool;
4. provide a visual representation of this work (this is included as an A3 diagram – see Appendix.

The following research areas are excluded from the scope of this review:

- social science KDP-funded research;
- mātauranga Māori KDP-funded research;
- external research funded outside the KDP.*

*While this review focuses on KDP-funded operational research, it would not provide an effective resource if research, funded by other agencies and undertaken by multiple other researchers including students, was not included where it added context to the KDP-funded research. Please note, a full reference list is provided for all research included in the review and this indicates where the KDP was the main research funder. Some KDP internal documents (Technical Advisory Group and Planning and Intelligence Team meeting minutes) are also referenced, where mentioned.

2 BACKGROUND

The delivery of a long-term disease management programme is a complex and difficult task, particularly when the disease is widespread, cryptic, has extended latency periods and is within the native estate.

The KDP began in 2009 (known at the time as the Kauri Dieback Joint Agency Long Term Management Programme), when it became clear that what is now known as *Phytophthora agathidicida* was causing significant disease in kauri across multiple sites. The KDP is a partnership programme involving the Tangata Whenua Roopu (a representative body for iwi and hapū with an interest in kauri lands), Biosecurity New Zealand (as part of the Ministry for Primary Industries), the Department of Conservation, Auckland Council and the Northland, Bay of Plenty and Waikato regional councils.

2.1 KAURI DIEBACK PROGRAMME RESEARCH STRUCTURE

Scientific research is delivered within the KDP through a planning and intelligence workstream that identifies research needs, gaps and priorities and how science knowledge is transferred to operations. That can be direct through provision of expert knowledge or through the procurement of research.

The Planning and Intelligence workstream is made up of representatives from the KDP as well as an external strategic adviser. As a collective, the workstream has strong technical knowledge in both Western science and mātauranga Māori and provides an end-user perspective in identifying kauri dieback management needs. As a result, the workstream has a strong focus on operational research. This report covers all the operational research procured from the KDP from 2009 until mid-2020.

Although some strategic research was funded, the level of investment decreased over time due to funding constraints and an increase in investment in applied science. The Planning and Intelligence workstream and KDP supported a large number of external strategic research proposals that were put forward annually to the Ministry of Business, Innovation and Employment (MBIE) research funding rounds from 2012 to 2019, with in-kind support provided if the research was funded. Ultimately, only one proposal was successful in getting funded, with $10 million going to the Healthy Trees, Healthy Future Programme from 2013 to 2019 (which the KDP also co-funded). The Biological Heritage National Science Challenge also invested in several kauri dieback research areas, including Kauri Rescue, a citizen science approach to control tools in 2017 and 2018. In 2019, Auckland Council provided additional funding support. In addition, Crown research institutes invested funding into strategic research and, wherever possible, the results were discussed and incorporated into operational outcomes. University research including Master of Science and doctoral research projects were also considered for combining into operational outcomes wherever the KDP was made aware of them.

To support the Planning and Intelligence workstream, an external technical advisory group (referred to as the Kauri Dieback TAG) was convened from time to time to provide technical advice on science research. In addition, the Strategic Science Advisory Group (SSAG) was set up in 2018 to provide strategic advice around high-level priorities and research themes. This report also refers to the

scientific questions that were put forward to the Kauri Dieback TAGs and discussed at the Planning and Intelligence workstream meetings.

It is recommended the KDP or an equivalent agency undertakes annual face-to-face meetings with New Zealand Crown research institutes, universities and other research or mātauranga Māori groups to discuss what kauri or kauri dieback related research is being undertaken and to provide an active communication pathway of research results to land management agencies, iwi and hapū and other landowners. A co-ordinated implementation pathway to add new knowledge to operational management of kauri dieback is also recommended.

3 UNDERSTANDING THE DISEASE: ORIGIN, TAXONOMY, BIOLOGY AND IMPACTS

The KDP has invested in a range of research to try to understand the disease origin, taxonomy, biology, and its impacts. The most significant research was a project co-funded by Manaaki Whenua – Landcare Research called Specialist *Phytophthora* Research: biology, pathology, ecology and detection of PTA (Bellgard et al. 2013). This important research into understanding the disease was a three-year project completed in December 2013. It covered identification of different hosts, spread pathways, plot-scale impacts, distribution of *P. agathidicida* in root systems and under infected trees (Bellgard et al. 2013).

3.1 ORIGIN AND TAXONOMY

What is now known as *P. agathidicida* was first isolated by Gadgil (1974) at Great Barrier Island, Hauraki Gulf, in the Auckland region. Gadgil (1974) isolated the pathogen, which was initially identified in 1972 as *Phytophthora heveae* by J Stamps of the Commonwealth Mycological Institute. This may not be the earliest record, however, because Gadgil (1974) notes in his discussion that, according to the Commonwealth Mycological Institute records, a previous isolation of *P. heveae* from New Zealand had been made. Beever et al. (2009) investigated 2006 reports of tree dieback in the Waitākere Ranges and designated a disease name of kauri collar rot for what is now known as *P. agathidicida*. The disease name of kauri dieback was also coined around that time, and the technical advisory group was formed as the "Kauri Dieback TAG" in 2008 and this name has remained in wide usage.

The main reference on the origin and taxonomy of *P. agathidicida* is the Weir et al. (2015) paper (preliminary results were presented in the Bellgard et al. (2013) report), which shows that *P. agathidicida* sits within Clade 5 of the *Phytophthora* species. This clade has host and geographic associations that suggests a centre of diversity in the East Asia and Pacific region (Weir et al. 2015), which overlaps with the postulated centre of diversity of *Agathis* (Bellgard et al. 2013) and indicates the introduction of *P. agathidicida* into New Zealand. The initial hypothesis in 2008 (Kauri Dieback TAG1 2008) was that evidence indicated *P. agathidicida* was a relatively recent (mid-20th century) introduction. The view of the technical experts was that its virulence was so high that there would have been historical evidence of disease in the native system. At the time (2008) Waipoua Forest, Great Barrier Island, and Huia in the Waitākere Ranges, showed evidence of kauri dieback disease caused by *P. agathidicida* (*Phytophthora* taxon *Agathis* or PTA as it was described at that time) and these sites were linked to a Waipoua nursery. Twelve years on, the geographical distribution of *P. agathidicida* is significantly greater (see Figure 4-1). Beachman (2017) investigated the hypothesis that *P. agathidicida* was introduced into New Zealand on *Agathis*, *Araucaria* and *Phyllocladus* seeds imported from 1940–1952 to build an arboretum within the Waipoua Forest. Beachman found no evidence to support this introduction pathway, based on the absence of kauri dieback symptoms within the arboretum site on either New Zealand *Agathis* or the few surviving foreign *Agathis* (*A. robusta*). Beachman (2017) concluded that either *P. agathidicida* was never introduced with the imported seed or has completely faded out. Preliminary results from a Massey University study that indicated greater genetic diversity and an earlier introduction were noted as a pers. comm. from

Peter Lockhart in Black and Dickie (2016). That work is nearing publication and is based on complete sequencing of mitochondrial DNA (mtDNA) of 16 *P. agathidicida* isolates and representatives of the other members of Clade 5 (R Winkworth, pers. comm., 2020). The mtDNA exhibits lower genetic diversity than earlier reported in Black and Dickie (2016) but it is still likely higher than expected if a single genotype of *P. agathidicida* was introduced very recently (R Winkworth, pers. comm., 2020). Winkworth's results suggest that *P. agathidicida* is not a relatively recent introduction (that is, mid-20th century) but is also unlikely to have been present in New Zealand for many thousands or millions of years. Moreover, the observed geographic structure does not support introduction of a single genotype and nursery-based distribution in the mid-1950s (R Winkworth, pers. comm., 2020).

Reports of *Agathis* species showing dieback-like symptoms were also made in New Caledonia, and samples were sent to New Zealand for analysis. The identification was similar to *P. agathidicida* but not identical (I Horner, pers. comm. to T Ashcroft, 2016).

3.1.1 FUTURE RESEARCH ON ORIGIN

The mtDNA-based research supports introduction at some point, rather than an endemic origin, and provides initial evidence of an earlier introduction date. This needs confirmation with further research of the nuclear genome to be definitive in answering this fundamental question. Ideally this would include study of other *Phytophthora* isolates from East Asia and the Pacific. Regardless of additional research in this field, it still does not answer the fundamental question raised by Black and Dickie (2016) of whether it is widely distributed and undetected or restricted to highly disturbed sites only. This is discussed further in the detection and surveillance sections.

3.2 BIOLOGY

Pathogenicity tests were conducted by Gadgil (1974) from the original isolates found on Great Barrier Island, and he proved Koch's postulates during his isolations. Interestingly Gadgil (1974) found very high levels of pathogenicity on young Kauri seedlings in glasshouse trials. This was also observed on untreated stem and soil-inoculated kauri seedlings during phosphite studies, which exhibited a 100 percent death rate after 20 weeks (Horner & Hough 2011; Horner & Hough 2013b).

The life cycle of *P. agathidicida* has been well described by several researchers (Bellgard et al. 2013; Bellgard et al. 2016; Bradshaw et al. 2020). An excellent illustration of the life cycle of *P. agathidicida* is given on page 6 of Bradshaw et al. (2020), which is available free online: https://bsppjournals.onlinelibrary.wiley.com/doi/pdf/10.1111/ppa.13104.

P. agathidicida has several life states, which are similar to other soil-borne *Phytophthora.* These comprise hyphae (vegetative), short-lived and motile zoospores (asexual) that are released from sporangium that can form directly on infected roots or germinate from oospores (sexual), which are thick-celled and long-lived (Bellgard et al. 2013; Bradshaw et al. 2020). Hyphal aggregations known as stromata have been observed to be produced within host roots (Bellgard et al. 2016; Bradshaw et al. 2020). A preliminary assessment has found that *P. agathidicida* is present in both the fine root fragments and fine organic matter within infested kauri forest soils (I Horner, pers. comm. to T Ashcroft, 2020).

The motile zoospores are important in localised host-to-host transmission. The zoospores disperse in soil water and follow chemotactic gradients towards the roots of kauri (Lawrence et al. 2019;

Bradshaw et al. 2020). Whereas oospores, which are formed in infected tissue particularly roots, are released into soil from the root or during root decomposition. The oospore can be vectored long distances (Beever et al. 2010). The infection cycle then starts again when the germinating oospores form sporangia and release zoospores that encyst to the fine kauri root and form a penetration structure into the root epidermis, before colonising the root cortex and forming stromata, oospores and surface sporangia (Bellgard et al. 2016; Bradshaw et al. 2020).

The natural spread rate of *P. agathidicida* was intensively investigated in a single site (Twin Peaks, Huia, in the Waitākere Ranges) from 2009 to 2012 by Bellgard et al. (2013). The authors extrapolated from their data that the linear distance of spread since the first record of disease at the site in 2007 until 2013 was 3.41 ± 0.52 metres. Bellgard et al. (2013) do not give an annual spread rate based on this data because it was complicated by the presence of other *Phytophthora* species that may interact with *P. agathidicida*. Their average over the six years, however, equates to an estimated average of 0.57 metres per year, which is well below that predicted by (Beever et al. 2009) of 3 metres per year based on the extension of disease at the Great Barrier Island "Gadgil site". The data from these two small case studies is insufficient to extrapolate the natural rate of spread of disease, other than to state that it is highly variable, likely to be site specific and influenced by multiple biotic and abiotic factors. The Bellgard et al. (2013) research represents an important comparison group for future studies investigating natural spread rates.

The disease (symptomatic trees) prevalence in regenerating large rickers calculated on the data reported by Bellgard et al. (2013) was 21 percent, 26 percent and 24 percent in 2006, 2009 and 2012 respectively (excluding dead trees). Disease prevalence, including dead trees (assumes death is related to *P. agathidicida* infection), was 29 percent, 35 percent and 42 percent for 2006, 2009 and 2012 respectively. The incidence rate calculated on the data reported by Bellgard et al. (2013), that is, the number of new cases developing over time, was 0.1 case per year from 2006–2009, which increased significantly to 5.6 new cases per year from 2009–2012. At that higher rate, the remaining 89 trees would be estimated to be lost to disease within 16 years, however, the incidence rate may accelerate over this time, which would reduce the period for all trees to be lost to disease. It would be valuable to reassess this site and update the incidence rate.

3.3 ALTERNATIVE HOSTS

At the first TAG, in 2008, it was recommended *Agathis robusta* was tested for susceptibility, which could infer the possibility that *P. agathidicida* is present in Australia (Kauri Dieback TAG1, 2008), as part of the determination of origin. In addition, the TAG recommended *Araucaria spp.* (monkey puzzles) and *Wollemia nobilis* were considered for testing as alternative hosts, but these were prioritised below kauri-associated alternative host testing. Host range testing of *Agathis robusta* (Queensland kauri) conducted by Bellgard et al. (2013) found no indication of susceptibility, and no kauri dieback symptoms were evident on the remaining few *A. robusta* trees at the Waipoua arboretum site examined by Beachman (2017).

In addition, Bellgard et al. (2013) investigated 19 native plant species to see if any were susceptible to *P. agathidicida*. The study was glasshouse based using young plants and soil-borne inoculum. The results showed that rimu, māmāngi (*Coprosma arborea*), pōhutukawa, rewarewa, mānuka, kānuka, pigeonwood and tawa all displayed a significant decrease in shoot and root weight, whereas taraire

and korokio, while infected, did not show any affects. This investigation was under controlled and ideal infection conditions, the authors note that no evidence had been found at the time that *P. agathidicida* infects non-kauri hosts in the field. A potential risk to other New Zealand native hosts has been indicated but field confirmation has not occurred.

As of 2019, observations have been made (J Craw, pers. comm., 2019) that tānekaha (*Phyllocladus trichomanoides*) growing near infected kauri were showing similar symptoms to kauri dieback, however, no *P. agathidicida* has been isolated from trees. Testing of tānekaha at Taheke Scenic Reserve near Whangarei, isolated only *P. cinnamomi* (A Beauchamp, pers. comm., 2020). A small trial showed evidence of glasshouse soil inoculation infecting tānekaha seedlings, where the outer layer of fine roots was observed to be absent (Ryder et al. 2016), however, it was insufficient to indicate this occurs in the field.

Knowledge of whether alternative hosts are infected and produce oospores will inform decisions around hygiene measures and may have implications in how areas that contain alternative hosts are managed. This will also inform risk management of a potential vector pathway of movement of non-kauri seedlings for restoration plantations. In addition, an understanding of the *P. agathidicida* inoculum loads (if any) associated with non-kauri hosts versus kauri hosts will influence the magnitude of risk. Initial research into alternative hosts was funded by the KDP despite the research appearing more fundamental than operational in focus, however, the operational management of tracks and restoration could be strongly influenced by this research. Alternative host research is under way and is expected to be completed in September 2023.

An important research gap remains in knowing if other native plants are acting as reservoirs of *P. agathidicida* and if inoculum is being released into the soil from non-kauri hosts; this was identified as a critical gap by Black and Dickie (2016) and Bradshaw et al. (2020). In addition, research by Lewis et al. (2019) found that soils collected from pasture and pine forest and inoculated with *P. agathidicida* supported higher oospore production than kauri soil. The authors concluded that these soil types could potentially act as a reservoir for the pathogen (Lewis et al. 2019).

Further research is required to understand this relationship as well as the host potential for clover and other pasture species. This is particularly significant in terms of rural vectoring in areas surrounding kauri and kauri forests.

3.4 EPIDEMIOLOGY

3.4.1 CASE DEFINITION

Kauri dieback is an infectious **disease** caused by the **pathogen** *P. agathidicida*. It is common for the disease (kauri dieback) and the pathogen (*P. agathidicida*) to be used interchangeably; however, it is important that the difference between these two terms is understood.

The disease is the visible symptoms observed on a host, which are characteristic of one or more specific disorders caused by a pathogen. In the case of kauri dieback, the disease includes bleeding lesions on the basal trunk or roots, canopy thinning, yellowing foliage and, in severe cases, tree death. The pathogen is the microorganism that infects the host and subsequently affects the physiology of the host to cause the disease symptoms. The pathogen can be present in the absence

of disease, and disease symptoms can all be present in the absence of the specific pathogen because the physiological changes can be caused by other biotic (different pathogens) or abiotic (physical, environmental or climate) factors.

Workshops to draft an agreed case definition were held in 2019 (Stevenson & Froud 2019), and the recommended case definitions below are taken from Stevenson and Froud (2020).

3.4.1.1 RECOMMENDED CASE DEFINITION DISTINCTIONS

Distinction is made between *P. agathidicida* **sites** based on pathogen presence in samples (soil, tissue, water etc.) and kauri dieback **trees** based on disease presence (that is, visible symptoms of disease).

P. agathidicida sites are useful data points for measuring disease spread and risk management.

Kauri dieback trees are useful data to document the prevalence and geographic extent of disease and to monitor disease progression and responses to controls or interventions.

3.4.1.2 *PHYTOPHTHORA AGATHIDICIDA* SITES

P. agathidicida sites are geospatial locations where the **pathogen** is confirmed or suspected to be present. How inclusive management agencies are with suspect *P. agathidicida* sites will be dependent on their objectives. For measuring disease spread into new regions, only confirmed cases are likely to be acceptable, whereas for risk management, agencies may include suspect *P. agathidicida* sites to enable site management under the Biosecurity Act 1993.

A **confirmed *P. agathidicida* site** is a point location where the presence of *P. agathidicida* has been confirmed (from a tree, soil or other substrate) using a national programme approved test at an approved laboratory.

A **suspect *P. agathidicida* site** is a point location where the presence of *P. agathidicida* is suspected on the basis that probable or suspect cases of kauri dieback (disease) have been recorded. Suspect *P. agathidicida* sites are recorded at the same point locations as probable or suspect cases of kauri dieback.

3.4.2 KAURI DIEBACK CASES (THREE CLASSES)

A kauri dieback tree is a kauri (*Agathis australis*, Araucariaceae) that meets the **symptomatic criteria** and may meet the **epidemiological criteria**, as described below, of having kauri dieback (disease). There are three classes of kauri dieback-trees: confirmed, probable or suspect depending on agreement with the epidemiological criteria (see Table 3-1 for a summary).

How inclusive management agencies are with suspect cases will be dependent on their objectives.

3.4.3 SYMPTOMATIC CRITERIA

The symptomatic criteria for kauri dieback on a kauri tree is meet if a national programme approved trained observer detects one or more of the following symptoms that are consistent with kauri dieback: bleeding lesions on the basal trunk, lesions on roots, the presence of canopy thinning, yellowing of the foliage, tree death.

3.4.4 EPIDEMIOLOGICAL CRITERIA

The **epidemiological criteria** for kauri dieback are meet if the tree is located within a radius of 50 metres of a **confirmed *P. agathidicida* site** (point location).

The epidemiological criteria differ significantly from the draft criteria, based on feedback received during consultation.

3.4.5 CASE CLASSIFICATION – KAURI DIEBACK CASES

3.4.5.1 CONFIRMED CASE

A kauri dieback **confirmed** case is a tree that meets the symptomatic criteria AND *P. agathidicida* has been confirmed from the tree or soil sampling specifically around the tree using the National Programme approved soil sampling protocol and approved test at an approved laboratory.

3.4.5.2 PROBABLE CASE

A kauri dieback **probable** case is a tree that meets the symptomatic criteria AND the epidemiological criteria (that is, a tree that has symptoms but **no laboratory confirmation** (either no test or an undetected test) but is within 50 metres of a confirmed *P. agathidicida*-positive site).

3.4.5.3 SUSPECT CASE

A **suspect** case of kauri dieback is a tree that meets the symptomatic criteria listed above but DOES NOT meet the epidemiological criteria (that is, a tree that has symptoms, **no laboratory confirmation** (either no test or an undetected test) and is not within 50 metres of a confirmed *P. agathidicida* site).

3.4.6 CASE CLASSIFICATION – NON-CASES

Note: a non-case relates to absence of disease NOT to presence or absence of the pathogen.

Unhealthy kauri – other causes, is a tree that may meet the symptomatic criteria, and possibly even the epidemiological criteria, but in the expert opinion of the trained observer the cause of ill-health is not kauri dieback related and rather is associated with other causes such as lightning strike, drought, flooding etc. It is useful to classify these trees separately to non-cases.

Non-cases – are kauri trees that **do not** meet any of the symptomatic criteria but may meet the epidemiological criteria.

Table 3-1: Proposed criteria for *confirmed*, *probable*, *suspect* case and non-cases (*unhealthy* and *non-cases*) of kauri dieback

Case classification	Test positive	Symptomatic criteria	Epidemiological criteria	Approved observer
Confirmed	Yes	Yes	Yes or no	Yes
Probable	No	Yes	Yes	Yes
Suspect	No	Yes	No	Yes
Unhealthy kauri	No	Maybe	Yes or no	Yes
Non-cases	No	No	Yes or no	No

Stevenson and Froud (2020) recommended several actions for adopting the case definitions, with two initial important steps: undertaking reclassification of existing KDP surveillance data then testing these definitions in a workshop on using them for operational decision-making.

3.4.7 DISEASE SEVERITY MEASURES

Disease severity measures were first described by (Dick & Bellgard 2010) who detailed a five-point scale for disease based on canopy health, as shown in Figure 3-1. Dick and Bellgard (2010) also described a binary resin category to identify basal lesion activity, to classify between fresh resin bleeds and old resin (that is, pus-like, soft and squishy versus hard to the touch). Data on canopy symptoms and basal lesions has been collected consistently by multiple agencies over the past 10 years of surveillance, and this will provide the symptomatic criteria for reclassification into the recommended case definitions (Stevenson & Froud 2020).

Figure 3-1: Canopy symptom class and severity rating: 1) healthy crown with no visible signs of dieback; 2) canopy thinning; 3) thinning and some branch dieback; 4) severe dieback; 5) dead (Dick & Bellgard 2010).

3.4.8 CAUSAL FACTORS OF DISEASE

From as early as 2008, the Kauri Dieback TAG1 (2008) suggested that field and experimental evidence indicated *P. agathidicida* was a primary pathogen, however, the TAG also suggested environmental factors may influence the pathogen system, and research into this was important.

The need to understand the fundamental biology of *P. agathidicida* was flagged in the 2010 TAG meeting (Kauri Dieback TAG2 2010), particularly because an early bid to undertake this research was not funded by the then Foundation for Research, Science and Technology (now MBIE). The research gaps around causal or contributing factors identified by the TAG (Kauri Dieback TAG1 2008) were:

- undertake research to determine what climatic conditions *P. agathidicida* requires;

- determine if *P. agathidicida* is associated only with previously stressed kauri versus healthy kauri;
- gain an understanding of the impact of climate change and environmental factors on disease;
- undertake research on the effect of hydrology changes to kauri (health) and *P. agathidicida*.

The need to look more holistically beyond the pathogen, host and climate was identified by Black and Dickie (2016), who stated (p 4):

> "… the focus of the KDP has been almost exclusively on *P. agathidicida* as the sole causal agent, rather than on other drivers of dieback or the status of kauri overall."

And recommended (p 5):

> "A larger-scale research programme around much better understanding of environmental factors driving the spread, adaptation, and virulence of *P. agathidicida* and of kauri dieback in the absence of *P. agathidicida*."

In the past four years, the KDP has increased its research focus on building knowledge and tools to identify areas of disease freedom and to undertake long-term monitoring of spread, impacts and epidemiological factors of disease development. This includes understanding test performance, designing baseline monitoring, and developing an agreed case definition for all agencies and researchers to use (Ashcroft 2016; Cogger et al. 2016; Vallee & Cogger 2019; Vallee et al. 2019; Stevenson & Froud 2020).

As an example, Cogger et al. (2016) developed a draft causal diagram showing the main factors that had been reported by researchers that may contribute to kauri dieback (Figure 3-2).

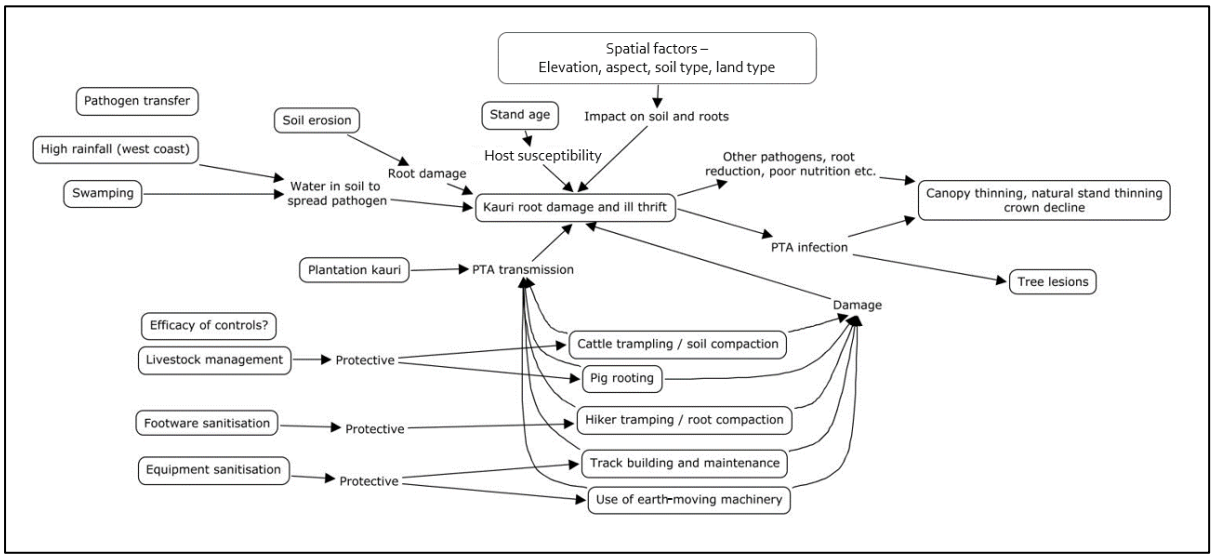

Figure 3-2: Example of a causal diagram indicating potential and known interrelating factors that could contribute to kauri dieback

Source: Cogger et al. (2016)

The KDP surveillance data was assessed before 2016 to see if epidemiological analysis of risk factors for disease development could be undertaken (Cogger et al. 2016). The research concluded that, in most cases, the surveillance was purposeful rather than random, which limits the data's usefulness

for risk factor assessment, and an essential requirement for data collection from BOTH diseased and non-diseased trees was recommended so statistical comparisons can be made (Cogger et al. 2016). Future epidemiological analysis relies on: development of baseline monitoring of diseased and healthy trees; collection or extraction of data on all important postulated risk factors; and wider involvement with mātauranga Māori and other researchers to determine what these should be. This is highly aligned with cultural health indicators (Lambert et al. 2018) and could be led within the current cultural health indicator research (Chetham & Shortland 2013) and the Biological Heritage National Science Challenge Ngā Rākau Taketake Mātauranga Māori Framework for Surveillance research.

3.5 IMPACTS

The initial study by Beever et al. (2009) into *P. agathidicida* on kauri in New Zealand concluded that there was sufficient evidence that *P. agathidicida* posed a threat as a primary pathogen to large iconic kauri trees leading to premature death over a prolonged period of years and was a significant threat to regenerating stands of kauri rickers. Beever et al. (2009) suggested the effect of kauri tree loss in regenerating stands may lead to a change in forest composition from one dominated by kauri to one dominated by podocarps. This is supported by Wyse et al. (2018) who note that the loss of kauri as an ecosystem engineer has the potential to affect the whole community composition and function of kauri forests.

Cultural and ecological impact research has been undertaken within a mātauranga Māori framework (Te Roroa cultural effects assessment), and pilot studies have been done on defining cultural health indicators for monitoring impacts of kauri dieback (Chetham & Shortland 2013). This review does not look at the cultural or social research so the cultural and social impacts of kauri dieback are excluded from this discussion. However, Lambert et al. (2018) and Bradshaw et al. (2020) provide a summary of both cultural and social impacts of kauri dieback. Research into the environmental impact of kauri dieback was recommended at the first TAG meeting where they stated “set up transects perpendicular to disease front to determine presence vs. absence and spread over time – baseline survey needs to be put in situ” (Pg 6. Kauri Dieback TAG1 2008)

By the second TAG meeting in 2010, it was suggested research on the long term ecosystem impacts should be considered and establishing monitoring plots was recommended as a high priority (Kauri Dieback TAG2 2010). However, the TAG also noted that wider ecosystem ecological research was more fundamental than operational and not a primary focus of the KDP, and that the KDP should help other ways to fund fundamental research (Kauri Dieback TAG2 2010).

At the third TAG meeting, it was noted that kauri health data on negative samples needed to be captured and issues of access and management of *P. agathidicida* data needed to be resolved, including developing a central repository of information for researchers and land managers (Kauri Dieback TAG3 2010). The fourth TAG was a research proposal prioritisation meeting. TAG members were concerned no ecological research proposals had been received and again indicated that long term plot based kauri ecological monitoring and research was needed (Kauri Dieback TAG4 2010). The fifth TAG meeting was held two-and-a-half years later, and, again, long-term monitoring of symptomology expression and canopy regrowth was mentioned as important future research (Kauri Dieback TAG5 2013).

Some 20 metre x 20 metre research and carbon plots relating to existing ecological studies are located within Trounson Kauri Park and Waipoua Forest. In addition, funding was used to set up 40 x 50 metre plots in the Waitākere Ranges by Bruce Burns from Auckland University (a member of the TAG). However, two of the three control plots were subsequently found to be *P. agathidicida* sites and a sampling strategy to move the plots sideways was designed using a block sampling method (A Beauchamp pers. comm., 2020). These plots could be used for long-term monitoring in the future.

The purpose of the final TAG meeting in 2015 was to discuss future and priorities for kauri ecosystem and ecological based research to inform kauri dieback management. The TAG prioritised ecological variation in symptomology as the top priority followed by host population demography and ecosystem consequences (Kauri Dieback TAG6 2015).

Despite a small amount of funding being invested in long-term monitoring, ongoing research was not commissioned by the KDP, and Black and Dickie (2016) were unable to find evidence of whether the kauri population was increasing, stable or decreasing regardless of presence or absence of *P. agathidicida*. They recommended "long-term demographic modelling of kauri populations allowing scenario modelling of different disease levels and management strategies" (Pg 4. Black & Dickie 2016)

The main reason why kauri dieback impact research has not been funded, despite the high priority assigned to it, is due to the KDP being unable to prioritise fundamental research under limited operational research budgets (in some years, this was as low as $50,000). In addition, ministerial expectations were that more investment in management tools was needed and less focus on research work that does not have a direct bearing on immediate disease management.

Results from a Master's study at University of Auckland were presented on the effects of *P. agathidicida* on kauri forest ecosystem processes (van der Westhuizen et al. 2013). The study looked at 13 sites, 6 with high to medium infection and 7 with minimal infection. It assessed litter fall and fractions, carbon loss and regenerative vegetation between these sites. van der Westhuizen et al. (2013) observed a decrease in total litter biomass below the more infected trees, particularly reproductive litter, which may indicate a decreased reproductive capacity for kauri. They found no difference in soil carbon dioxide but did observe differences in surrounding vegetation. More recently, a study on 10 kauri trees indicated a reduction in carbon and nitrogen under *P. agathidicida*-infected trees that was correlated with increasing *P. agathidicida* DNA concentration (Schwendenmann & Michalzik 2019). A follow-up study of the physiological response of both kauri and understorey species with much larger sample sizes and a comparison of uninfected trees would be useful to gain a full understanding of the causes and impacts of the observed correlations.

Recent research to agree a case definition and design a methodology for baseline monitoring in the future is the first step in being able to monitor the impact of kauri dieback at the population level and measure the impact of mitigation, such as phosphite treatment, track upgrades, public awareness, cleaning stations and other measures (Stevenson & Froud 2020). The SSAG stated "the causes and factors associated with the spread of the disease, and the dynamics and significance of these factors within kauri forests, need to be better understood to inform effective long-term management approaches. We still do not know what the overall direct and indirect impacts of kauri dieback will be." (Pg 1. Kauri Dieback Strategic Science Advisory Group 2018)

The SSAG also identified that the lack of knowledge on the functional and ecological health of kauri ecosystems was a fundamental science gap (Kauri Dieback Strategic Science Advisory Group 2018). In response to the Biological Heritage National Science Challenge Ngā Rākau Taketake programme science planning, the KDP indicated as a priority the importance of environmental risk mapping investigating disease expression, latency period, healthy forests (in the absence of symptoms) versus infected forests and long-term demographic modelling of kauri populations, especially under climate change scenarios (Froud et al. 2019). The Ngā Rākau Taketake programme has now initiated a risk assessment and ecosystem impacts team to quantify the affect kauri dieback has on the wider ecosystem. This research is closely tied to the Ngā Rākau Taketake surveillance research and progression of long-term disease monitoring.

3.6 KNOWLEDGE GAPS FOR BIOLOGY

Significant knowledge gaps remain around the fundamental biology of the pathogen *P. agathidicida*. These gaps were outside the mandate of operational research, however, the KDP did contribute funding to larger research programmes to investigate the biology and ecology of kauri dieback.

The main gaps in knowledge that would contribute significantly to improving operational objectives for managing kauri dieback in New Zealand are:

- understanding the biological mechanisms that control oospore dormancy and options to break dormancy to improve surveillance testing and control of the most robust dispersal structure of *P. agathidicida*;
- understanding whether *P. agathidicida* is present throughout the kauri lands of New Zealand at levels below the level of detection using existing diagnostic tools, or if it is only present where disease is being detected (this is a fundamental question that could inform management of spread versus management of ecosystem level factors contributing to disease);
- understanding the long-term cultural and ecological impact of kauri dieback on forest health and the effects of mitigation measures both in managing kauri dieback and on the forest;
- understanding the role of alternative hosts in *P. agathidicida* distribution and spread.

4 SURVEILLANCE, KAURI MAPPING, DETECTION, DIAGNOSTICS

One of the KDP's important goals is to improve the understanding of disease distribution to inform where to apply operational management tools. The KDP invested in research to operationalise detection of the pathogen *P. agathidicida*, kauri dieback disease and the host population. Research on pathogen detection focused on soil, from lesions and in water. Host and disease detection were investigated via aerial, remote sensing and ground visual survey.

This review describes the research conducted to support kauri dieback and *P. agathidicida* surveillance programmes (but not the results of these programmes).

4.1 OPERATIONAL DELIVERY OF SURVEILLANCE

Surveillance reports available within the KDP are recorded in Table 4-1. Twenty reports were completed between 1974 and 2015, but a comprehensive review of them has not been done. It is strongly recommended a review be done of the results from all forms of surveillance for *P. agathidicida*, kauri dieback (disease symptoms) and hosts. The data should be collated in full (excluding private landowners' data where Privacy Act considerations prohibit inclusion) into a single or regional-based geospatial relational database, and appropriate cultural approval be sought to collect and use the data. The review should clearly describe the survey methods and case definition applied for different datasets. It should also describe the surveillance results both temporally and spatially, based on search effort from all KDP partners, research samples and any other surveillance data that the KDP has access to.

Currently programme partners manage data that they collect. In addition, MPI houses a geospatial database which contains most programme collected data excluding private landowner data where permission by the landowner has not been given to share the data.

Table 4-1: Kauri Dieback Programme surveillance activities and references to surveillance reports

Year	Name	Survey method	Reference
1974	Great Barrier	Ground	Gadgil (1974)
2009	Northland	Ground – research	Beever et al. (2010).
2011	Surveillance 1 – Wide area	Ground	(Beauchamp 2010, 2011)
2011	Waipoua	Ground	(Beauchamp 2012c, d)
2012	Waitākere	Aerial	Jamieson (2012c)
2012	Hunua Ranges and environs	Aerial and ground	Jamieson et al. (2012)
2012	Coromandel (including Kaimai Ranges)	Aerial	Jamieson (2012d)
2012	Coromandel	Aerial	Jamieson (2012a)
2012	Aotea/Great Barrier	Aerial	Jamieson (2012b)
2012	Surveillance 2 – Wide area	Ground, aerial, forward traces	(Beauchamp 2012a; Dick & Bellgard 2012; Beauchamp 2013b, a; Beauchamp & Waipara 2014)
2014	Kawau Island	Aerial	(Jamieson 2014b; Jamieson et al. 2014)
2014	Hauturu (Little Barrier)	Aerial	(Jamieson 2014c; Jamieson et al. 2014)
2014	Waiheke and Ponui islands	Aerial	Jamieson (2014a)

Year	Name	Survey method	Reference
2014	Aotea/Great Barrier	Ground truthing from 2012 aerial	Hill et al. (2014)
2015	Northland	Aerial	Macdonald (2015)
2017	Waitākere	Aerial and ground	Hill et al. (2017b)
2017	North-western Waikato 2016/17	Aerial	Macdonald (2017b)
2017	Northern Waikato (Eastern Waikato, Hunua, Kaimai Range into Bay of Plenty and Hauraki Plains)	Aerial	Macdonald (2017a)
2018	Northland/Auckland 2017/18	Aerial	Macdonald (2018)
2019	Coromandel (Tairua)	Aerial	Macdonald (2019b)

Figure 4-1 and Figure 4-2 show the current (as of June 2020) distribution of kauri forest and confirmed *P. agathidicida* sites in New Zealand. In these mapped distributions, the case definition is a positive soil test result for *P. agathidicida.* Clear statements of where *P. agathidicida*-positive sites are, in comparison with where kauri dieback has been observed, that are consistent with the proposed case definitions of (Stevenson & Froud 2019) are recommended. The geospatial maps of KDP partners are excellent at a regional level and well suited to operational planning. However, the map on the kauri dieback website (Figure 4-2) shows a high level *P. agathidicida* distribution and the estimated range of the underlying population at risk, but it excludes data on where a soil sample was not taken but clear signs of kauri dieback (disease) were observed. The map is also not of a scale suitable for researchers and the public to understand at a glance the risk that kauri dieback poses to New Zealand's kauri forests.

Areas covered by fixed wing aerial surveillance from 2011 to 2018 are shown in Figure 4-3 and from 2016 to 2019 in Figure 4-4 (Andrew Macdonald, pers. comm., July 2019). Drone and helicopter aerial surveillance also occurred during this period.

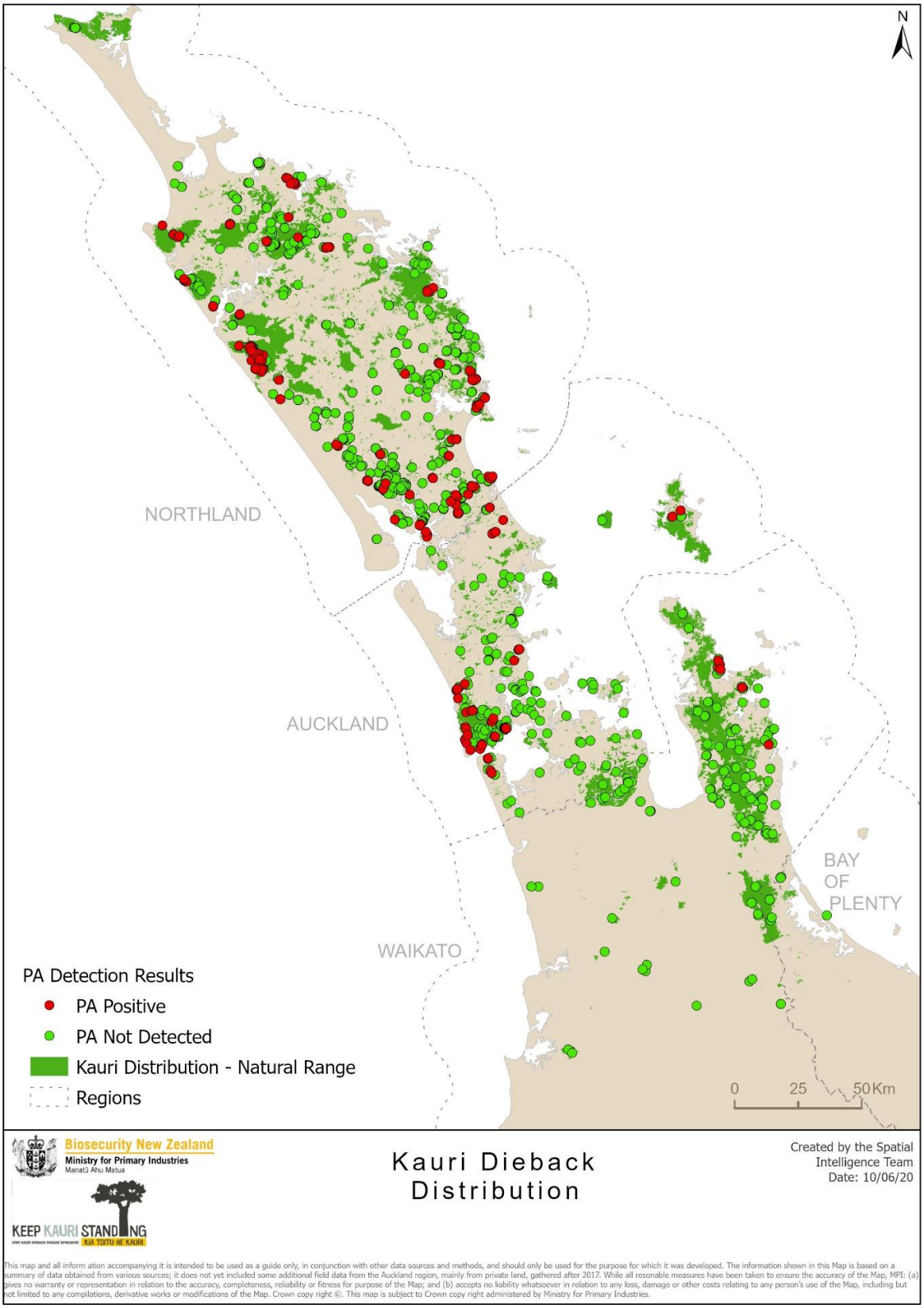

Figure 4-1: *P. agathidicida* distribution as of 10 June 2020, excluding private landowner data held at Auckland Council

Note: PA Positive = *P. agathidicida* detected from soil sample via lab test; PA Not Detected = *P. agathidicida* not found from soil sample via lab test.

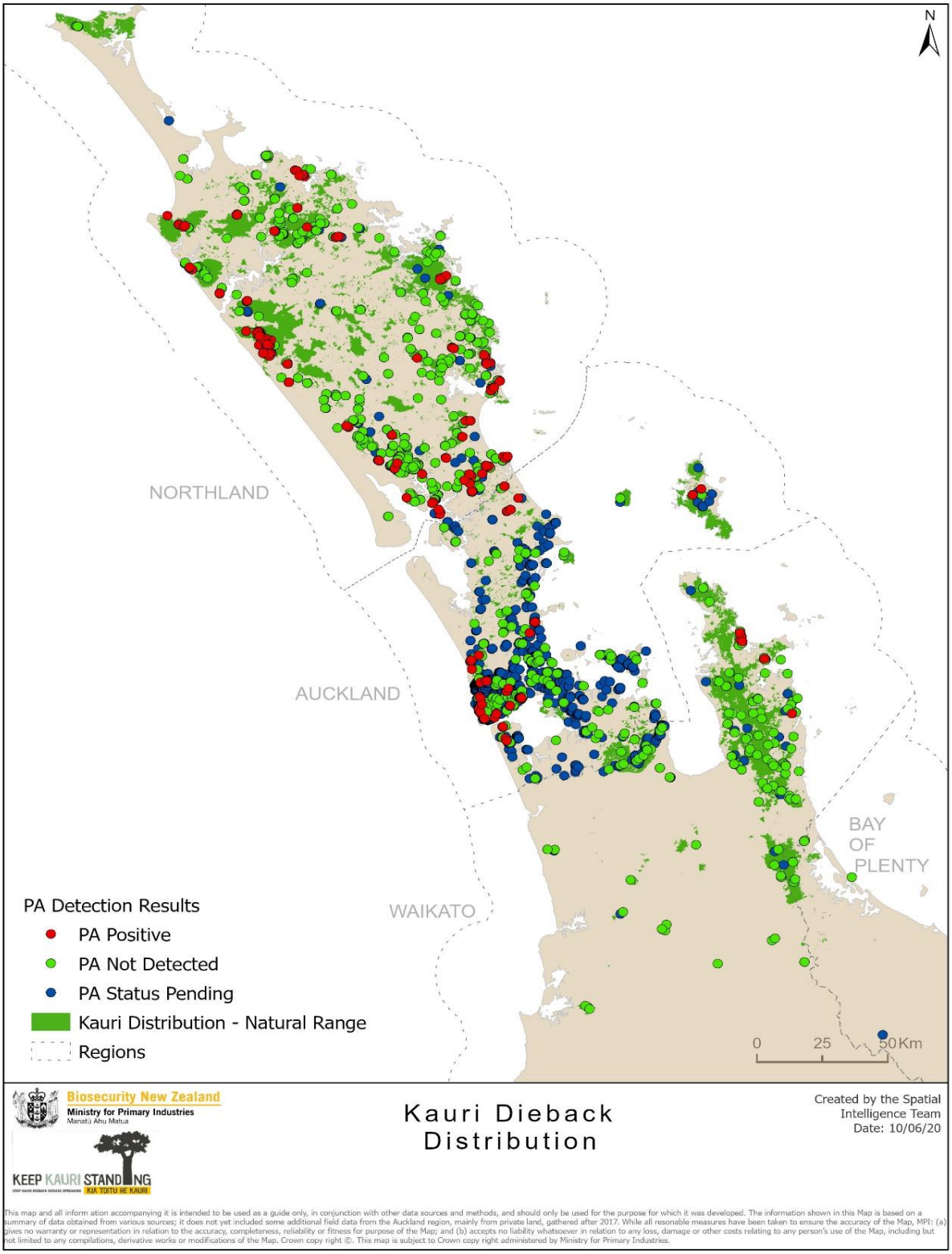

Figure 4-2: *P. agathidicida* sampling locations as of 10 June 2020 (excluding Auckland Council data from 2015 onwards)

Note: PA Positive = *P. agathidicida* detected from soil sample via lab test; PA Not Detected = *P. agathidicida* not found from soil sample via lab test; PA Status Pending = sample collected, pending lab test results OR pending soil sampling.

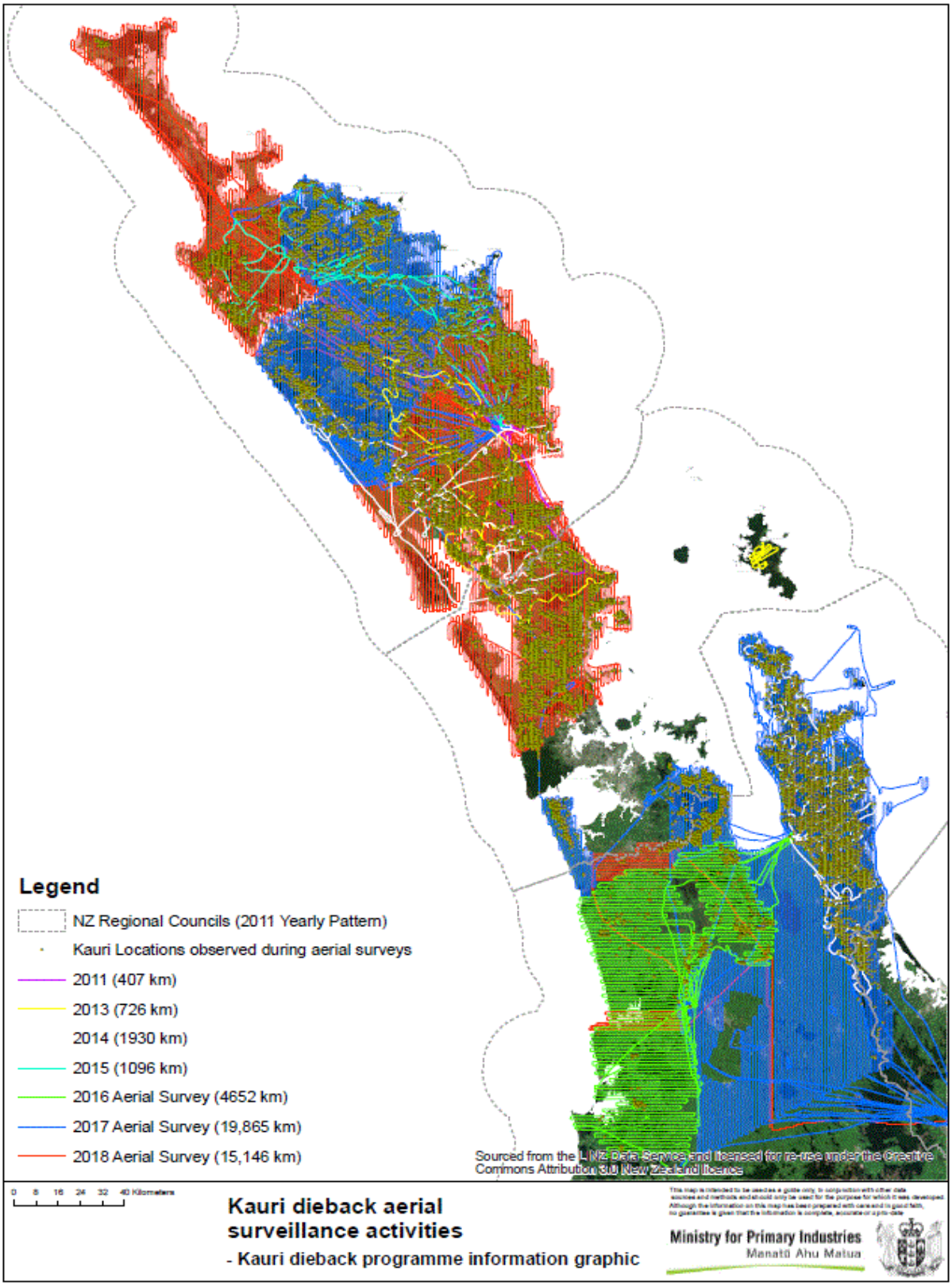

Figure 4-3: Flight paths of fixed wing aerial surveillance projects from 2011 to 2018

Source: Originally from Andrew MacDonald, Biospatial Ltd (2019) (www.kauridieback.co.nz/media/2038/aerialsurveys_2019_350_dpi.jpg)

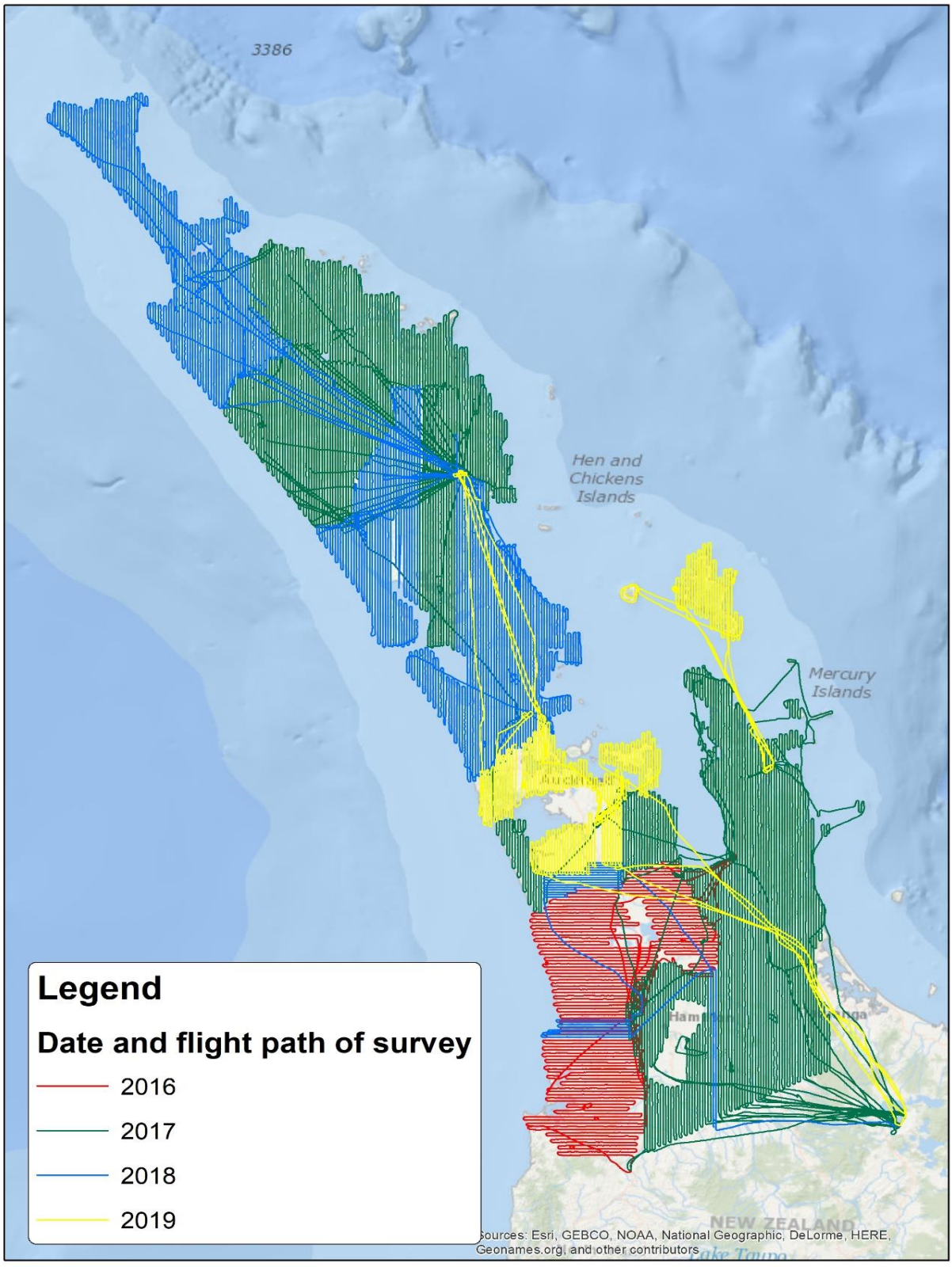

Figure 4-4: Flight paths of fixed wing aerial surveillance projects from 2016 to 2019

Source: Andrew MacDonald, Biospatial Ltd (2020).

Note: The yellow marked areas are in addition to areas covered in the previous figure.

4.2 HOST POPULATION DETECTION (KAURI TREES)

Research in host population detection has aimed over the past decade to identify and map where kauri trees are present at a regional, forest, within forest and individual tree level.

At a regional forest level, research into wide-scale kauri and disease detection has found that wide-area oblique aerial photography provides good identification of the baseline host population at risk and assessment of kauri crown health (Macdonald 2016b). This was used as an equivalent of a gold standard for the reference population of trees for remote sensing research (Meiforth 2018; Meiforth et al. 2019; Meiforth et al. 2020). This approach has successfully helped ground surveillance teams locate and sample kauri trees showing the canopy ill thrift typical of kauri dieback, over relatively large areas. Although it has been cost-effective for the KDP partners, it relies on a manual visual assessment of crown health, which offers opportunity for human error and is not a practical option for developing a full baseline of the state of New Zealand kauri health and monitoring changes in health over time.

KDP investment in remote sensing for kauri host and stress detection shows promise and is in the pre-implementation phase (Meiforth 2018). The remote sensing research objectives were to remotely identify kauri trees as the host population at risk and identify kauri stress symptoms to inform the possible presence of kauri dieback disease using Light Detection and Ranging (LiDAR), RGB aerial images, a WorldView02 image and an AISA Fenix hyperspectral image. The research described kauri phenology in depth, including shape, colour and stand variation, and compared the full spectral characteristics of kauri with 21 other canopy species common in the Waitākere Ranges (Meiforth 2018).

As with the kauri stress study, which is detailed in Section 4.4, the main purpose of the host population detection study was to use hyperspectral data, which is acquired over narrow swaths (and is therefore expensive), and analyse hundreds of spectral bands to identify the four to six most important spectral bands for identifying healthy and stressed kauri trees. Those index spectral bands can then be used in multispectral sensors on aircraft that have a wide swath width (so are cheaper to use to acquire data) and can be combined with other remote-sensing technology, such as LiDAR, to map the host population across a wide area (Meiforth et al. 2019). The research by (Meiforth et al. 2019) found that a five-band multispectral sensor with indices in the visible (VIS) to near-infrared (NIR2) range (electromagnetic wavelength bands up to 1,209 nanometres (nm)), which are not usually included in standard multispectral sensors, had the highest overall pixel-based accuracy (91.7 percent) on trees (or dense stands) with crowns larger than 3 metres. Accuracy was further improved when dead or dying kauri were combined with kauri in one class (93.8 percent) stratifying for high and low forest stands and combining bandwidths to 10 nm for analysis (Meiforth et al. 2019).

The five spectral bands outperformed satellite imagery, which achieved 80.3 percent accuracy in host detection (Meiforth 2018). Kauri trees have distinct spatial characteristics, and current unpublished results show that LiDAR data can significantly improve the performance of multispectral images for kauri detection, when the characteristic “kauri bands” in the NIR2 region are not available (J Meiforth, pers. comm., 2020). A test on 1,216 crowns with the three classes “kauri”, “dead/dying

trees" and "other" increased the crown-based accuracy from 80 percent for only WorldView02 data to 90 percent, when LiDAR attributes were added (J Meiforth, pers. comm., 2020).

Meiforth et al. (2019) concluded that the five-band multispectral method allowed accurate and cost-efficient mapping of kauri trees and was suitable for area-wide mapping within the forest ecosystem represented in the Waitākere Ranges. Meiforth et al. (2019) recommended that a manual decision tree be developed to help implementation of the research, due to complexity of the Random Forest classifier model. They also recommended that the indices and model be tested in other kauri forests, particularly where forest composition differs (Meiforth et al. 2019). This testing could be referenced against the oblique aerial photography that now has wide coverage in areas with different forest composition.

Meiforth et al. (2020) also assessed methods to segregate the crowns of individual trees to enable crown-based polygons to be spatially described for long-term monitoring. They found that multi-resolution segmentation in eCognition gave the best results and recommended that over-segmentation (several polygons per crown) was a better approach in dense stands (Meiforth 2018). Completion of the segregation research is yet to be published, but this could be useful for describing the minimal unit of interest from which a change in disease state over time could be monitored (refer to Stevenson and Froud (2019)).

In 2015, the KDP engaged Wildlands Consultants Ltd. to develop a geospatial database (geodatabase) to describe the current distribution, abundance and maturity of kauri and kauri ecosystems in New Zealand (Ranger et al. 2019). Wildlands Consultants undertook a review of data sources and these are detailed in its draft report.

Ranger et al. (2019) mapped the southern limit of naturally occurring kauri (Figure 4-5) based on literature and herbarium records. They also categorised tree maturity using three classes based on aerial observation of canopy diameter (ricker less than 5 metres, mature 5–20 metres and old-growth more than 20 metres), however, discretion was used for class classification for rickers in open areas where the authors observed that canopy diameter could extend up to 7 metres (Ranger et al. 2019). This was undertaken for representative forests in Waipoua (Northland), Parry Kauri Park (Warkworth), Cascades (Waitākere Ranges) and Tapu–Coroglen (Coromandel) using Google Earth Pro™ and oblique photos from Google Street View™.

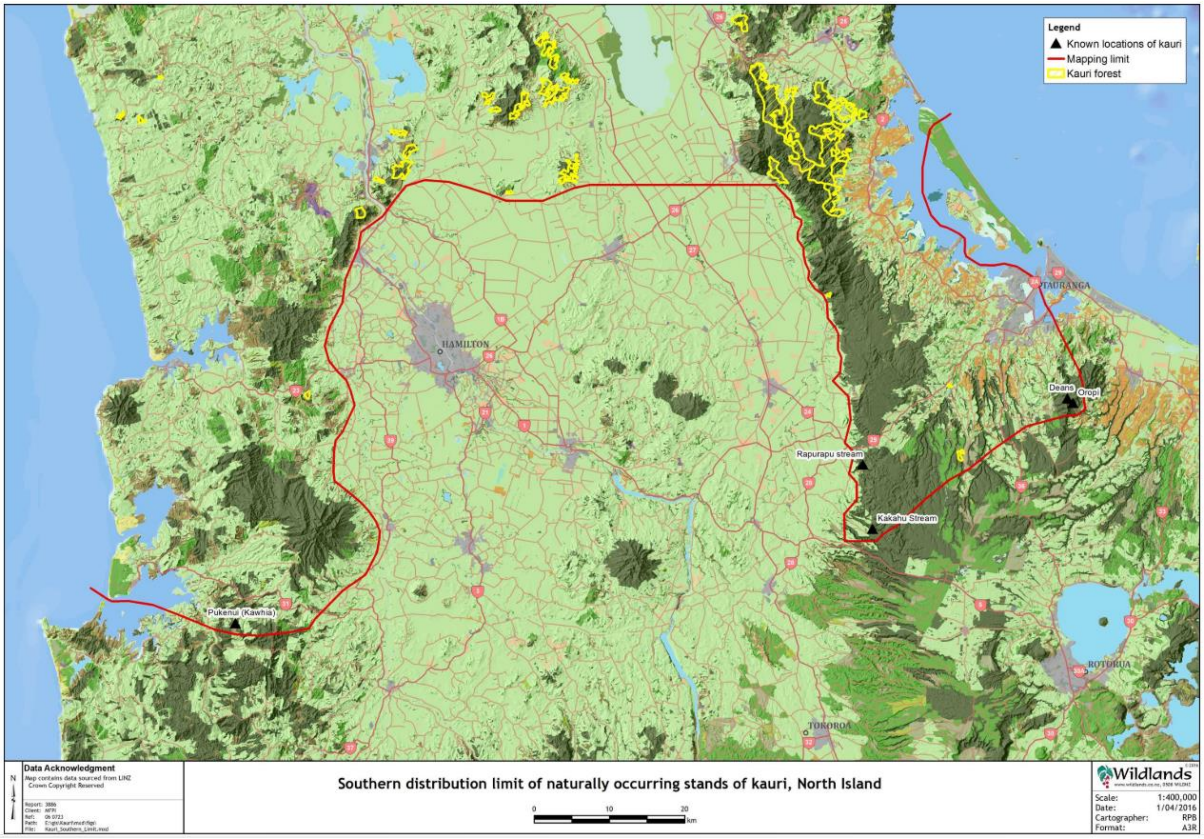

Figure 4-5: Southern distribution limit for naturally occurring kauri in New Zealand

Source: Ranger et al. (2019)

Spatial distribution was assigned for each polygon where kauri were deemed "present" based on various information sources, including expert opinion, reports, herbarium records, oblique aerial photographs and global positioning system spatial data from the Ministry for Primary Industries (MPI) and other KDP partner organisations, and Google Earth™ (Ranger et al. 2019). The geodatabase contains 8,899 "kauri present" polygons covering 540,940 hectares but excludes large areas exempted, such as rural land.

This is the first systematic assessment in New Zealand of the current extent of kauri forest and old-growth kauri (Ranger et al. 2019). For every "kauri present" polygon an attributes table was developed to show abundance (cover), maturity, distribution, ecosystem and anthropogenic attributes (Ranger et al. 2019). Cover was estimated visually as the proportion of kauri canopy within a kauri present polygon to the nearest 5 percent (Ranger et al. 2019). The main evidence source and likelihood estimate for each type is detailed by Ranger et al. (2019). The proportions of polygons or areas mapped that were estimated from each evidence source are also detailed; for example, MPI oblique aerial images accounted for 44 percent of polygons and 28 percent of the total kauri area mapped, whereas expert opinion accounted for 7 percent of polygons and 32 percent of the area mapped, and field observation only accounted for 1 percent of both polygons and area mapped (Ranger et al. 2019). Even though confidence levels are stipulated for each polygon, in terms of accuracy, the geodatabase has information gaps and is not accurate enough to define the population at risk for baseline monitoring because uncertainty remains whether some areas classified as "kauri present" contain hosts and what the true abundance (cover) of hosts is within polygons.

KDP partners and community groups have made several other attempts to map kauri forest areas and individual trees for surveillance. Kauri health remote sensing was conducted in Waipoua Forest by ArborCarbon (Taoho Patuawa, pers. comm., Te Roroa remote sensing workshop presentation, 13 September 2019).

The Waikato Regional Council has been working on a dataset for kauri point location in the Waikato and Bay of Plenty regions, which has used oblique aerial imagery point location surveillance (Macdonald 2016a, 2017b, a, 2019b) and an "old growth" kauri geospatial layer to enable strategic resource allocation to these kauri stands (K Parker, pers. comm., 2020). In addition, a research group from the University of Auckland has explored the use of convolutional neural network technology to segment kauri trees from aerial images, and results indicate 93 percent accuracy (Han et al. 2017). Further investigation is required to see how that research could be incorporated into a national framework.

Population level data, and existing and future remote sensing data, need to be accessible regionally and nationally before national baseline monitoring can begin. In addition, the constraint of how cultural knowledge can be protected and shared in a culturally appropriate way is the focus of the Biological Heritage National Science Challenge research project "Mātauranga Māori Framework for Surveillance" under the Ngā Rākau Taketake (Saving Our Iconic Trees) banner.

The geodatabase host population's level of accuracy is at the polygon within forest level rather than individual tree level, so it is preferable to use existing point location methods or implement remote sensing to describe the population of interest. Although highly complex, remote sensing could be operationalised to accurately spatially describe the emergent canopy population at risk (referred to as the "kauri mask" by Meiforth et al (2019)) and build a sampling frame for baseline monitoring of kauri dieback (Stevenson & Froud 2019). A sampling frame is a dataset of all known hosts and their location, from which a random or targeted sample group can be extracted for surveillance. The size of the sample group is informed by the purpose of the surveillance (for example, freedom from disease), the expected prevalence of disease and the sensitivity and specificity of the test used to detect disease. These concepts are discussed in the next section.

Existing geospatial maps of the kauri forest areas for Northland, Auckland and Waikato could be sufficient, in the interim, to describe the population and the area (hectares) at risk of severe effects due to Kauri dieback at a forest level. The geospatial layers could be linked to the ability to map individual trees, as shown using oblique aerial imagery and multispectral remote sensing (larger than 3 metre canopy diameter). If the proposed Mātauranga Māori Framework for Surveillance is proven to be effective, then inclusion of its framework and approach should be considered for future monitoring. At that stage, the KDP may be in a position to set a baseline against which to address a high priority gap raised by Black and Dickie (2016) and Bradshaw et al. (2020), which was to investigate the long-term population dynamics of kauri with and without *P. agathidicida* and the long-term impacts of kauri dieback.

4.3 PATHOGEN DETECTION

4.3.1 EARLY ISOLATIONS AND DISEASE SYMPTOM DESCRIPTIONS

As noted in Section 4.1, the first isolations of what is now known to be *P. agathidicida* were by Gadgil (1974) at a site showing collar rot and a yellowing and thinning canopy of kauri on Great Barrier Island, Hauraki Gulf, in the Auckland Region. Gadgil (1974) isolated *P. agathidicida* in soil and infected tissues (trunk and root lesions) from diseased trees and from soil and roots taken from asymptomatic trees around 4 kilometres away from the symptomatic site. Gadgil (1974) also proved Koch's postulates for *P. agathidicida* from seedlings during pathogenicity testing.

Beever et al. (2010) undertook kauri disease surveillance soil sampling (and baiting using lupin for *Phytophthora*) in Waipoua Forest and Trounson Kauri Park in 2003. They detected *P. cinnamomi* in 47 percent of the 36 soil samples in the Waipoua Forest and 25 percent of the 25 soil samples in Trounson Kauri Park, but *P. agathidicida* was not detected at that time (Beever et al. 2010). It is suggested that *P. agathidicida* was not historically detected in soil baiting because the extraction methods are favoured other *Phytophthora* species which outcompete *P. agathidicida* (Beauchamp 2014).

The first report of kauri dieback on mainland New Zealand was in 2006 on diseased trees on Maungaroa Ridge in the Waitākere Ranges, west of Auckland (Beever et al. 2009) and from Trounson Kauri Park in terminal pre-human kauri forest (Beauchamp 2014). Kauri at the Trounson site showed symptoms consistent with the Great Barrier Island site of yellow foliage, a thinning canopy and lower trunk and root lesions (Beever et al. 2009). *P. agathidicida* was isolated and, because it did not fit existing species descriptions at the time, was given the tentative name of *Phytophthora* taxon *Agathis* (abbreviated to PTA) (Beever et al. 2009). It was formally described as *P. agathidicida* by Weir et al. (2015) nine years later (Black & Dickie 2016).

Gadgil (1974) and Beever et al. (2009) both showed that *P. agathidicida* could be isolated from soil and lesions. Both studies were, however, detailed investigations of small sites and research was needed to develop detection methodologies that could be rolled out for larger scale surveillance. Asymptomatic trees next to diseased trees at a Great Barrier Island site had *P. agathidicida* detected in soil and on roots, and also at an apparently healthy site some distance away (Gadgil 1974). Gadgil (1974) concluded the pathogenic activity must be governed by unknown environmental factors.

4.3.2 SOIL SAMPLING FOR PATHOGEN DETECTION

In 2009, the KDP invested in research to define the symptoms of *P. agathidicida*; to develop a method to optimise detection of *P. agathidicida* in soil and from lesions; and to optimise soil and lesion sampling methodologies (Beever et al. 2010). This research gave the KDP confidence in the methodology for baiting *Phytophthora* from soil where Manaaki Whenua – Landcare Research, Plant and Food Research and Scion all tested common *Phytophthora* baits, along with kauri leaves. A consensus was formed that lupin radicles (blue lupin cultivar, *Lupinus angustifolius* cv. "fest bitter") and trimmed Himalayan cedar needles (*Cedrus deodara*) were the most consistent baits (Beever et al. 2010). Interestingly, kauri leaves, along with rhododendron, were the least effective (Beever et al. 2010).

Soil sampling was optimised before the initial large-scale surveillance rounds. An eight cardinal-point collection of soil was devised, that is, collecting soil samples from four points close to the trunk and four points further towards the dripline of the tree (Dick & Bellgard 2012) (see Figure 4-6). Soil baiting, while optimised, was still inconsistent between diagnostic laboratories, particularly during the early years of surveillance, and remains an important issue with soil sampling. By the fourth TAG, the problem of not getting positive soil samples around clearly diseased trees was of major concern. Significant effort was made to improve field collection methodologies and detection probabilities (Beauchamp 2012b, c, d, a, 2013b), including improving temperature management of samples (Beauchamp 2012c; Beauchamp & Waipara 2014). This research culminated in the reissuing of the soil surveillance guidelines for KDP partners in early 2016 (Beauchamp 2016; Kauri Dieback Programme 2017). This included:

- limiting sampling when soil was too dry, to avoid high oospore dormancy and when too wet, to avoid the risk of sampler transmission to other kauri trees;
- managing temperature during sampling, transport and storing of samples to between 4 degrees Celsius and 26 degrees Celsius;
- increasing sampling to a cluster of three trees (within 15–50 metres of each other) rather than individual trees;
- sampling trees with symptomology consistent with kauri dieback first, then asymptomatic if no symptomatic trees present;
- insisting that eight cardinal points per tree were used with the inclusion of LIVE fine kauri root material wherever possible;
- using lesion and canopy images for all samples to assess not detected results.

The inclusion of lesion and canopy data in historical sample collections may be useful for contributing to the symptomatic criteria of the proposed case definition and to reclassify results without positive *P. agathidicida* tests.

Technical experts also suggested the possible need to find out if dormant spores were still within a negative sample following the extraction process (Kauri Dieback TAG4 2010). Bellgard et al. (2013) stated that a challenge with the soil bioassay is that, if dormant spores fail to germinate, *P. agathidicida* will not be detected. In laboratory cultures, Bellgard et al. (2013) noted that around 80 percent of oospores produced were dormant. Further optimisation of laboratory methods was researched to address the issue of extended dormancy, which led to additional steps in the baiting process to optimise extraction of *P. agathidicida*. These included setting up samples for processing on day 1, moistening soil on day 3 and flooding and baiting with lupins on days 4 and 7, followed by plating baited material on day 9 and checking plates on days 11 to 17 (Ganley 2015).

The biological factors contributing to breaking dormancy of oospores remain a significant gap in our knowledge and are discussed further around oospore deactivation in Section 6.2.4.

Of note is the observation Beever et al. (2010 Pg. 47) make about soil detection around heavily diseased trees:

> It must also be remembered that there is a lag-time between initial root infection and expression of PTA-disease symptoms in the collar and crown of the infected tree. When first symptoms become visible in the crown, the destruction of the fine root system may be

already at a very advanced stage. At this point the inoculum of the primary parasitic *Phytophthora* may have decreased to a low, nearly undetectable level.

Currently, surveillance undertaken by KDP partners either adheres to the Dick and Bellgard (2012) method of eight cardinal point soil sampling and sending samples to two diagnostic laboratories (Beauchamp 2016) or to a subset of these methods that takes four soil samples close to the trunk and eliminates the four dripline soil samples, followed by diagnostic testing at a single laboratory (Hill et al. 2017a) (see Figure 4-6).

One of the objectives of the KDP is to develop a diagnostic tool that is real time, cost effective and has a high degree of accuracy.

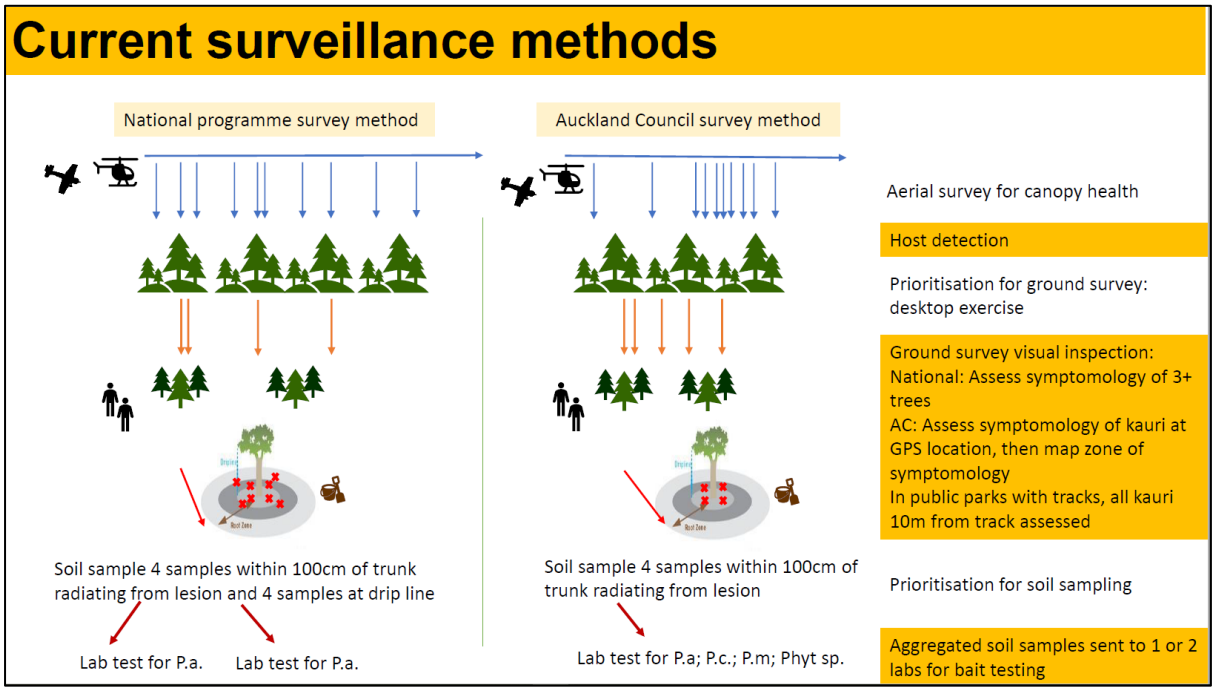

Figure 4-6: Kauri dieback programme surveillance methods as at 2019

Note: The eighth cardinal point method (4 + 4 samples) used in the National Programme was developed by Dick and Bellgard (2012). P.a = *Phytophthora agathidicida*; P.c = *Phytophthora cinnamomi*; P. m = *Phytophthora multivora*; Phyt sp. = Other *Phytophthora* species.

Image: K Froud, C Green and Y C Chin

4.3.3 LESION SAMPLING FOR PATHOGEN DETECTION

As early as 2008, it was suggested that DNA diagnostics could be useful but would likely be less cost effective than classical techniques of lesion sampling (Kauri Dieback TAG1 2008). Preliminary evidence showed that *Phytophthora*-specific antibody lateral flow devices (LFDs) could be effective, because *Phytophthora* from kauri lesions at the Huia (Waitākere Ranges) site had been detected using LFDs, which were isolated as *P. agathidicida* (Kauri Dieback TAG1 2008). This technique was further discussed in Beever et al. (2010) as a good screening test for which tissue samples should be prioritised for rapid plating for species detection. By the third TAG (Kauri Dieback TAG3 2010) tissue

sampling was discussed as an alternative to soil sampling, which was relatively unreliable at that point.

The focus on soil sampling was due to several factors, the first was the need to detect *P. agathidicida* from areas where trees were not showing symptoms or basal lesions. Scientists, KDP members and landowners were also concerned about the invasive nature of lesion sampling and the possible tree damage when taking a sample. These concerns were raised at the first 2008 TAG meeting questioning if lesion tissue samples may be an issue (Kauri Dieback TAG1 2008). The 2010 detection research, however, used both lesion and soil testing, including on Public Conservation Land under a Department of Conservation High Impact Research and Collection Permit (number # NO-27331-Res).

During Auckland Council sampling from 2008 to 2013, private landowners objected to lesion sampling, raising concerns of introducing infection. This in turn reduced the number of tissue samples taken (Waipara et al. 2013). Despite the minimal use of tissue sampling during this period, both LFD screening and tissue sampling were recommended as complementary diagnostic tools to soil sampling (Waipara et al. 2013). Further research to optimise tissue sampling was funded and resulted in reductions in the size of the incision from 5–10 centimetres down to 1–2 centimetres. Unpublished results indicated a small excision of around 1 square centimetre taken from freshly forming lesions on the leading edge was sufficient for in-field testing of *Phytophthora* detection using LFDs, and this could be directly isolated in the field (N Waipara, pers. comm., 2019). Figure 4-7 shows positions on a kauri dieback-infected tree where lesion excision is best done. This methodology would reduce the risks associated with soil contamination of sampling equipment and reduce the volume of test material. The main advantage of this method is confirmation of *P. agathidicida* status for sites with kauri trees exhibiting dieback. However, this test is not appropriate for screening surveillance of areas to determine pathogen freedom because it is not feasible to test asymptomatic trees without trunk lesions.

Enzyme-linked immunosorbent assays (ELISA) can have a role in rapid diagnostics of *Phytophthora* pathogens in direct association with symptoms and lesions, as a quick confirmation of the likely cause of infection. However, they are known to cross-react with other microbial species, and resin-laden material from kauri could confound the test (Scott et al. 2015). Further investigation using ELISA is being carried out under the KDP-funded Alternative Host Project led by Scion. As with all other current diagnostic methods, issues are involved with not knowing the sensitivity and specificity of the test in detecting *P. agathidicida* if it is present in the forest.

Figure 4-7: Kauri dieback basal trunk lesion, showing ideal lesion excision points

4.3.4 DNA AND OTHER DIAGNOSTIC TEST DEVELOPMENT FOR PATHOGEN DETECTION

Manaaki Whenua – Landcare Research developed a quantitative qPCR DNA PaqMan tool (Than et al. 2013) that was reported to improve the laboratory sensitivity and specificity of *P. agathidicida* detection from soil samples especially when both baiting and RT-PCR were used in series (Bellgard et al. 2013). The laboratory test performance for the RT-PCR was compared with the soil bioassay and had a diagnostic sensitivity of 75 percent and a diagnostic specificity of 68.8 percent (Bellgard et al. 2013). However, this assumed that the soil bioassay was a gold standard test and represented true disease status in the samples.

It is known that the soil bioassay does not have 100 percent sensitivity or specificity and so the calculation for the laboratory test performance is likely to be lower than that stated, as is the soil bioassay. Test performance is discussed in detail in Section 4.5.

The RT-PCR tool was also compared with the soil bioassay by McDougal et al. (2014) and again found to be similar in performance to the soil test. While the RT-PCR performs similarly to the soil bioassay, DNA-based tests were not incorporated into the revised soil surveillance guidelines in 2016 because of “inconsistent results with the bioassay” (Beauchamp 2014, 2016). It is possible the combined use of the soil bioassay and the RT-PCR test could have improved overall test performance for detection, but confidence issues remain in not understanding the true test performance.

Singh et al. (2017) tested qPCR and found it performed better than the soil baiting and culturing test. They have developed and tested a DNA sequence analysis tool to confirm qPCR results faster than culturing and morphological analysis. The biggest advantage in using qPCR is a shortened time between sampling and results (Barnwal et al. 2013). Cost comparisons by Singh et al. (2017) excluded staff costs so are not sufficient for determining if the qPCR method is more cost efficient. qPCR is being further investigated under the KDP-funded Alternative Host Project to find a real-time, cost-effective and accurate tool to use in nursery situations.

A loop-mediated isothermal amplification (LAMP) assay recently performed significantly better at detecting *P. agathidicida* on soil baited cedar baits than the culturing test, detecting *P. agathidicida* in five out of six versus one out of six soil baiting samples (Winkworth et al. 2020). The LAMP assay had higher laboratory-based sensitivity than the soil baiting method, as assessed by Winkworth et al. (2020), which investigated the limit of detection within a positive sample. The LAMP assay has advantages over common soil bioassays because it is likely to be more accurate, cost effective and quicker in producing results. LAMP testing could potentially be portable and conducted at the local level without involving a laboratory set up (Stan Bellgard, BioSense, pers. comm., 24 April 2020). It is important to note that this is not at all related to the calculation of field test sensitivity and specificity, which was not assessed.

The main point for all these DNA tools is that soil sampling using the existing protocol is still needed and, therefore, the inherent issues with soil collection, not understanding test sensitivity and specificity, hygiene and sample handling remain.

From the first TAG meeting, water detection of *P. agathidicida* was recommended for research to see if it could be used as a catchment-wide surveillance tool (Kauri Dieback TAG1 2008), which has been successful for other species of *Phytophthora (Sims et al. 2015)*. Auckland Council supported a Master's thesis that attempted to detect *Phytophthora* species within five streams in the Waitākere Ranges (Randall 2011). Randall (2011) deployed leaf baits into streams located within five catchments (two at Piha, two at Cascades and one at Nihotupu) for two weeks on seven occasions, roughly two months apart from October 2009 to November 2010. Each bait bag contained 10 leaves each of rhododendron, pittosporum, cedar, lupin and kauri (Randall 2011). Six *Phytophthora* species were detected throughout the study: *P. multivora*, *P. gonapodyides*, *P. kernoviae*, *P. aspargi*, *P.* taxon "*pg chlamydo*" (now known as *P. chlamydospora* (Hansen et al. 2015) and *P.* sp. "Waitakere" (still unnamed) (Randall 2011). Notably, neither *P. cinnamomi* nor *P. agathidicida* were detected during the research, despite soil sampling evidence that both were present in the catchment areas where the stream baiting was undertaken (Randall 2011). In addition, Randall (2011) used stream water filtration to detect *Phytophthora* but found it had a lower detection efficacy than baiting. The Randall (2011) study was comprehensive and the results show that *P. agathidicida* is not readily detected in stream water using the methods applied. Recent research in New Zealand has been successful in detecting *P. agathidicida* from water samples collected from streams (R Winkworth, Massey University, pers. comm., 22 July 2020), which indicates that water surveillance may have potential.

The use of metabarcoding to detect multiple species of *Phytophthora* has not been well demonstrated yet in New Zealand for *P. agathidicida*, although work has been done both on soil and in water (Stuart Fraser, Scion, pers. comm., 22 July 2020). This technique has been highly effective in

other countries for detecting and mitigating the risk of pathogenic *Phytophthora* species (Khaliq et al. 2018; Riddell et al. 2019; Green et al. 2020). The method still requires soil sampling, but it is at a lower volume than the existing KDP soil sampling protocol and has a higher sensitivity than baiting, for example, the Green et al (2020) study in the United Kingdom used four soil auger samples that were 2 centimetres wide x 30 centimetres deep, collected within 1 metre of the tree. The authors are using the same techniques to sample irrigation water in UK nurseries for biosecurity risk *Phytophthoras*, which could also be applied in New Zealand to identify risk and manage spread (Forest Research 2018). In fact, Scion, as part of the KDP-funded Alterative Host Project, tests *Phytophthora* presence in irrigation water in its nursery. Further research was recommended to the SSAG, in response to a request for review of the Kauri Dieback Science Plan (Kauri Dieback Strategic Science Advisory Group 2018), by Sarah Green from Forest Research in the United Kingdom.

Potential options for further research include use of metabolite profiling and detector dogs. Development of a leaf-based assay using metabolite profiling (metabolomics) that can be used to rapidly diagnose if a kauri tree is infected by *Phytophthora* has been explored, but unfortunately, due to funding constraints, it did not progress beyond the concept stage (T Ashcroft, pers. comm., 2020). The theory was that diseases cause disruption of biochemical pathways of the host organism, which change its metabolite profile (S Villas-Boas, pers. comm., February 2018). Metabolomics, is therefore a powerful post-genomics tool in biomarker discovery. The benefits of using a metabolomics approach in finding biomarkers include: its potential to increase test sensitivity, cost effectiveness and ability to detect infection at an early stage (S Villas-Boas, pers. comm. 23 February 2018). The Heathy Trees, Healthy Future Programme (run by Scion and co-funded by MBIE and the KDP), undertook metabolite profiling research to gain a better understanding of how *P. agathidicida* infects kauri and the changes in chemical compounds upon infection. Unfortunately funding of the programme finished in September 2019, and more research is required to explore the use of metabolomics as an early detection tool.

Preliminary studies on detector dogs showed good laboratory-based differentiation between *P. agathidicida* and two species of *Phytophthora* that are co-located in the field, namely *P. cinnamomi* and *P. multivora* (Bassett & Auckland Council Biosecurity 2016). The assessment was based on inoculated oat grains in small glass jars (including an uninoculated control) and undertaken in a confined containment facility (Bassett & Auckland Council Biosecurity 2016). A Labrador-breed dog performed at 87 percent correct identification of the *P. agathidicida* samples on its first attempt and achieved 100 percent detection on its second attempt, and its ability to ignore negative samples was 96 percent (Bassett & Auckland Council Biosecurity 2016). Issues were encountered with early intervention by the assessor on incorrect samples where concern was expressed that the dog was "trying it on", and time was not given to clarify if it was a strong positive signal or not. A second dog, which was trained in Argentine ant detection, outperformed the Labrador (Y C Chew, pers. comm., 2020) but no results were included in the report. The second stage of the pilot tried using inoculated seedlings to provide more complexity, but the dog was disinterested in the seedling trials, and it was concluded that the confined space (required to contain an unwanted organism) and repetition was not stimulating for the dog to focus on the task. The potential remains to progress this detection method, however, it is costly to train a dog and containment requirements would need to be addressed. Auckland Council is continuing with this research. Dog detection of *P. agathidicida* in soil in the field would reduce the risk of root damage during soil collection and save laboratory costs,

dogs could also be used to monitor compliance and efficacy of boot cleaning stations and in nurseries or at the ports of arrival.

4.4 KAURI DIEBACK (DISEASE) DETECTION

The symptoms of kauri dieback (bleeding lesions on the basal trunk or roots (Figure 4-7), canopy thinning (Figure 4-8 4-8), yellowing foliage and tree death (Beever et al. 2009)) may be caused by other biotic or abiotic agents, but experienced observers improve detection likelihood (Waipara et al. 2013).

Aerial surveillance started in 2011 and has been optimised over time (Jamieson et al. 2014; Macdonald 2016b) using a combination of canopy symptoms to detect suspected kauri dieback, followed by ground truthing using visual observation of canopy and basal trunk lesions and soil sampling, if required. For KDP sampling, ground truthing is undertaken if the site meets priority criteria, which include disease history, symptomology and forward vector risk of the site (Beauchamp 2015). Ground truth soil sampling either adheres to the eight cardinal point method with samples sent to two laboratories (KDP standard) or to a subset of four soil samples close to the trunk sent to one laboratory (Auckland Council standard) (see Figure 4-6).

Research into wide-scale disease detection has found that wide-area oblique aerial photography provides an accurate capture of kauri crown health at a point in time (Macdonald 2016b) but the data analysis is time consuming.

The KDP, together with several other organisations, invested in remote sensing for kauri stress detection (along with the previously mentioned host detection in Section 4.2). The objective was to remotely identify kauri trees with stress symptoms in the canopy to inform the possible presence of kauri dieback disease over a wide landscape scale (Meiforth 2018). An important factor in remote detection of tree stress is it is not specifically detecting kauri dieback. Meiforth et al. (2020) clearly state that stress symptoms can have multiple causes, including drought, insects, other diseases, difficult growing conditions with shallow soil and salt exposure from the ocean, which can be both independent causes of stress and additional stress factors that can exacerbate kauri dieback.
The research is based on airborne LiDAR data, a WorldView02 satellite image and an AISA Fenix hyperspectral image. The reference data was based on fieldwork and aerial image interpretation for over 3,800 tree crown positions (Meiforth 2018). The canopy condition of kauri crowns was classified into the same five crown symptom classes as used for KDP surveillance (1 to 5; see Figure 3-1) (Meiforth 2018).

The study focused on three sites within the Waitākere Ranges: the Cascades (10.5 square kilometres), Maungaroa (6.5 square kilometres) and Kauri Grove (1 square kilometre), which were visited between 2016 and 2017 (Meiforth 2018). The study's main purpose was to use hyperspectral data, which is expensive to acquire but gave 352 spectral bands over narrow swaths, to identify the four-to-six most important spectral bands for identifying healthy and stressed kauri trees (Meiforth 2018). The selected bands can then be used in multispectral sensors on aircraft that have a wide swath width and are cheaper to use to acquire data (Meiforth et al. 2020). In addition, the performance of a WorldView02 satellite image and LiDAR data were tested for stress detection.

Meiforth (2018) investigated the optimal spectral index combinations from AISA Fenix hyperspectral images to characterise visible stress symptoms in kauri canopies within forests by distinguishing the classes “kauri”, “dead/dying trees” and “other” canopy vegetation.

While the best bands for kauri identification were located in the NIR2 spectral region, stress detection only required standard bands in the visible NIR1 spectral range up to 970 nm (Meiforth et al. 2020). A comparison analysis of stress detection between all methods found that (airborne) multispectral data performed better (the correlation coefficient was 0.91 for all crowns and 0.93 for crowns larger than 3 metres in diameter) than satellite data (0.85 for crowns larger than 3 metres in diameter). Meiforth (2020) concluded that stress monitoring could be based on satellite data, such as the WorldView02 image, as long as small crowns (less than 4 metres in diameter) are analysed in homogenous forest segments (that is, kauri stand segments and not individual rickers). Despite a lower correlation, the satellite data may still be a low-cost option for long-term monitoring of significant changes over time.

The six spectral index combinations identified in the study are suitable for large area monitoring of kauri health (Meiforth et al. 2020), and this research is ready for implementation. Remote sensing (either multispectral or satellite) could be incorporated into a surveillance sampling frame where only individual trees or stands of rickers greater than 4 metres in diameter are included in the sample selection for remote assessment (and ground truthing, if required). This would be suitable for most population-level research questions related to kauri dieback disease impacts, risk factors and efficacy of mitigation measures, because these are important tree age and stand classes. However, results of such studies may not be suitable to be extrapolated to individual rickers in mixed canopy forest or to understorey or sub-canopy kauri seedlings or saplings, and different ground-based study designs would be required to assess the effects over time on these host population classes.

The use of higher spatial resolution and smaller bandwidth of more expensive airborne multispectral data, which improves stress detection and allows for smaller crown sizes (J Meiforth, pers. comm., 2020), could be used for smaller area studies on rickers. Future research is recommended to compare the performance of selected bands from the hyperspectral sensor against a four-band HiRAMS sensor from airborne multispectral data acquired in March 2019 along with an aerial image (J Meiforth, pers. comm., 2020).

Only minor improvements in correlation were made when LiDAR data was used in combination with airborne multispectral imaging (from 0.91 to 0.92), and from 0.89 to 0.92 when LiDAR data was added to WorldView02 satellite images (for crowns greater than 4 metres in diameter) (Meiforth 2018, 2020). These minor improvements were outweighed by higher processing effort and cost (Meiforth 2018, 2020).

Another finding by Meiforth (2018) was that aerial images were better than ground truthing for assigning canopy classes for the reference data set in forest stands with high trees and dense undergrowth. However, the aerial image needs to have a pixel size of less than or equal to 15 centimetres, defined bandwidths, match the same season and be properly spatially aligned to the other remote sensing data (J Meiforth, pers. comm., 2020). It would be useful to record in the kauri dieback surveillance data whether ground or aerial observation (or both) was used to classify canopy symptoms.

In summary, multispectral, satellite and wide-area oblique imaging could potentially be used in a future monitoring programme both for host and tree health detection. The final results from research by Meiforth et al. (2020) are being assessed to determine how best to apply them to KDP operational objectives, and a workshop to progress them was held in Auckland in September 2019. The priority recommendation from the workshop was to collect baseline data over all kauri forests (pilot over the Hunua Ranges and Waipoua Forest) and across different ecosystem compositions, for example, tānekaha or beech. This is consistent with Meiforth et al. (2019) important research gap for this technology, which was to validate it more widely in other forest types outside the Waitākere Ranges to measure the level of variability and differentiate other non-kauri species from kauri that were not captured in the research. Meiforth also suggests new microsatellites could provide a lower-cost alternative to the WorldView02 satellite data because microsatellites feature a red-edge band in addition to the standard red-green-blue and NIR bands (J Meiforth, pers. comm. 2020, Source: Planet-Labs-Inc. Planet imagery product specifications).

Figure 4-8: Canopy thinning, a symptom of kauri dieback, caused by *Phytophthora agathidicida*

Source: K Froud, August 2019, photo taken from the Huia Dam, Waitākere Ranges, Auckland

4.5 SURVEILLANCE TEST PERFORMANCE

In 2016, the KDP requested additional research into determining the test performance of soil samples by calculating test sensitivity and specificity based on existing surveillance data (Cogger et al. 2016).

Sensitivity refers to the proportion of sites **with** *P. agathidicida* present that test positive and specificity is the proportion of sites **without** *P. agathidicida* present that test negative (Cogger et al. 2016). The research results showed that test performance was not able to be calculated with the existing data, and a recommendation was made to undertake additional surveillance specifically to calculate these values (Cogger et al. 2016).

For the purpose of managing kauri dieback-free areas, test performance research was initiated in 2019 to address the ongoing concern about the inability to prove an area was free of *P. agathidicida*. A scoping exercise was conducted to determine what was required to calculate test performance of the soil bioassay and remote sensing (Vallee & Cogger 2019; Vallee et al. 2019). The second stage of undertaking sampling to calculate test performance has not progressed due to funding constraints, but it is considered a priority research area. This knowledge gap was identified at the first TAG meeting in 2008 (Kauri Dieback TAG1 2008) and has been referenced by several sources as a research priority (Black and Dickie, 2016; (Kauri Dieback Strategic Science Advisory Group 2018).

Key to any freedom from disease survey is the ability to calculate the sample size required to have confidence in freedom, and this calculation requires sensitivity and specificity values for test performance. It is important to note that test performance is the measurement of how the entire test methodology performs, from sample collection to laboratory confirmation, not how well a test performs in the laboratory compared with controls. That is, is the pathogen present in the site sampled versus is the pathogen present in the sample collected?

A simple way to think about test performance is by asking the question, is the test valid? Cameron et al. (2014 Pg 33) state:

> The validity of a test is the probability that it will get the classification correct. Validity is expressed in terms of sensitivity and specificity.

Test sensitivity and specificity with regard to *P. agathidicida* and kauri dieback were defined by Stevenson and Froud (2020 Pg 21) as:

Sensitivity (Se) Proportion of trees with the disease that will test positive

i.e.

$$\frac{True\ positives}{True\ positives + false\ negatives}$$

Where false negatives are trees that test negative but do have disease. Highly sensitive tests can be used to rule-out disease because they will have few or no false negatives e.g. if we fail to detect *P. agathidicida* from the leading edge of a fresh lesion where the lateral flow device has indicated phytophthora, it is most likely that it truly isn't *P. agathidicida*. Less sensitive tests such as soil samples may fail to detect *P. agathidicida* even when it is present. Typically, if a test has high sensitivity it will have lower specificity (i.e. you will find almost all cases of disease (high Se), but you will also call lots of things diseased that are not (low Sp).

Specificity (Sp) Proportion of healthy trees that will test negative

i.e.

$$\frac{True\ negatives}{True\ negatives + false\ positives}$$

Where false positives are trees that test positive but do not have disease. Highly specific tests will have very few or no false positives e.g. if we detect *P. agathidicida* in a soil sample using culture and sequencing it is almost certainly *P. agathidicida*. Less specific tests may detect '*P. agathidicida'* but actually be a cross-reaction detecting a different species of *Phytophthora*. Typically, if a test has high specificity it will have lower sensitivity (i.e. the cases you find are truly diseased, but you will miss quite a few cases of disease).

For the purposes of detecting *P. agathidicida* using the KDP's current soil testing methods, sensitivity is the probability that a tree infected by *P. agathidicida* will be identified as positive by the soil test. This is also referred to as the true positive rate. Whereas, the specificity is the probability that a non-diseased tree will not test positive for *P. agathidicida*. Because a method is used that cultures the pathogen, it is considered there is a high specificity, that is *P. agathidicida* will not be isolated and morphologically identified if it is not there (the probability of false positives). Although it is possible that an inexperienced diagnostician may misclassify a different species of Phytophthora as *P. agathidicida*, resulting in a false positive. What is less certain is if it will be able to be detected it if it is there (the probability of false negatives, test sensitivity), and therefore will it be erroneously stated that an infected tree or site is negative?

The KDP is aware of this risk, and all negative soil samples are classified as "Not Detected" to indicate the uncertainty. Figure 4-9 shows the differences between tests that have 100 percent sensitivity compared with tests with 100 percent specificity. Typically, a trade-off occurs between high sensitivity versus high specificity tests, and a 100 percent valid (100 percent Se and 100 percent Sp) test is not realistic (Lalkhen & McCluskey 2008). High sensitivity tests are effective as a screening tool, where a wide net needs to be cast to make sure cases are not missed, whereas high specificity tests are effective as confirmatory tools, applied to samples that have been identified as positive during screening (Lalkhen & McCluskey 2008; Cogger et al. 2016).

Stage 1 of the KDP research into evaluating the test performance of the standard surveillance method has been completed, and the statistical priors required for Bayesian analysis of test performance have been collected from diagnostic and detection experts (E Vallee, pers. comm., 2019). From this, researchers have designed a plan to obtain the posterior values from field testing in areas of high and low disease prevalence (Vallee et al. 2019). Because this requires many hundreds of soil samples, the KDP is currently unable to fund this research (T Ashcroft, pers. comm., 2020). In the future, as additional diagnostic technologies are implemented or developed, understanding how new tests perform (that is, the sensitivity and specificity of the tests) and comparing them with the existing tests will be essential and a high research priority. For example, the use of DNA-based methods will likely have a higher sensitivity and lower specificity than a culture test, with a higher probability of false positives. High sensitivity and lower specificity in a test could have significant impacts on land management agencies and land managers (A Beauchamp, pers. comm., 2020) because *P. agathidicida* is an "unwanted organism" under the Biosecurity Act 1993 and no person shall knowingly spread (termed communicate) the organism (under sections 52 and 53 of the Biosecurity Act 1993). Therefore, a test with high sensitivity and lower specificity may lead to costly actions and obligations where none may be warranted, if the original diagnosis was a false positive.

Having values for sensitivity and specificity enables test results to be assessed appropriately. For example, any DNA test positive should be assessed based on the expected disease prevalence in an area and the number of samples taken and could be combined with a high specificity test, for example, culture to validate a result. This is important in situations where a *P. agathidicida* detection is found in a new area.

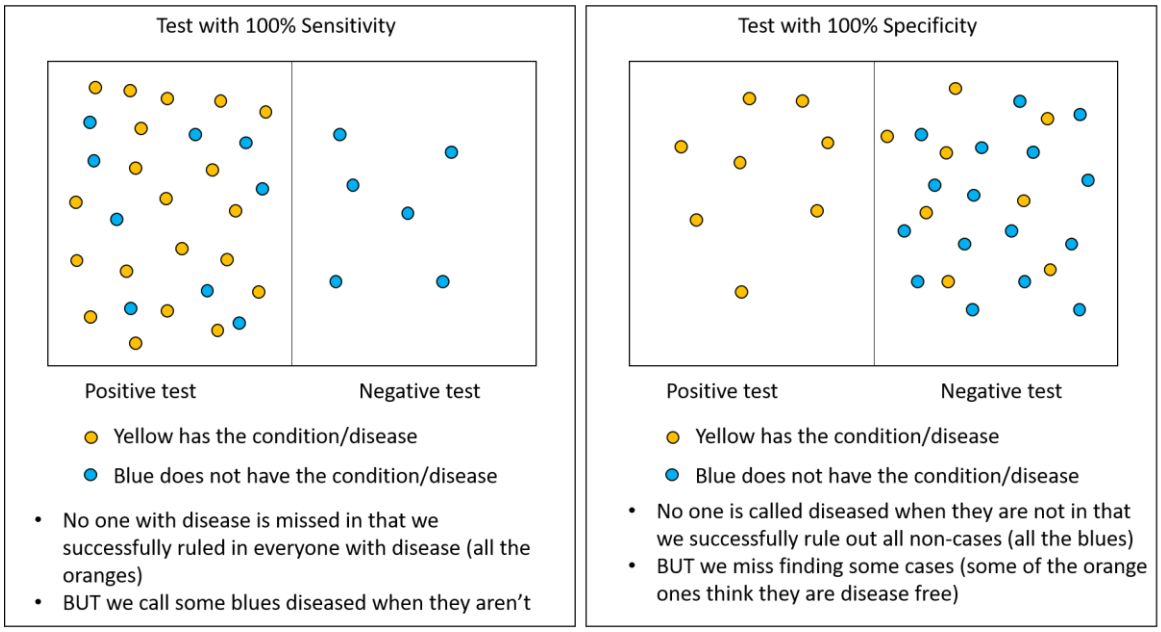

Figure 4-9: Diagram of test performance showing tests with either 100 percent sensitivity or 100 percent specificity

The Rapid Implementation Group under the Biological Heritage National Science Challenge Ngā Rākau Taketake programme stated that diagnostic test evaluation was essential for underpinning long-term monitoring of impacts, to compare current, unimplemented and future diagnostic methods and to inform confidence of the current distribution of disease, including areas of disease freedom (Froud et al. 2019).

4.6 FUTURE SURVEILLANCE AND MONITORING

The surveillance research gaps identified by the SSAG were prioritised into the Kauri Dieback Science Plan in 2018 (Kauri Dieback Strategic Science Advisory Group 2018). In 2019, the Ngā Rākau Taketake programme under the Biological Heritage National Science Challenge, reviewed the of research to date and work in progress against the priorities in the science plan and provided the following recommendations for priority research gaps for surveillance (Froud et al. 2019):

> "We need to work with agencies and communities that are implementing surveillance and determine their data and communication needs, particularly around spatial reporting to enable management of their Ngahere. A spatially based long-term monitoring and surveillance system needs to be designed, based on detecting areas currently free of PA pathogen and/or disease to enable protection zones, and monitoring the change of disease incidence and pathogen spread over time, and impacts on tree and forest health. This needs to incorporate statistically robust sample sizes, based on known diagnostic test performance."

> "We need to include long-term impact factors and disease factors postulated by scientists and Mātauranga Māori into the monitoring and surveillance design, collected from diseased and non-diseased trees and areas, along with spatially referenced baseline population density and current interventions (location of track upgrades etc). We need to reclassify all existing surveillance data against a single set of criteria so that we can map and analyse this data, and work with agencies to see what can be publicly shared with land managers and how best to do that. Then we need to work with land managers to identify areas where insufficient surveillance has been done to inform decisions. Key to all this is working out how to report consistent surveillance results across agencies and partners to enable land management decisions. This needs to be co-designed with these partners. This will underpin future analysis of surveillance data to assess existing interventions, roll out treatments and identify other factors that could be manipulated to reduce disease impacts. Note some of this work will enable assessment of natural spread through spatial analysis of topography, soil, vegetation etc."

These priorities all remain, and research on a mātauranga Māori focused approach to surveillance in the future is under way (Waitangi Woods, pers. comm., 2019). In addition, the Cultural Health Indicators Monitoring Framework developed (Chetham & Shortland 2013) is being tested by selected mana whenua communities in kauri lands. This framework, underpinned by te ao Māori, lets mana whenua assess and measure the health, resilience and disease status of kauri forests using cultural indicators.

4.7 SURVEILLANCE RECOMMENDATIONS

- It is recommended a review be done of the results from all forms of surveillance for *P. agathidicida*, kauri dieback (disease symptoms) and hosts, that the data be collated in full into a single geospatial relational database and appropriate cultural approval is sought to use the data.
- The case definition used for each surveillance activity needs to be clearly stated.
- A more informative map that includes *P. agathidicida* sites and kauri dieback cases should be produced, at a scale that lets researchers and stakeholders immediately understand the risk that kauri dieback poses to New Zealand's kauri forests.
- Reclassification is recommended of where *P. agathidicida* positive sites are in comparison with where kauri dieback (disease) has been observed, using existing surveillance data consistent with the proposed case definitions of Stevenson and Froud (2019).
- Further research needs to be done on the biological factors that contribute to breaking dormancy of oospores and how this influences diagnostics.
- The proposed test performance research needs to be done, to obtain the sensitivity and specificity values from field testing in areas of high and low disease prevalence. Assessment of test sensitivity and specificity should be included in the development of any new methods and provided for existing methods, so methods can be applied, and results interpreted appropriately.
- The potential for metabarcoding should be investigated as a rapid detection method for *P. agathidicida* in soil, root samples and irrigation water.

- Options for an in-expensive remote disease detection methodology should be investigated by integrating the implementation of aerial, multispectral, satellite, vertical (helicopter) and oblique angle photography methods for canopy health assessment.
- Healthy kauri forests with disease undetected need to be identified and research should be done to clarify whether *P. agathidicida* is present, even where disease is not observed, and prioritise for protection.
- The potential to use metabolite profiling as an early detection tool should be investigated.

5 PATHWAYS AND VECTORS

5.1 OVERVIEW

The KDP is working on a precautionary principle that *P. agathidicida* is new to New Zealand and therefore its spread is a significant concern. Localised spread of *P. agathidicida* was investigated by Gadgil in 1974 and other researchers more recently. Natural spread rate was calculated at the Great Barrier Island site as around 3 metres per annum (Beever et al. 2009). Gadgil (1974) found that *P. agathidicida* could be isolated from the soil and infected tissues (trunk and root lesions) from symptomatic trees and from soil and roots taken from asymptomatic trees around 4 kilometres away from the affected site. The main vector mechanism for *P. agathidicida*, a soil-borne pathogen, is soil and soil–water contaminated with zoospores or the long-lived oospores (Beever et al. 2009).

Section 1.3 of the Kauri Dieback Science Plan (Kauri Dieback Strategic Science Advisory Group 2018) identifies understanding vectors to inform management as a priority research area. It states (p 10):

- "Determine the level of risk associated with key vectors, including different types of …(human)… users. Determine the primary and secondary vectors. Determine what vectors can realistically be managed. Identify and assess rural and urban vectors.
- Role of nurseries, especially in spread in terms of revegetation. Can we certify that materials are pathogen free to inform management?
- Mode of natural spread: Mechanisms and rates across landscapes, topography, soil types, vegetation types, etc."

This section reviews the current knowledge of pathways and vectors and concentrates mainly on soil because this is a soil-borne pathogen. Foliage and seed cones are not known to contain *P. agathidicida*, however, the pathogen has been detected from roots, soil and the lower trunk of infected trees (in basal lesions) (Beever et al. 2010; Bellgard et al. 2013).

5.2 HUMAN FOOTWEAR VECTORING

The fourth TAG considered vector-related spread of *P. agathidicida* and general consensus was reached that humans move soil and soil spreads pathogens. A recommendation was made to assume that humans are a vector and measures to stop human-vectored spread was required (Kauri Dieback TAG4 2010).

The KDP webpage states:

> "By far the greatest amount of movement of the disease is attributed to human activities. A number of observational studies imply that the movement of contaminated soil on people and associated vehicles & equipment, represent the greatest risk of spread. Given the high frequency of visitors to the forest and the type of activities being carried out, increases the likelihood of large amounts of contaminated soil being removed inadvertently from within and between kauri forests."

And:

> "Risk is proportional to the volume of soil moved and the frequency and distance of movement. The relative importance of these various pathways will be proportional to the volume of soil moved and the frequency and distance of such movement"

A small study by Pau'uvale et al. (2011) isolated several species of *Phytophthora* (not *P. agathidicida)* from two boot-wash stations in the Waitākere Ranges and from fresh soil on boots from a Waitākere track, which confirmed *Phytophthora* species could be picked up and potentially transferred to other sites, with the assumed implication that *P. agathidicida* could also be transmitted in this manner. Ian Horner (pers. comm. to the KDP Planning and Intelligence Team) has successfully isolated *P. agathidicida* from boots worn in an infected area during sampling. While the Pau'uvale et al. (2011) study did not directly show *P. agathidicida* contamination, the isolation report from Ian Horner provides sufficient evidence of contamination. In addition, while no direct evidence is available of transmission of *P. agathidicida* or other *Phytophthora* species in New Zealand via contaminated footwear, evidence is available of transmission for other *Phytophthora* and soil-borne pathogen species worldwide, so it is appropriate to assume this is likely and would apply to *P. agathidicida*. Examples include *P. kernoviae* and *P. ramorum* in the United Kingdom (Webber & Rose 2008; Elliot et al. 2015).

The observational studies that show a correlation between walking tracks and kauri dieback include several surveillance reports (Dick & Bellgard 2010; Hill et al. 2017a). The first KDP surveillance programme, referred to as Surveillance One, was a small survey to determine the presence of *P. agathidicida* in forests. The survey's design was based on the assumption that *P. agathidicida* was present in New Zealand in the 1950s (www.kauridieback.co.nz/science-and-research/understanding-the-disease/) and that initial spread was human-mediated because an unquantified correlation has been observed between *P. agathidicida* detection and proximity to tracks and roads (Dick & Bellgard 2010). This correlation may be directly causal in that the pathogen was vectored by humans or may be confounded by kauri root damage during track construction, which contributed to disease development from an existing reservoir of *P. agathidicida* in the soil. The survey strategy relied on proximity to tracks or roads, which excluded the option of analysing the correlation of disease with track proximity because no comparison data was available for trees or sites not exposed to tracks (Cogger et al. 2016). The Waitākere Ranges surveillance also indicated an increased risk of kauri dieback within 50 metres of walking tracks and in association with bait lines and waterways (Hill et al. 2017a), further statistical analysis is needed, however, to fully quantify the increased risk.

It is recommended the relative risk of human vectoring via footwear is robustly quantified to fully evaluate the risks associated with human vectors and inform mitigation. Methods to assess this are provided in the (Stevenson & Froud 2020) baseline monitoring report. The following information is required to quantify track network risk.

		Disease (kauri dieback)	
		Yes	No
Risk (exposure)	Yes	a – Number of kauri dieback cases (confirmed, probable and suspected) within 50 m of a track	b – Number of kauri dieback non-cases (not diseased) within 50 m of a track

		Disease (kauri dieback)	
		Yes	No
	No	c – Number of kauri dieback cases (confirmed, probable and suspected) greater than 50 m away from a track	d – Number of kauri dieback non-cases (not diseased) greater than 50 m away from a track

$$Where\ the\ odds\ ratio = \frac{a/b\ (odds\ of\ the\ disease\ in\ the\ risk\ exposed\ group)}{c/d\ (odds\ of\ the\ disease\ in\ the\ non\ exposed\ group)}$$

For example, using dummy data and calculating 95 percent confidence intervals for apples having cases of bite marks within 50 metres of a school playground:

		Apple bite mark cases	
		Yes	No
Risk exposure of within 50 m of a school playground	Yes	135	1,660
	No	25	720

$$Odds\ ratio = \frac{0.08}{0.03} = 2.67\ (1.52 - 3.62\ ;95\%\ CIs)$$

This would be interpreted as:

> Apples within 50 metres of a school playground are around 2.7 times as likely to have bite marks compared with those that are further away from a school playground and the true risk lies between 1.5 times and 3.6 times as likely.

So risk can be quantified, it is recommended future surveillance activities include the measurement of cases within the non-exposed group (c + d in the table above), along with any other variables that could potentially confound this association.

5.3 KAURI WOOD

Attempts to clarify "where in the wood" *P. agathidicida* was present, to determine the vector risk associated with cultural harvest and arborist activities of infected trees, proved difficult to undertake. Research done on a single, recently dead, kauri tree with *P. agathidicida* (confirmed from soil samples) had wood discs cut every 2 metres and samples collected from the bark, cambium, resin and wood layers (Scott et al. 2015). The researchers found that *P. agathidicida* was detected in only 2 of 41 wood samples, taken from the collar directly below the resin layer (Scott et al. 2015). It was determined that the results were inconclusive and chemical properties within the wood may have affected the processing of samples (Scott et al. 2015). Isolations by Beever et al. (2010) from xylem associated with basal lesions also produced *P. agathidicida*, however, the data is insufficient

to state that *P. agathidicida* is absent from timber wood with lesions in the lower trunk. This is discussed further in Section 7.4 about best practice guidelines for kauri tree removal and pruning.

5.4 NURSERIES AND FORESTRY PLANTATIONS

While specific research into quantifying the risk of nurseries in the spread of *P. agathidicida* has not been funded, the fact that they represent a risk pathway is of significant concern. Evidence from Europe shows nurseries are a significant vector of *Phytophthora* into native areas (Jung et al. 2016). *P. agathidicida* has high pathogenicity on kauri seedlings (Gadgil 1974; Beever et al. 2010; Horner & Hough 2013b), however, indications show variability in host susceptibility (discussed fully in the control section (Section 6)). In addition, alternative hosts may harbour *P. agathidicida* (discussed in Section 3.2) so the risk of transmitting *P. agathidicida* through a range of native seedlings, including kauri, for restoration projects is considered feasible.

The 2020 introduction of the Plant Production Biosecurity Scheme, led by the New Zealand Plant Producers Incorporated (NZPPI), with core nursery hygiene standards (NZPPI 2020a) and a specific *Phytophthora* module and *P. agathidicida* schedule for kauri dieback, once fully adopted, will mitigate future risks of nursery-related vectoring (NZPPI 2020b).

An extensive investigation by Beachman (2017) looked at the historical role New Zealand Forest Service (NZFS) kauri management activities may have had in both the introduction and vectored spread of *P. agathidicida* into and between forests in New Zealand. As mentioned in section 3.1, Beachman (2017) found no evidence to support the introduction of *P. agathidicida* into New Zealand on *Agathis*, *Araucaria* and *Phyllocladus* seeds imported from 1940–1952. However, Beachman (2017) suggests that earlier activities may have been implicated in the introduction and subsequent spread of *P. agathidicida*, such as European kauri gum collection from root extraction and tree trunk notching that started around 1840, extensive logging (in Waipoua Forest) up until 1952 and the development of the state highway (12) through Waipoua Forest in the late 1920s.

Beachman (2017) concluded no link existed between the extensive practice of tree stand improvement, where non-timber trees were managed to favour straight, and improved growth of kauri and other native timber and the presence of kauri dieback disease. Nor was any correlation seen between the Sweetwater Nursery, engineering works undertaken by the NZFS (roading, quarrying and land clearance) or NZFS staff rotations and the presence of kauri dieback. Of interest is the note Beachman makes that, due to the lack of evidence of spread through these means, it may be more difficult to spread *P. agathidicida* than previously thought.

Beachman (2017) found a correlation between Waipoua Nursery seedling batches from 1953–1956 and four-to-five sites that are known to be affected by kauri dieback. However, many other sites showed no such correlation, so the link may be circumstantial. An excellent outcome of Beachman (2017) search of the historical archives is a Geographic Information System record for many of the historical sites, including kauri plantations and nurseries (Figure 5-1), which has been incorporated as a layer in the KDP geospatial database. Each site, represented as a polygon or point location, has information on the area size, year of operation, number of plants planted or produced and the source or area the plants (and approximate numbers) were sent to. This information could help in trace-back or trace-forward investigations (Macdonald 2019a).

In the future, once the baseline surveillance methodology is standardised, and test sensitivity and specificity are well understood, the presence and/or absence of *P. agathidicida* and analysis of isolate sequences could be undertaken at these and other sites (a comparison group). This work would help quantify the risk of large-scale forestry management and provide more evidence for mitigation measures if necessary.

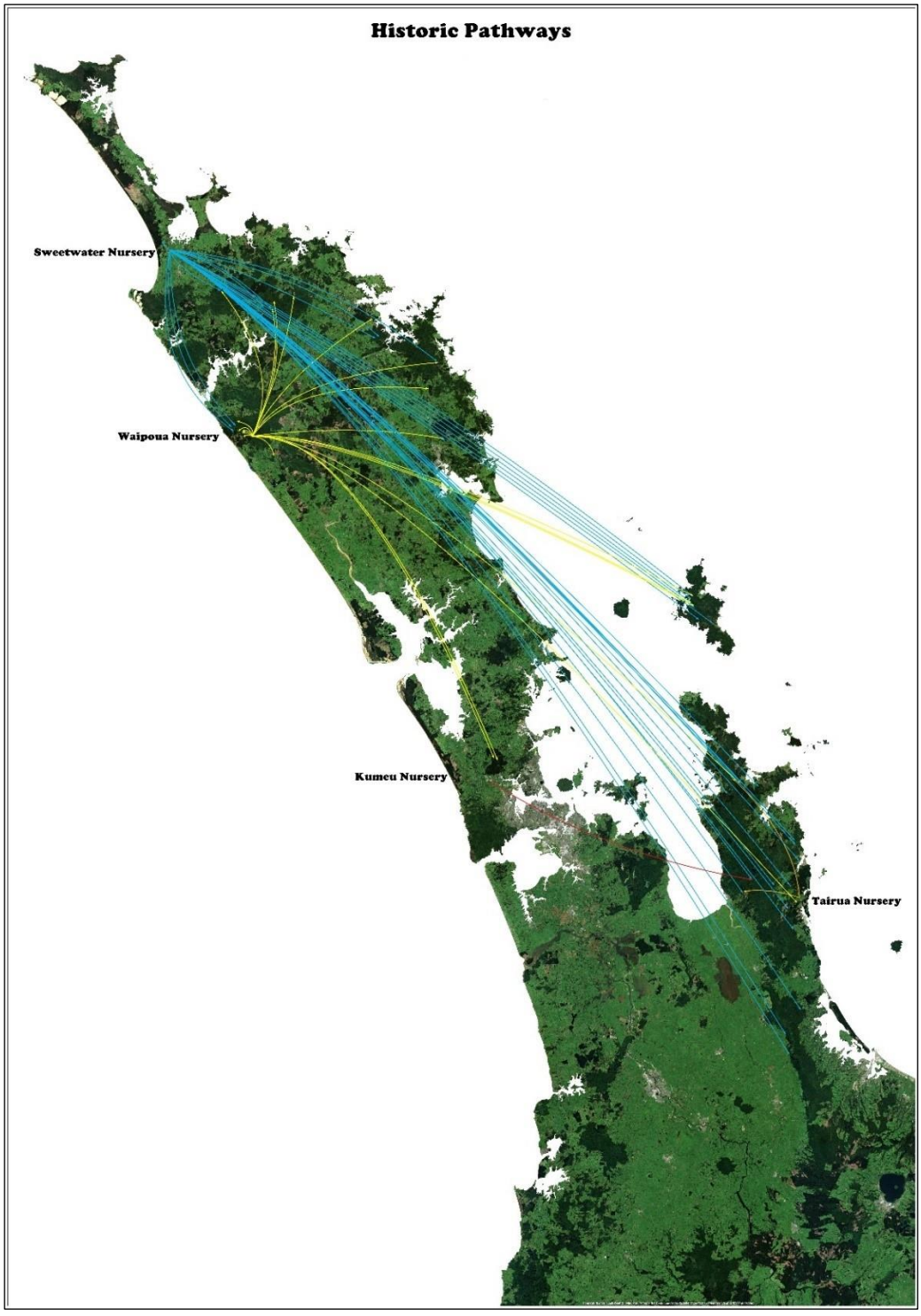

Figure 5-1: Map depicting the main New Zealand Forestry Service kauri nurseries and distribution of kauri seedlings to plantation sites

Image: Biospatial Ltd.

5.5 ANIMAL VECTORS

Understanding the vectors of long-distance spread of *P. agathidicida* was identified as a priority for the management of kauri dieback. Although this was very human-centric for the first few years of the KDP, minor mentions of animals were made as potential sources of infection. The surveillance form developed by Dick and Bellgard (2010) included observations of soil disturbance from pigs, cattle and other animals.

The first specific mention of research for animal vectors was at the fourth TAG meeting in reference to a student at Auckland University who was investigating pigs (Kauri Dieback TAG4 2010) In 2011, investigating animal vectors was noted as "may be required" when research priorities were being identified (Beachman et al. 2011).

Podger and Newhook (1971) suggested the extensive distribution of *P. cinnamomi* in New Zealand was helped by pigs and other cloven-hoofed feral animals, such as goats and deer. The KDP has also been concerned that unfenced stock (cattle and sheep) and feral animals, such as goats and particularly pigs, can spread *P. agathidicida* on soil and cause root damage to kauri. Other animal vectors have also been mentioned, such as earthworms, kauri snails, kiwi, other birds, rats and possums, but were considered lower risk (Kauri Dieback TAG4 2010). During the second round of kauri dieback surveillance, *P. agathidicida* was detected in soil from a cattle race in Kaiwaka, Northland (Beauchamp 2013a). Also, Northland Regional Council has observed evidence of cattle presence and damage in forest areas outside of or some distance from paddocks and has extracted *P. agathidicida* positive sample results from these locations (G Clapperton, pers. comm., 2020). The practice of under-grazing kauri forest remnants on farms puts cattle vectoring high on the KDP's list of concerns (A Beauchamp, pers. comm., 2020).

To date, only pigs have been researched because of a longstanding concern they are a significant vector of *P. agathidicida*, along with being one of the main species of large vertebrates, in kauri forests (Bassett et al. 2017). Several suggested mechanisms through which pigs can affect the development of kauri dieback are:

1) through direct transfer of external soil contamination;
2) through ingestion and excretion of viable spores;
3) through damage to fine feeder roots caused by rooting and trampling;
4) through an attraction for rubbing against bleeding lesions;
5) through enabling prolonged root wetness through flooding of wallowing pits next to kauri trees.

Direct transfer and spread of *P. agathidicida* via soil adhered to pigs was investigated by screening soil from the snout and trotters of 457 pigs from the Waitākere Ranges (Krull et al. 2013). The authors detected *P. cinnamomi* washed from a trotter sample and multiple other pathogens but did not detect *P. agathidicida* from any of the samples. However, due to poor test sensitivity, a sample size of over 1,000 was required to be able to reliably claim that *P. agathidicida* would not be detected from pigs (Krull et al. 2013). Krull et al. (2013) stated that, while *P. agathidicida* was not detected, they were unable to rule out pigs as likely vectors and concluded, based on circumstantial evidence, they probably could vector *P. agathidicida*.

Bassett et al. (2017) have tested the ingestion and excretion theory in research conducted on captive pigs and assessed the stomach contents of feral pigs from the Waitākere Ranges culled between 2008–2011. Bassett et al. (2017) were able to extract *P. cinnamomi* and several other *Phytophthora* species from 184 feral pigs, confirming Podger and Newhook's (1971) theory, but they did not detect *P. agathidicida* in any samples. In addition, faecal samples of 12 pigs fed with *P. agathidicida* on kauri roots, oats and millet resulted in only one detection of viable *P. agathidicida* from a small kauri root 24 hours after ingestion (Bassett et al. 2017). This is in contrast to the successful recovery of viable *P. cinnamomi* from pig faeces in Australian trials using a similar methodology (Li et al. 2014).

The conclusion from these trials was that, although there was a proof of concept that pigs could spread *P. agathidicida* through ingestion and excretion of kauri roots, it was likely to be a minor vector pathway in comparison with other *Phytophthora* species such as *P. cinnamomi* (Bassett et al. 2017).

Both the Krull et al. (2013) and Bassett et al. (2017) studies provide little evidence that *P. agathidicida* is vectored externally or internally by pigs. However, based on both expert opinion (from a range of researchers) and previous research proving that pigs are an important vector of other *Phytophthora* species, information that pigs are a vector for *P. agathidicida* is presented in several websites and articles:

RNZ (3 July 2018) *Kauri dieback in Waipoua Forest a 'tragedy' – scientist*. www.rnz.co.nz/news/national/360955/kauri-dieback-in-waipoua-forest-a-tragedy-scientist. Accessed 2 June 2020.

Barton, M; Waipara, N; Craw, J (December 2007) *Kauri dieback science frequently asked questions*. New Zealand Plant Conservation Network. http://nzpcn.org.nz/publications/Kauri-Dieback-Science-FAQ_dec17.pdf. Accessed 2 June 2020.

Forest and Bird (1 May 2018*) Kauri dieback: why it matters*. www.forestandbird.org.nz/campaigns/kauri-dieback. Accessed 2 June 2020.

Biosecurity New Zealand (16 September 2019) *Protection and response: kauri dieback*. www.mpi.govt.nz/protection-and-response/long-term-pest-management/kauri-dieback/. Accessed 2 June 2020.

Wikipedia (10 August 2020) *Kauri dieback*. https://en.wikipedia.org/wiki/Kauri_dieback. Accessed 2 June 2020.

Root and basal trunk damage caused by pig wallowing and rooting has been observed (**Error! Reference source not found.** and Figure 5-23) and recorded during kauri dieback surveillance. However, the association between pig damage and disease was not able to be assessed, nor was the presence and/or absence of cattle, because of a lack of comparison data (Cogger et al. 2016).

Figure 5-2: Evidence of pig wallowing next to a kauri tree

Photo: Ian Horner, Plant and Food Research Ltd

Figure 5-2: Evidence of pig damage to a kauri tree trunk

Photo: Ian Horner, Plant and Food Research Ltd

The contrast between vector success of *P. cinnamomi* (clade 7) compared with *P. agathidicida* (clade 5) is interesting. It is possible both pathogens have had similar incursion periods of several hundred years, yet the distribution and abundance of *P. cinnamomi* is much greater than *P. agathidicida* (Krull et al. 2013). Although the components of this are not well understood, it could be due to effective vectoring on soil associated with humans, animals, birds and invertebrates (Keast & Walsh 1979; Krull et al. 2013); a much wider host range (Studholme et al. 2016); and a wider temperature tolerance of *P. cinnamomi* compared with *P. agathidicida* (Bassett et al. 2017).

As identified by Black and Dickie (2016), a significant knowledge gap remains between the assumption that pigs are contributing to the infection, spread and severity of disease and the ability to prove an association. Further research is required to provide evidence robust enough to get support to actively manage feral pigs for the purposes of reducing the impact and spread of kauri

dieback (Black & Dickie 2016). The recommendations made by Cogger et al. (2016) to record the absence as well as presence of pig signs (and all other ecological factors) remain important if future analysis on an association is to be attempted. The following statement was made during the fourth TAG meeting where *P. agathidicida* vectoring was first discussed in depth:

> "Need to learn how the hunters feel about the issue and why before you can hope to change their behaviour" (Kauri Dieback TAG4 2010)

The KDP has long recognised the socio-cultural implications of eradicating pigs, and this statement has been echoed throughout the programme and within the research community with limited social science to test these concerns. The removal of damaging invasive vertebrate species from native forest may enhance the resiliency of the forest and kauri, and a lack of compelling evidence for vectoring should not undermine control of pigs and other vertebrates. Research is recommended into the social and cultural desirability of vertebrate control and stock exclusion in the context of possible kauri forest loss. Evaluation is also needed of the comparative risk of vertebrate pest control operations (off-track hunting) versus vertebrate pests as potential vectors of *P. agathidicida* spread.

5.6 ROOT-TO-ROOT TRANSFER

TAG members suggested *P. agathidicida* may be root transferred between trees (Kauri Dieback TAG1 2008) and research was needed to understand the temporal component of root-to-root transfer. This research area was not invested in and is probably biologically unimportant because the motility of the pathogen is at a similar scale as root-to-root transmission. Although motility is potentially constrained by water-flow dynamics and therefore up-slope spread may be enhanced by root grafting and root-to-root transmission. Recent non-programme funded research showed that a single kauri tree-stump in the Auckland region was being supported through root grafting to a nearby living kauri tree (Bader & Leuzinger 2019). While this individual case study shows a clear physiological association within the two trees, no evidence was observed of a physical root graft or for pathogen transmission between trees, or that root grafting is a common phenomenon within kauri trees.

Ecroyd (1982) mentions that a colleague (I L Barton) observed kauri stumps surviving following selective logging, indicating root grafting with adjacent trees, and Bergin and Steward (2004) state that fusion of large lateral roots is seen in mature trees and roots occasionally graft with neighbouring trees and stumps. Subsequently, Steward and Beveridge (2010) state that root grafting is presumed to occur, citing Beddie (1942) who mentions kauri root grafting is "strongly suspected" but has no definite proof. A brief mention is made in a 1944 Botanical Society bulletin that kauri root grafts "were specifically mentioned" during a talk on the vegetation of the Waitākere Ranges (Hillary 1944). No evidence is provided for these early reports. Currently, root grafting has been suggested by multiple authors, observed but not formally published by several authors and physiologically, but not physically, shown in one case (Bader & Leuzinger 2019). On balance, given its common occurrence in many tree species, and unpublished observations, it is likely to occur occasionally in kauri. No evidence exists, however, that it is biologically important as a means of spread, in comparison with spread through zoospores to nearby overlapping root structures. If root grafting

does occur it may have minor implications for kauri dieback management, such as transmission of phosphite between trees (Horner 2016a) and minor localised uphill transmission.

5.7 KNOWLEDGE GAPS FOR SURVEILLANCE

Clarification of whether *P. agathidicida* is ubiquitous and present at undetectable levels where disease is not observed remains a fundamentally important research gap for the management of kauri dieback. This knowledge has been identified in the Kauri Dieback Science Plan (Kauri Dieback Strategic Science Advisory Group 2018):

> "Many of the current Kauri Dieback Programme management tactics (boot sanitation, track closure, pig control, etc) were developed on the assumption that the pathogen is relatively new and has a discrete distribution. Addressing this fundamental assumption will inform the future direction of the Kauri Dieback Programme, i.e., whether we will invest in pathogen containment or put effort into improved forest health and control tools."

The wide and patchy distribution of *P. agathidicida* in New Zealand indicates both localised and long-distance spread patterns typical of many plant disease outbreaks (for example, Rosanowski et al. (2013)). Clade grouping research by Winkworth et al (R Winkworth, pers. comm., 2020) and further work on 12 whole genome sequences of *P. agathidicida* (R Bradshaw, pers. comm., 2020) may provide more evidence of the timeframe for *P. agathidicida* introduction. Results from the whole genome sequences to date indicate a low level of diversity (R Bradshaw, pers. comm., 2020 referring to Guo et al, 2020, In press), which is similar to that seen for the mtDNA. These results are consistent with slow natural spread and limited long-distance spread rather than an endemic, ubiquitous distribution, but this is not the only possible interpretation (R Winkworth, pers. comm., 2020). Therefore, the precautionary approach of managing long-distance spread mechanisms and pathways remains important. The KDP has developed several risk-based and science-led best practice guidelines to lessen pathway and vector spread risks, and these are discussed in Chapter 7, Decision support.

5.7.1 SIGNIFICANT RESEARCH GAPS

The following points have been noted as significant research gaps:

- quantify the risk of human and invasive pig vectoring of *P. agathidicida* using proximity to track networks and pig density counts against kauri dieback cases and non-cases;
- investigate the efficacy and uptake of vector mitigation measures (for example, hygiene, track closures, stock exclusion, best practice guidelines and pest control) and the impact of these measures on the distribution and severity of disease;
- research the social and cultural desirability of vertebrate control and stock exclusion in the context of possible kauri forest loss;
- research low-risk pest control options to reduce human-mediated spread of *P. agathidicida* (for example, through off-track activities such as bait lines or pig hunting).

6 CONTROLLING THE DISEASE – PHOSPHITE, HYGIENE AND OOSPORE CONTROL, BIOLOGICAL CONTROL, GENETIC RESISTANCE AND/OR TOLERANCE

6.1 PHOSPHITE

Phosphite (also known as phosphorous acid) refers to the salts of phosphonic acid (H_3PO_3). It is a systemic fungicide that is translocated through both phloem and xylem vessels (Hardy et al. 2001). Phosphite works both as a direct inhibitor of pathogen growth and indirectly by stimulating host defence responses that inhibit pathogen growth (Hardy et al. 2001).

6.1.1 LABORATORY TRIALS 2011

The potential application of phosphite was discussed at the first TAG meeting in 2008, and investigations into injection or aerial sprays on kauri were recommended based on successful use against *Phytophthora* infections in horticulture (Kauri Dieback TAG1 2008). The need for this research was reiterated in the second TAG meeting and identified as a priority for funding, especially because of the long-term nature of such trials (Kauri Dieback TAG2 2010). The KDP started funding phosphite control trials in late 2010 and early 2011. The initial trials investigated the efficacy of phosphite *in vitro* and in seedlings. The *in vitro* results were promising, with significant inhibition of *P. agathidicida* mycelial growth and a delay in sporulation on media with phosphite, which showed a clear dose response and a greater response than other *Phytophthora* that are commonly controlled by phosphite (Horner & Hough 2011; Horner & Hough 2013b).

Main finding: Phosphite is active against *P. agathidicida in vitro*.

6.1.2 GLASSHOUSE TRIALS 2011

Following the success of the *in vitro* studies, glasshouse seedling tests were undertaken to determine the efficacy of phosphite on stem- or soil-inoculated *P. agathidicida* in two-year old seedlings (Horner & Hough 2013b). Stem injection of phosphite had 100 percent survival of seedlings and high efficacy against lesion development, foliage and root symptoms (Horner & Hough 2013b). In comparison, phosphite soil drench had 45 percent survival, phosphite spray had 20 percent survival and the fungicide Ridomil® (metalaxyl granules) had 30 percent survival, all of which were better than untreated controls at 0 percent survival after 20 weeks (Horner & Hough 2011; Horner & Hough 2013b).

The issue with a lack of efficacy in using phosphite sprays, may be due to a lack of penetration of the phosphite into the upper surface of the leaves. Horgan (2017) found a higher uptake of phosphite occurred through the lower leaf surface of kauri, with negligible uptake into the upper leaf. Even if further research proved successful, the difficulty in operationalising the research and the risk of non-target impacts due to spray drift are factors that need to be considered.

The use of phosphite plugs in Australia to combat *P. cinnamomi* has been discussed by the KDP as a non-invasive way to administer phosphite into trees. However, this line of enquiry has not been explored further and may be worth future investigation.

Main finding: Stem injections of phosphite have high efficacy against *P. agathidicida* infection in stems and roots in glasshouse seedlings. Phosphite soil drench and phosphite sprays had low efficacy.

6.1.3 FIELD TRIALS – PHOSPHITE RATE TRIALS 2012

Trials to develop a control protocol that carefully balanced efficacy with phytotoxicity for ricker trees were the next step for phosphite research. Horner and Hough (2011) recommended caution when transferring glasshouse results to the forest environment and suggested a staged approach. First to determine ricker tree tolerance to different rates of phosphite in the absence of disease and, second, to investigate phosphite efficacy in diseased ricker trees in a confirmed *P. agathidicida* site (Horner & Hough 2011).

In January 2012, trials were done to determine a 'safe' rate to avoid phytotoxicity of phosphite injections into trunks of healthy trees. Researchers selected two sites (Waipoua Forest and Huia, in the Waitākere Ranges) and used phosphite concentrations of 7.5 percent, 10 percent, 15 percent, 20 percent and a maximum dose of 60 percent (undiluted) at trunk injection spacings of 20 centimetres, 30 centimetres or 40 centimetres on healthy trees (Horner & Hough 2014b). Their results showed no obvious phytotoxic effects after two-to-three weeks. At the 6 week, 8 week and 20 week assessments, however, phytotoxicity symptoms of leaf yellowing, leaf drop and small branch drop were observed, particularly above a high rate of 20 percent phosphite (Horner & Hough 2013a, 2014b). Tree health improved within 12 to 18 months post-treatment and, after two years, green canopies and new growth were evident (Horner & Hough 2013a, 2014b). The typical field rate of phosphite used in New Zealand horticulture is 15 percent per 1 metre canopy diameter, with evenly spaced trunk injections (Horner & Hough 2014b). However, as a result of the observed phytotoxic effects during the rate trials, Horner and Hough (2014b) recommended that 7.5 percent phosphite be adopted for future field trials on diseased trees and that rates be optimised in the future.

Main finding: Trunk injections of phosphite have the potential to cause phytotoxicity to healthy kauri at field rates of more than 10 percent. A 7.5 percent field rate at a 20 centimetre injection spacing is recommended.

6.1.4 FIELD TRIALS – PHOSPHITE FOREST TRIALS ON RICKER TREES 2012–2017

The first phosphite field trials on diseased trees (that is, symptomatic trees at *P. agathidicida* positive sites) were on ricker-sized stands in the Waitākere Ranges (Huia and Whatipu) in January 2012 (Horner & Hough 2013a). Two rates were used, a high 20 percent rate and low 7.5 percent rate, all at 20 centimetre injection intervals, some with repeat 7.5 percent injections 12 months later (Horner & Hough 2013a).

Treatments were evenly stratified against differing disease severity, and trials were assessed based on canopy health and lesion dimension and activity (Horner & Hough 2013a, 2014b). As seen with the rate trials, trees treated with 20 percent phosphite showed phytotoxicity and the higher rate was dropped for the two Northland forest replicates treated in March 2012 (plantation kauri, Raetea and Omahuta forests, in the Mangamuka Ranges) and for the repeat injections for the Waitākere Ranges sites the following year (Horner & Hough 2013a, 2014b).

By June 2014, in addition to the phytotoxicity symptoms seen in the rate trials at 20 percent, vertical bark cracks were observed in some treated trees and 12 percent of treated trees in the Waitākere Ranges replicates had died, compared with 0 percent of the control trees as of June 2014 (Horner & Hough 2014b). In comparison, no trees died at Omahuta Forest, and 0 percent of treated trees and 29 percent of untreated trees in the Raetea Forest had died (Horner & Hough 2014b). Horner and Hough (2014b) noted that trees that died were in an advanced state of disease and typically (but not all) received the higher rate of phosphite and so suggested treatment may have accelerated their decline.

Canopy health in most cases continued to decline slightly (including the untreated controls) except at the Omahuta site, where no decline occurred in the treated trees but did occur in the untreated trees (Horner & Hough 2014b). In contrast, a reduction in lesion activity and lesion dimension values was significantly better for treated trees, compared with untreated controls at all sites (Horner & Hough 2014b). A pilot twig assay for assessing phosphite efficacy was not successful nor was a leaf and twig assay refinement study aimed at detecting biologically active concentrations of phosphite to guide reapplication times (Horner & Hough 2017).

Despite advanced disease (canopy scores 3+) tree death, phytotoxicity effects and canopy decline, results from the initial phosphite trials showed promise for disease suppression, specifically for lesion suppression (Horner & Hough 2014b; Horner et al. 2015). The trials raised questions around operational implementation of the control tool for larger trees, for trees with advanced disease, use of lower rates and differing injection frequencies, and timing of treatment to optimise the use of phosphite (Horner & Hough 2014b).

These trees have continued to be assessed for disease suppression, and the latest results (July 2017) show all injection points and lesions of surviving treated trees have healed and, in most cases, the bark has peeled off to display clean bark beneath (Horner et al. 2017a). In contrast, most lesions on untreated control trees continued to advance, although a few healed (Horner et al. 2017a). The canopy scores of untreated trees continued to decline, and for most treated trees an initial decline was obvious after one year, followed by stable canopy scores over the five-year trial, with some improvement on individual trees, especially in the Northland sites (Horner et al. 2017a). It is not known how long after treatment the canopy score will remain unimproved on heavily diseased trees, despite lesion healing (Horner et al. 2017a). This is important to consider when designing baseline monitoring using aerial canopy health scores to investigate impacts of interventions.

Due to issues with phytotoxicity and trunk cracking, the 7.5 percent at 20 centimetre intervals treatment is difficult to extrapolate to larger trees and further research, specifically on large trees, was recommended (Horner et al. 2017a).

Main findings: Low rate trunk injections of phosphite have high efficacy for lesion suppression in naturally infected ricker trees in the forest. The risk that treatment may accelerate death in advanced disease trees is outweighed by the fact these trees would have died eventually in the absence of treatment and in the benefit of saving less severe trees. A significant delay occurs in canopy recovery, which may affect baseline comparisons of interventions that are canopy disease-severity based.

6.1.5 FIELD TRIALS – PHOSPHITE FOREST TRIALS ON LARGE TREES 2016–2020

The main objective of the KDP and proposed National (Kauri Dieback) Pest Management Plan is to protect kauri trees and stands with special values from kauri dieback (Kauri Dieback Governance Group 2019). Following on from the success of lesion suppression in ricker trees using phosphite, the KDP funded investigations into treating large trees with phosphite. Due to phytotoxicity issues with ricker-sized trees, lower-dose research was required to determine a safe dosage of phosphite to treat large and iconic trees.

The large tree trials started in 2016 on 42 diseased trees at three sites (Cascades in the Waitākere Ranges (n=18), Puketotora Road, Kerikeri (n=9) and Trounson Kauri Park in Northland (n=15)) on trees with a trunk diameter ranging between 0.4 metres and 2.4 metres (Horner & Arnet 2019). To avoid the risk of phytotoxicity and possible tree death, a low rate of 4 percent phosphite was used at 40 centimetre and 80 centimetre injection spacings around the trunk, treatments were stratified against disease severity and assessed every six months (Horner & Arnet 2019). Treatments were repeated for the 80 centimetre and 40 centimetre spacings in 2018 and 2019, respectively, following discussion with the KDP (Horner & Arnet 2019).

After three years of assessments, interim results show no signs of phytotoxicity (Horner & Arnet 2019), indicating that 4 percent phosphite was below the rate required for visible phytotoxicity. Several treated and untreated control trees died at the Cascades, Waitākere Ranges, site and two trees had reduced canopies, one treated, one not, at the Puketotora Road and Trounson Kauri Park sites respectively (Horner & Arnet 2019). A trend could be seen towards less activity in lesions in treated trees compared with untreated controls 12 to 18 months after the first treatment, however, lesions had not fully healed at any site, prompting a recommendation for a second treatment (Horner & Arnet 2019). A second round of treatment was applied at Puketotora Road in 2018 and in April 2019 at the other sites and, within two months, a noticeable improvement could be seen in lesion reduction (Horner & Arnet 2019).

Eight months after the second application, a decrease has occurred in the number of active lesions in treated trees across all sites and lesion activity has reduced, although canopy health shows no major changes (Horner & Arnet 2020). In untreated control trees, on average, lesion activity has remained similar with no changes in canopy health, and additional trials with higher rates (4 percent) at smaller injection intervals (20 centimetres) are recommended (Horner & Arnet 2020).

The KDP Planning and Intelligence Team identified several remaining knowledge gaps for large tree phosphite injections:

- We do not know if phosphite reaches all parts of the tree or more importantly whether it reaches the root system, where *P. agathidicida* first infects kauri.
- If phosphite does not reach the roots in sufficient concentrations, then efficacy may be low.
- Undertaking root assays may inform translocation of phosphite to roots and inform when phosphite injections should be reapplied to maintain efficacy.
- Research to trial a higher concentration of phosphite is required.
- A pre-emptive treatment, where the objective is prevention compared with mitigation, may prove more cost effective over the long term, and observational research into a phosphite barrier approach (injecting all kauri regardless of symptomology) is recommended.

Main findings: Very low rate single trunk injections of phosphite have some limited efficacy for lesion suppression in infected large trees in the forest. A second application of phosphite showed improved results, but it is too soon to confirm this. No canopy recovery occurred within the period assessed to date. Increased rates at reduced injection intervals were recommended for future research. This research is ongoing, however, there is sufficient data to show that repeated low rate injections are unlikely to kill large trees in comparison with *P. agathidicida* (which may kill an infected tree). Attempts to save large iconic or highly valued trees using phosphite injections could be undertaken, even in the absence of finalised results, while bearing in mind the risk of success is uncertain.

6.1.6 FIELD TRIALS – PHOSPHITE LOW RATE AND TRUNK SPRAYS 2016–2019

Due to phytotoxicity concerns from the ricker field trials using 7.5 percent and 20 percent phosphite rates from 2012–2015, the KDP funded additional research into lower rates for injection as well as investigating the efficacy of phosphite as a spray on trunk lesions. Trials at two Dargaville sites and one site at Huia (Waitākere Ranges) were set up involving 72 advanced rickerearly mature-sized trees (Horner et al. 2019a). In March 2016, phosphite injection rates of 4 percent at 20 centimetres and 40 centimetres, the standard 7.5 percent at 20 centimetres and 10 percent trunk sprays (with and without bark penetrant) were applied (Horner et al. 2019a). As with previous phosphite field trials, treatments were randomly assigned to trees stratified by disease severity (Horner et al. 2017b). After three years of observations, six trees have died, evenly spread between untreated and trunk-treated trees across two sites (Huia and one Dargaville site), whereas no injected trees have died (Horner et al. 2019a). No canopy phytotoxicity was observed but the trunk-sprayed trees exhibited peeling of bark and the injected trees had minor bleeding and “stretch marks” (Horner et al. 2019a).

While some lesion reduction was observed for trunk-spray treated trees, it was not consistent nor sufficient to show efficacy after the first application, however, lesions did improve after a second application in early 2018 (Horner et al. 2019a). The lack of effectiveness may be due to the phosphite formulation and/or the surfactant being used as a spray (Plant Protection Chemistry NZ 2015; Horner et al. 2019a). Horner et al. (2019a) indicate that a different surfactant that is more compatible with the current phosphite formulation could be used in future research and note that trunk sprays could be a useful tool for superficial trunk lesions. However, the biology of infection suggests that basal trunk lesions are unlikely to be superficial because infection is through the roots not the trunk (Bellgard et al. 2013) and there is no evidence of systemic movement of phosphite in the area where trunk sprays are applied (Horner et al. 2019a). Exploring improved uptake by using another surfactant and/or phosphite formulation may, however, be worthwhile, because this is a much less invasive treatment than trunk injection.

The lower rate 4 percent phosphite injections at 20 centimetre intervals showed similar efficacy to the standard 7.5 percent rate after three years and indicate that lower rates may be sufficient to suppress lesions, the wider intervals also showed efficacy, which was in contrast to results from the large tree trial using the same rates and intervals (Horner et al. 2019a). The KDP is keen to continue assessment of these trials for a further two years (with the first year being funded by Auckland Council and the second year pending funding) to determine if the 4 percent rate continues to provide similar suppression to the higher rate, and to observe if stem cracking occurs. Severely

diseased trees may potentially be treated with a low rate phosphite injection initially to avoid accelerating death through phytotoxicity, followed by a higher rate a few years later to completely heal lesions.

Nothing is known about the optimal time for injecting, and whether factors such as season, time of day or weather conditions have any effect on uptake. Trials were initially done using water injections. Uptake of water showed no differences based on time of day, season, rain, tree health, tree girth or aspect of trunk injected (Horner 2016b). The research was subsequently discontinued by the KDP, due to other priorities.

Main findings: Phosphite injection of ricker trees at 4 percent at 20 centimetre intervals has efficacy for lesion suppression after three years. Trunk sprays are not favourable under the trialled formulations, but scope exists for additional surfactants to be tested to improve efficacy.

6.1.7 LANDOWNER TRIALS – PHOSPHITE CITIZEN SCIENCE

As early as 2013, questions were raised about the use of phosphite injections by private landowners, who were under pressure to save trees on their properties and were being offered a range of untested treatments by various companies and consultants (Kauri Dieback TAG5 2013). In 2017, the Biological Heritage National Science Challenge, in collaboration with councils, initiated the citizen science project, Kauri Rescue™, trialling the use of phosphite injections by the public (www.kaurirescue.org.nz). In the first two years, 50 participants were recruited into the project and a further 26 joined in 2019. The enrolment criterion was a positive soil test for *P. agathidicida*, and, to date, 1,250 trees have been treated with varying rates of phosphite under the project (Kauri Rescue 2019). Pre-application and six-monthly post-application monitoring data is being collected by participants (Horner et al. 2019c). Participants were given the option to choose the dosage (low–40 centimetre and high–25 centimetre) and concentration (6 percent or 4 percent). Where lesions were present, most went with high concentration and high rate and vice versa. Their decision was dependent on what the tree looked like instead of using the canopy score for the assessment (I Horner, pers. comm., 2019). Detection and treatment data collected in this project represents an observational dataset rather than an experimental dataset, although it is not evident whether comparison data has been collected (untreated trees). The data is yet to be thoroughly analysed (I Horner, pers. comm., 2020).

6.1.8 PHOSPHITE BARRIERS SCOPING

In 2016, the KDP commissioned a scoping report on the potential to use phosphite as a barrier treatment for managing the natural spread of *P. agathidicida* (Horner 2016a). This was the first step in operationalising the potential of phosphite to manage disease at a forest or landscape level, rather than individual tree level. The use of phosphite barriers using foliar sprays and injections to contain disease foci and prevent new incursions assumes the area has recently been infected by the pathogen and is locally isolated. As Horner (2016a) notes, the concept of phosphite barriers has been proven successful in one Australian study (Shearer et al. 2004). Several technical and financial impediments would need to be addressed for the research to be funded in New Zealand, such as the high initial cost and an estimated research length of 10 to 15 years.

The extensive site requirements listed in the scoping report indicate that investigation of phosphite barriers is not suited to an experimental trial design and using an observational study design should be considered. The scoping report (Horner 2016a) met several criteria where an observational study design is better suited than an experimental design (Froud & Cogger 2015). For example, interactions between multiple factors are of interest but are too complex to manipulate experimentally, and the sample size required to obtain statistical power is likely to be cost prohibitive. Additionally, the uncertainty around soil test performance suggests that disease front is a better measure than infection front for a large-scale long-term study in the current absence of a highly sensitive test for *P. agathidicida* in the soil.

In 2019, Auckland Council, Waikato Regional Council, iwi and private landowners (under the Kauri Rescue programme), invested in trialling the operational delivery of a mass roll out of phosphite injections in the Waitākere Ranges, Coromandel and Northland (Biosense, Unpublished data, Hill 2019) (see Figure 6-1). This roll out provides a natural experiment data set (observational data), which could be used to assess the efficacy of phosphite barriers and study the confounding factors that affect efficacy if phosphite injections were extended to asymptomatic trees in a buffer zone or if diseased control trees were left untreated.

6.1.9 OPERATIONAL USE OF PHOSPHITE

As of September 2019, Auckland Council reported that 3,700 and 7,019 kauri trees had been treated with phosphite in 2018 and 2019 respectively (P. Hulse, Auckland Council, pers. comm., 2019). In this Auckland Council funded initiative, trees within a previously identified area of high disease incidence were treated (see Figure 6-1). The trees were between 25 centimetres and 350 centimetres diameter at breast height (DBH), and most did not show basal lesions. Trees were injected with 20 millilitres of 4 percent phosphite at 25 centimetre trunk spacings. Kauri dieback symptoms and tree health data were collected for all trees, and 5 percent were selected for long-term monitoring (Auckland Council, pers. comm., 2019).

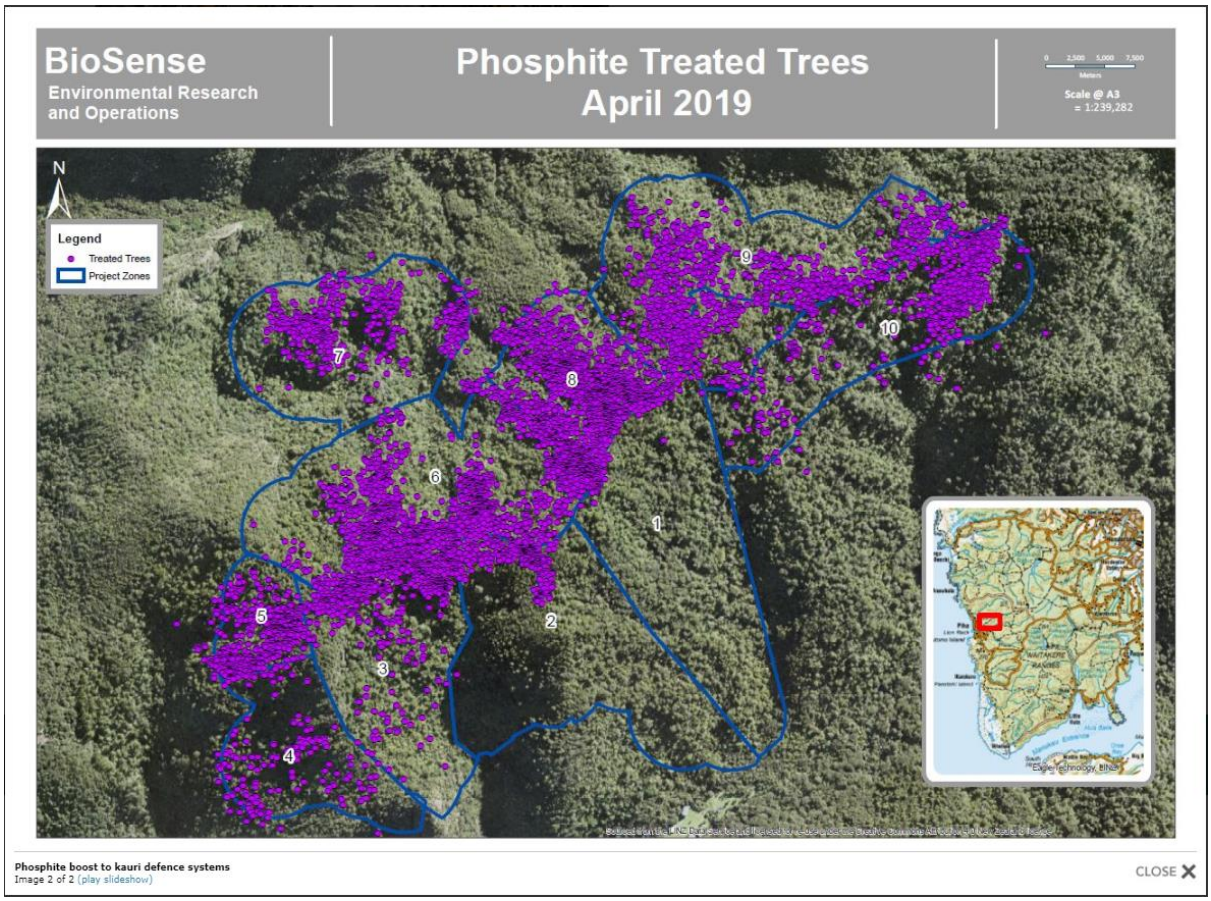

Figure 6-1: Image accessed from the piha.org.nz website showing where phosphite treatment trees are located in the Waitākere Ranges

Waikato Regional Council injected phosphite into more than 2,415 ricker and emergent kauri trees in the Whangapoua catchment in Coromandel (Hill 2019; Waikato Regional Council 2019). The phosphite delivery area was decided based on 2017 aerial imagery, where the disease front was clearly visible, and translated into a polygon for the work to be undertaken (K Parker, pers. comm., 2020). Trees were more than 25 centimetre DBH and all had visible evidence of canopy decline. Trees were injected with 20 millilitres of 4 percent phosphite at 25 centimetre trunk spacings. Kauri dieback symptoms and tree health data were collected for most trees and 275 (10 percent) were selected for long-term monitoring. No asymptomatic or symptomatic untreated trees were included in the long-term monitoring group. Their inclusion would be necessary as a comparison group, if future analysis of these treated trees is intended beyond efficacy of treatment across different disease severity. The opportunity remains to include comparison trees in the programme. In 2019, drone images were collected over the monitoring trees and surrounding areas and, in 2020, images and full motion video were captured (K Parker, pers. comm., 2020).

With the large-scale operationalisation of phosphite injections, non-target or ecosystem level impacts could occur over time as well as phosphite resistance with continued use (Scott et al. 2016). However, these will likely be limited by use of injection rather than foliar or trunk sprays but should be monitored. In addition, evidence shows that phosphite use can affect tree reproduction (Hardy et al. 2001), which has not been investigated in kauri, although the effect of *P. agathidicida* left untreated may be worse.

6.1.10 PHOSPHITE SUMMARY AND DISCUSSION

Stem cracking, frequency of injection and understanding the economic viability of large-scale treatments were identified as significant concerns for phosphite treatment by Black and Dickie (2016). Lower rate trials have shown efficacy in rickers without severe phytotoxicity issues, although observations have not been long enough to see stem cracking yet. Lower rates for large trees had no phytotoxic effects but were not enough, after one round of injections, to suppress lesions, so higher rates or repeat treatments may be required to save larger trees (Horner & Arnet 2019).

As of 2019, no economic modelling had been done of the long-term practicality and efficacy of phosphite use at the forest level, as recommended by Black and Dickie (2016), and this remains a knowledge gap. As phosphite treatments are rolled out across kauri lands, an opportunity is also available to transfer from experimental trials, to observational trials, to fine-tune rates and frequency of treatment, and, more importantly, record non-target effects. To do this, well-structured data collection should be set up immediately to monitor the pre-treatment condition of trees, post-treatment outcomes, ecological factors of kauri-associated species, along with any potential confounding variables that may affect the efficacy of phosphite treatments in the field. Another advantage of using an observational study design for phosphite treatment is to measure the treatment's impact on a large scale by a range of operators (including the public) rather than how it performs under experimental application (Froud & Cogger 2015). Completion of existing experimental research is still important and may give more certainty around rates for large trees, longevity of lesion suppression and canopy recovery.

It is still not known how long single injection phosphite control can be maintained and whether complete canopy recovery can be achieved, or what the risk of non-target impacts may be. Sufficient evidence is available, however, that 20 millilitre phosphite injections of 4 percent at 20 centimetre spacings can suppress lesions in ricker trees, and multiple treatments of phosphite of 4 percent at 40 centimetres can suppress lesions in large trees with relatively minor phytotoxicity. Potential non-target impacts may well be less than loss of the dominant tree species if no treatment is applied, and should not restrict the roll out of this tool, but should be closely monitored. Land managers should also be ready to stop use of phosphite if adverse ecological effects are observed.

6.2 HYGIENE AND OOSPORE CONTROL

6.2.1 INTRODUCTION TO OOSPORES AND HYGIENE

As mentioned in Section 3.2, oospores form in diseased plant tissue and are the main survival state for *Phytophthora* species, such as *P. agathidicida*, that do not produce chlamydospores (Ribeiro 1978; Bellgard et al. 2013). Oospores can be present on infected plant material and in decaying plant material in the soil, and, once mature, can remain dormant for months to years (Dick & Kimberley 2013). Preliminary results from Plant and Food Research indicate that the primary inoculum source of *P. agathidicida* within soil is not free-living spores but small organic root fragments (T Ashcroft, pers. comm., 2020). These results, once confirmed, will inform future research in determining the efficacy of disinfection treatments by specifically targeting the persistent inoculum source of *P. agathidicida* from naturally infested soils (T Ashcroft, pers. comm., 2020).

Of significant concern is that not all dormant oospores break dormancy and germinate when conditions are favourable (Dick & Kimberley 2013). Those that do break dormancy become part of the "infective capacity" referred to by Bellgard et al. (2009), and those that do not, may remain at risk of transfer in soil for long periods. Not only is oospore dormancy a major concern for hygiene efficacy, as mentioned in Section 4.3, it is also concerning that dormant oospores may remain within a soil sample that returns a "not detected" result following the leaf-baiting process (Kauri Dieback TAG4 2010) and so would constitute a false negative result.

The ideal hygiene product for use in the KDP is one that is effective against all life stages of *P. agathidicida*, especially oospores, and has minimal human health and environmental impacts. This is critical given the number of forest users who will be exposed to the disinfectant during the cleaning process at hygiene stations and the volume of disinfectant used in the environment.

Hygiene is defined by the *Oxford Dictionary* as*:*

> Conditions or practices conducive to maintaining health and preventing disease, especially through cleanliness.

In regard to kauri dieback, hygiene refers to the practice of removing soil and disinfecting boots, hands, tools and other items to prevent the opportunity for soil-borne infectious material (zoospores, oospores, mycelium and sporangia) to be transmitted (vectored) from one host to another (Dick & Kimberley 2013). It also includes water and plant material contamination, for example, stream water and sawdust from felled trees. An early example of *P. agathidicida* hygiene is the measures Beever et al. (2010) applied between sites during their detection research. That included applying 95 percent alcohol to chisels for lesion sampling and 2 percent TriGene™ (II) Advance to hand-trowels and footwear away from tree-root zones (Beever et al. 2010).

6.2.2 Efficacy of biocides against *Phytophthora agathidicida*

The first TAG meeting in 2008 observed that no literature was available on the efficacy of TriGene™ Advanced (marketed as SteriGENE® in New Zealand) against *P. agathidicida*, specifically the resistant spores (oospores), and recommended more research into it (Kauri Dieback TAG1 2008). At the same meeting, experts stated it was important that researchers were careful with hygiene measures, to avoid spreading *P. agathidicida*, and recommended that research protocols and appropriate techniques were required (Kauri Dieback TAG1 2008).

In 2009, Auckland Council installed hygiene stations at the start of walking tracks in the Waitākere regional parks and provided hygiene prescriptions stating people must clean shoes, tyres and equipment before entering kauri forest, and adhering soil must be cleaned after every visit (Bellgard et al. 2009). These are consistent with the current (2018) hygiene measures recommended by the KDP (www.kauridieback.co.nz/media/1857/2018-kauri-dieback-hygiene-procedures.pdf) and with agency-specific standards.

Also of note is a Better Border Biosecurity (B3) funded project where Cheah et al. (2009) looked at various disinfectants for microbial decontamination of footwear. They noted that sodium hypochlorite (bleach) out-performed Virkon® and recommended further tests specifically for *Phytophthora*.

The first KDP hygiene research looked at the efficacy of TriGene™ (II) Advance (SteriGENE®), Phytoclean™, Virkon® S, Janola® and Citricidal® on mycelium, zoospores and oospores *in vitro*, in soil and from soil on boots (Bellgard et al. 2009). The authors also investigated the infective capacity of *P. agathidicida* oospores where the infective capacity was represented by the ability of soil spiked with 2,000 oospores to germinate, form sporangia, release zoospores and colonise leaf baits (Bellgard et al. 2009). Infection occurred on 11 out of 30 (37 percent) of leaf baits before treatment (Bellgard et al. 2009). This suggests the majority of oospores remained dormant in most of these assays (Dick & Kimberley 2013).

The research results were interesting because mycelium and zoospores were well controlled, along with active oospores (defined as oospores breaking dormancy and germinating), by most treatments, except Citricidal, with TriGene and Phytoclean performing well, and most consistently, under the various treatments (Bellgard et al. 2009). However, the authors did not discuss the findings of a high percentage of viability of dormant spores across all treatments (for example, of 200 oospores, 184 remained dormant, none were active and 16 were non-viable for TriGene, compared with 158 dormant, 19 active and 23 non-viable in the control treatment). In addition, subsequent experiments (soil and boots) within the same study were dependent on measuring active zoospores in soil using a baiting assay following treatment (Bellgard et al. 2009) and did not investigate the presence of dormant oospores. Bellgard et al. (2009) recommendations were that TriGene (II) Advance was suitable for hygiene, effectively killing *P. agathidicida* propagules, and qualified this by saying it reduced the "infective capacity" of soil containing *P. agathidicida*. While these experiments indicate that mycelium, zoospores and active oospores (those that have broken their thickened cell wall to germinate) can be controlled using TriGene, they provide no evidence that dormant oospores (where the cell wall remains intact) can be controlled.

Dick and Kimberley (2013) note that zoospores, mycelium and sporangia are readily killed by biocides and disinfectants, but oospores are more challenging. Dick and Kimberley (2013) undertook the next significant KDP research into oospore hygiene measures. They investigated TriGene™(II) Advance, salt-water, fumigation (metam sodium (Fumasol®)), pH on oospores taken from three Waipoua Forest isolates in 2011. They used a tetrazolium bromide stain to show viability of oospores following treatment. Their results showed that TriGene at 2 percent (label rate), 5 percent and 10 percent for 30 to 120 minutes, and short salt-water immersions or fumigation with metam sodium, was ineffective in deactivating oospores. Solutions of different pH levels between 3 and 10 were effective only at low or high pH levels (3, 9, 10), and even then were not 100 percent effective at high levels until after 48 hours of exposure (Dick & Kimberley 2013).

Due to its high pH, crushed limestone is being used as a base substrate for tracks (termed green bridging), to help reduce the spread of *P. cinnamomi* in Australia. Given similar results were shown against *P. agathidicida* under laboratory conditions (Dick & Kimberley 2013), the KDP funded a feasibility review into further research on developing a pH tool and what barriers may impede operational implementation. Bellgard and Probst (2018) showed significant constraints were involved with using pH compounds in native forests, but limestone could potentially be investigated. In general, potential non-target impacts in a natural ecosystem were seen as a major constraint, so pH modification was considered a lower priority for further research by the KDP.

Even though TriGene (SteriGENE®) was less effective against oospores, compared with Janola or Virkon, the product is biodegradable in the soil and has minimal human health effects, compared with other products, so its application in a forest by forest users was considered to be more suitable. As a result, the KDP adopted SteriGENE® as the disinfectant of choice for use in hygiene stations.

6.2.3 HEAT TREATMENTS

It has been shown that *Phytophthora* species can be killed by exposure to high temperatures for a sufficient length of time (Bellgard et al. 2018). The heat treatment mechanism is based on intracellular cell proteins being exposed to high temperatures and breaking down, causing failure of cells and killing the organism (Lippmann et al. 1974).

Specific research into the effects of temperature on the viability and growth of *P. agathidicida* was done by Bellgard et al (2013), Dick and Kimberley (2013), Bellgard et al. (2013); Horner et al. (2019b) and Williams (2015). Bellgard et al (2013) found that the temperature for optimal growth of *P. agathidicida* was 22 degrees Celsius, with no growth occurring at 30 degrees Celsius.

Dick and Kimberley (2013) investigated heat treatments in soil and in solution on oospores taken from three Waipoua Forest isolates. They found exposure of artificially inoculated oospores to temperatures of 55 degrees Celsius or greater for four hours in solution reduced viability to extremely low levels (Dick & Kimberley 2013). When soil was exposed to wet heat at 60 degrees Celsius and 70 degrees Celsius for four hours, oospore viability was reduced by 95 percent (Dick & Kimberley 2013). In contrast, exposure to dry heat at 70 degrees Celsius for four hours resulted in only a 30 percent reduction in viability emphasising the importance of moisture in achieving the desired results (Dick & Kimberley 2013).

Williams (2015) extended the Dick and Kimberley (2013) research to review the viability assessments using additional methods and include a post-treatment storage period. The results reconfirmed Dick and Kimberley (2013) that heating above 50 degrees Celsius had the highest efficacy in deactivating oospores, and baiting was the best assessment method, in comparison with qPCR, germination and staining (Williams 2015). However, an important observation was that laboratory-cultured oospores were variable and oospores from naturally contaminated soil or plant material may respond differently (Williams 2015). Use of outdoor shower bags for soil solarisation was assessed as a proof of concept, and temperatures between 46–49 degrees Celsius were achieved when the clear side of the shower bags were exposed to the sun in summer, and were recommended for further assessment. Both the Dick and Kimberley (2013) and Williams (2015) heat-treatment research focused on short exposure times of up to 24 hours. Given that low numbers of oospores remained viable above 50 degrees Celsius (Williams 2015), further research is required to assess lethal temperature-time combinations over longer periods.

Horner et al (2019b) investigated temperature *in vitro* in a range of soil types and on inoculated kauri roots, and used germination (involving direct plating and baiting) to assess oospore viability. The results showed that exposure to 45 degrees Celsius for 4 hours; 40 degrees Celsius for 2 days or 35 degrees Celsius for 14 days, resulted in 100 percent oospore deactivation in roots (Horner et al. 2019b). In addition, exposure of artificial inoculum in different soil types (with different volumes and percentages of water content) to 50 degrees Celsius (with a 20–30 percent water content) for 72 hours reduced oospore viability to zero (Horner et al. 2019b). These results indicate that natural

oospore inoculum was more sensitive to temperature, compared with oospores produced artificially (Horner et al. 2019b). Possible reasons why natural inoculum may have less tolerance to temperature, compared with those produced *in vitro*, include lower inoculum numbers in soil; the presence of antagonistic microorganisms; and soil enzyme stimuli that may break dormancy (Dick & Kimberley 2013; Horner et al. 2019b). *P. agathidicida* oospores are present in decaying infected plant material in the soil and this suggests that results from oospores enclosed in substrate (for example, kauri roots), and naturally occurring oospores in kauri soils, are likely to be more accurate in predicting field efficacy than *in vitro* trials.

The accuracy is also uncertain of using vital stains to measure oospore viability in the artificially inoculated oospore trials conducted by Horner et al. (2019b). Other techniques, such as baiting, are likely to be more accurate (Williams 2015).

Further research into refining the temperature thresholds in the range of 35–55 degrees Celsius would be helpful. From an operational perspective, however, it would not add value if further research suggests a temperature threshold shift of ±5 degrees Celsius from current results or that exposure time is only slightly reduced or increased.

These results formed the basis for further discussion of the development of operational guidelines around heat treatment of soil, potting mix and plant material. To inform final temperature-time combinations as well as certain operational aspects of these guidelines, a literature review was undertaken (Ashcroft 2020b). Ashcroft (2020b) tabulated all the temperature results, to help develop a best practice guideline, and these are shown in Table 6-2. The results were also combined into a temperature versus time heat-treatment efficacy figure (adapted from S Bellgard and I Horner, pers. comm., 2019), with a 5 degrees Celsius safety margin, to take into consideration uncertainties in measurement and potential operational errors in attaining the correct temperature for the full period (see Figure 6-2).

Moisture can affect oospore susceptibility to heat, as shown by Dick and Kimberley (2013) and Horner et al (2019b), where wet heat and soil moisture reduced oospore viability compared with dry or a dry–medium heat. Horner et al (2019b) found that soil, with a 10 percent moisture content, had viable oospores after exposure at 50 degrees Celsius, whereas soil with 20–40 percent moisture resulted in zero viability, apart from a low survivorship in loam soil.

Results also showed that mycelium had a much narrower thermal tolerance than older cultures that contained oospores. No mycelia survived at –14 degrees Celsius for 24 hours, whereas 1 out of 10 oospores survived at –14 degrees Celsius for 48 hours, which was the lowest temperature of the trial (Bellgard et al. 2018; Horner et al. 2019b). Horner et al. (2019b) found that the lower thermal tolerance of *P. agathidicida* oospores grown on millet in soil and in inoculated kauri roots in soil was not reached at –20 degrees Celsius, whereas the upper thermal tolerance for mycelium was 35 degrees Celsius for 24 hours, 40 degrees Celsius for 24 hours and 45 degrees Celsius for 4 hours (Horner et al. 2019b). Interestingly, Horner et al. (2019b) noted isolates containing oospores treated at 35 degrees Celsius for 24 hours showed a delay in regrowth of over a week and towards the thermal extremes. The delay may indicate that those that survived remained in a dormant state during the treatment period.

Table 6-1: Temperature and time heat–treatment results from Kauri Dieback Programme-funded and other research

Temp (°C)	Time	Type of experiment	Heat source	Viability (%)	Reference
70	30 m	*In vitro* solution	Direct heat	4	Dick and Kimberley (2013)
70	1 h	In soil – Mesh	Dry	18	Dick and Kimberley (2013)
70	1 h	In soil – Mesh	Steam	10	Dick and Kimberley (2013)
70	1 h	In solution	Direct heat	2	Dick and Kimberley (2013)
70	1 h	In soil – Mesh	Incubator	77	Dick and Kimberley (2013)
70	1 h	In soil – Dry	Incubator	14	Dick and Kimberley (2013)
70	1 h	In soil – Wet	Incubator	3	Dick and Kimberley (2013)
70	2 h	In solution	Direct heat	1	Dick and Kimberley (2013)
70	4 h	In soil – Mesh	Dry	2	Dick and Kimberley (2013)
70	4 h	In soil – Mesh	Steam	1	Dick and Kimberley (2013)
70	4 h	In soil – Mesh	Incubator	60	Dick and Kimberley (2013)
70	4 h	In soil – Dry	Incubator	4	Dick and Kimberley (2013)
70	4 h	In soil – Wet	Incubator	4	Dick and Kimberley (2013)
70	4 h	*In vitro* solution	Direct heat	5–40	(Williams 2015)
70	4 h	*In vitro* solution	Direct heat	20–40	(Williams 2015)
70	4 h	*In vitro* solution	Direct heat	<5	(Williams 2015)
70	24 h	In soil – Mesh	Dry	2	Dick and Kimberley (2013)
70	24 h	In soil – Mesh	Steam	7	Dick and Kimberley (2013)
60	15 m	*In vitro* soil/slurry mix	Wet heat	5–40	(Williams 2015)
60	15 m	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
60	1 h	In soil – Mesh	Dry	1	Dick and Kimberley (2013)
60	1 h	In soil – Mesh	Steam	3	Dick and Kimberley (2013)
60	1 h	In soil – Dry – Mesh	Incubator	72	Dick and Kimberley (2013)
60	1 h	In soil – Wet – Mesh	Incubator	87	Dick and Kimberley (2013)
60	2 h	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
60	2 h	*In vitro* soil/slurry mix	Wet heat	<20	(Williams 2015)
60	4 h	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
60	4 h	In soil – Mesh	Incubator	83	Dick and Kimberley (2013)
60	4 h	In soil – Mesh	Dry	33	Dick and Kimberley (2013)
60	4 h	In soil – Dry – Mesh	Incubator	6	Dick and Kimberley (2013)
60	4 h	*In vitro* soil/slurry mix	Wet heat	<10	(Williams 2015)
60	4 h	In soil – Wet – Mesh	Incubator	3	Dick and Kimberley (2013)
60	4 h	*In vitro* solution	Direct heat	25–90	(Williams 2015)
60	4 h	In soil – Mesh	Steam	0.3	Dick and Kimberley (2013)
60	4 h	In solution	Direct heat	5	Dick and Kimberley (2013)
60	4 h	*In vitro* solution	Direct heat	20–60	(Williams 2015)
60	4 h	*In vitro* solution	Direct heat	<5	(Williams 2015)
60	6 h	In solution	Direct heat	3	Dick and Kimberley (2013)
60	9 h	In solution	Direct heat	5	Dick and Kimberley (2013)
60	19 h	In solution	Direct heat	0.2	Dick and Kimberley (2013)
60	24 h	In soil – Mesh	Dry	13	Dick and Kimberley (2013)
60	24 h	In soil – Mesh	Steam	2	Dick and Kimberley (2013)
55	2 h	Enclosed millet – Sand	Incubator	48	Horner et al. (2019b)
55	4 h	In solution	Direct heat	0.2	Dick and Kimberley (2013)

Temp (°C)	Time	Type of experiment	Heat source	Viability (%)	Reference
55	4 h	Soil/sand	Incubator	38	(Horner et al. 2019b)
55	6 h	In solution	Direct heat	5	Dick and Kimberley (2013)
55	12 h	In solution	Direct heat	0	Dick and Kimberley (2013)
55	1 day	In solution	Direct heat	2	Dick and Kimberley (2013)
55	1 day	Enclosed millet – Sand	Incubator	27.4	Horner et al. (2019b)
55	2 days	Enclosed millet – Sand	Incubator	9	Horner et al. (2019b)
55	3 days	Enclosed millet – Sand	Incubator	0	Horner et al. (2019b)
55	7 days	Enclosed millet – Sand	Incubator	0	Horner et al. (2019b)
55	14 days	Enclosed millet – Sand	Incubator	0	Horner et al. (2019b)
55	28 days	Enclosed millet – Sand	Incubator	0	Horner et al. (2019b)
50	15 m	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
50	15 m	*In vitro* soil/slurry mix	Wet heat	10–40	(Williams 2015)
50	2 h	*In vitro* soil/slurry mix	Wet heat	10–30	(Williams 2015)
50	2 h	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
50	4 h	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
50	4 h	*In vitro* soil/slurry mix	Wet heat	<20	(Williams 2015)
50	4 h	In soil – Mesh	Dry (lid on/off)	8	Dick and Kimberley (2013)
50	4 h	In soil – Mesh	Steam	4	Dick and Kimberley (2013)
50	4 h	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
50	4 h	*In vitro* solution	Direct heat	>60	(Williams 2015)
50	4 h	*In vitro* solution	Direct heat	20–70	(Williams 2015)
50	4 h	*In vitro* solution	Direct heat	0–70	(Williams 2015)
50	1 day	In soil – Mesh	Dry	5	Dick and Kimberley (2013)
50	1 day	In soil – Mesh	Steam	0	Dick and Kimberley (2013)
50	1 day	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
50	2 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
50	3 days	Soil (all types)	Incubator	0	Horner et al. (2019b)
50	3 days	Soil (10% water)	Incubator	Viable	Horner et al. (2019b)
50	3 days	Soil (20% water)	Incubator	0	Horner et al. (2019b)
50	3 days	Soil (30% water)	Incubator	0	Horner et al. (2019b)
50	3 days	Soil (40% water)	Incubator	~1	Horner et al. (2019b)
50	3 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
50	4 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
50	7 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
50	14 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
50	21 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
45	4 h	*In vitro*	Incubator	0	Horner et al. (2019b)
45	1 day	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
45	2 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
45	4 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
45	7 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
45	14 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
45	21 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
40	15 m	*In vitro* soil/slurry mix	Wet heat	10–50	(Williams 2015)
40	15 m	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
40	2 h	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)

Temp (°C)	Time	Type of experiment	Heat source	Viability (%)	Reference
40	2 h	*In vitro* soil/slurry mix	Wet heat	20–30	(Williams 2015)
40	4 h	*In vitro* soil/slurry mix	Wet heat	0	(Williams 2015)
40	4 h	*In vitro* soil/slurry mix	Wet heat	10–40	(Williams 2015)
40	4 h	*In vitro*	Incubator	Viable	Horner et al. (2019b)
40	1 day	*In vitro*		0	Horner et al. (2019b)
40	1 day	Natural soil inoculum	Incubator	Viable	Horner et al. (2019b)
40	2 days	*In vitro*	Incubator	0	Horner et al. (2019b)
40	4 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
40	7 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
40	14 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
40	21 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
37	1 day		Incubator	Viable	M. Gerth, pers. comm., 2020
35	4 h	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
35	4 days	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
35	7 days	Natural soil inoculum	Incubator	Viable	Horner et al. (2019b)
35	14 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
35	21 days	Inoculated roots/soil/agar	Incubator	0	Horner et al. (2019b)
30	4 h	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
30	1 day	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
30	2 days	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
30	4 days	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
30	7 days	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
30	14 days	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)
30	21 days	Inoculated roots/soil/agar	Incubator	Viable	Horner et al. (2019b)

Note: Viability results (percentage of viable oospores) adapted from Williams (2015) are estimates derived from graphic interpretation. H = hours and m = minutes.

Source: Ashcroft (2020b)

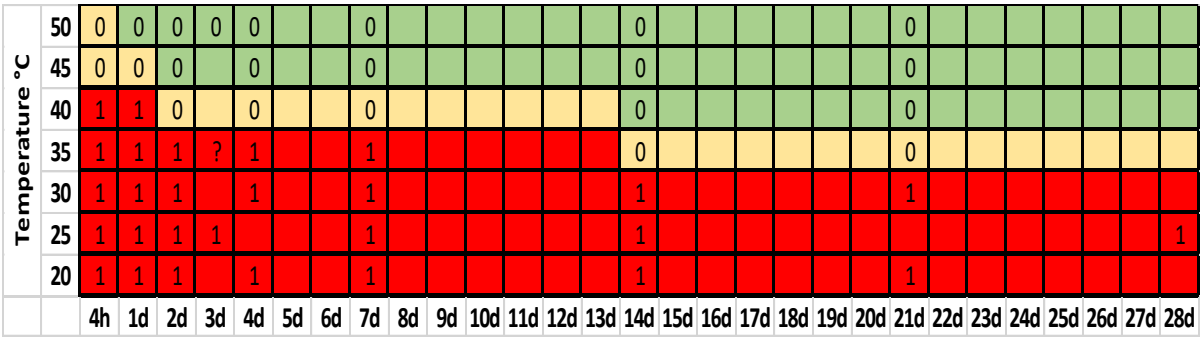

Figure 6-2: Temperature versus time heat–treatment efficacy results

Note: Red cells indicate that oospores survived heat treatment, and green cells indicate that oospores were deactivated. Binary values of 0 or 1 indicate a specific temperature time combination where the experimental result was either lethal (0) or not lethal (1). Yellow cells provide a 5 degrees Celsius buffer safety margin. Empty cells are conservatively estimated across increments in temperature and time where experimental results are not available. h = hours and d = days.

Source: Adapted from S Bellgard and I Horner, pers. comm., 2019

6.2.4 Discussion on oospore deactivation and hygiene knowledge gaps

Based on oospore deactivation research, cleaning station hygiene measures using SteriGENE® (TriGene II Advanced) will control all non-dormant propagule stages (zoospores, sporangia and mycelium) but will not control oospores of *P. agathidicida* in soil, unless the oospore germinates before or during treatment. Methylated spirits could potentially be used, which is common in Australia for hygiene against *P. cinnamomi* (G Clapperton, pers. comm., 2020) but has not been tested against *P. agathidicida*.

A research gap is understanding the role of stromata (hyphal aggregations) within kauri roots. To date, this reservoir of survival structures of *P. agathidicida* has been given little attention (N Williams, pers. comm., 2020) yet is fundamental to determining the efficacy of surface and volumetric disinfestation treatments.

Effective removal or avoidance of soil that may contain oospores (for example, using improved (gravel or board-walked), dry or re-routed tracks), followed by use of SteriGENE® is still valid to reduce inoculum loads and, therefore, the risk of transfer of *P. agathidicida*. Where the risk of oospore contamination is high, such as with off-track work and machinery use in kauri forests, and thorough cleaning is not feasible, temperature deactivation (through heat treatment) shows the most promise for a pragmatic control tool.

Several important gaps remain in the hygiene and oospore deactivation research area, for example:

1. investigating the biology of, and options to break, dormancy, that is, how to force **all** oospores to germinate so chemical or bioactive agents are given the opportunity to control the resulting susceptible life stages;
2. understanding isolate variation and treatment responses;
3. field testing high temperature oospore deactivation and validation to check that all oospores are deactivated using temperature protocols (that is, are not retained in a dormant state);
4. investigating how long oospores survive in soil, roots and hygiene stations;
5. investigating the efficacy of methylated spirits against oospores;
6. investigating the use of chlorine to disinfect water;
7. investigating the role of hyphal aggregations, known as stromata, within kauri roots and how that influences future research design;
8. determining the operational implementation potential of high temperature deactivation;
9. investigating further oospore deactivation testing of high-temperature, short-duration combinations (for example, steam sterilisation, boiling).

Gaps are also echoed in the SSAG Kauri Dieback Science Plan (Kauri Dieback Strategic Science Advisory Group 2018) where oospore deactivation is noted as a high priority. In addition, the KDP Planning and Intelligence Team identified it as an important priority for future research, and the Biological Heritage National Science Challenge Ngā Rākau Taketake programme's investment in tools

and technologies statement prioritised research into validated or alternative disinfectants (Biological Heritage 2019).

6.3 EXCLUSION AND TRACK MANAGEMENT

Managing the spread of *P. agathidicida* has extended beyond hygiene stations, and KDP partners are using tools such as Rahui, Controlled Area Notices, track closures and track upgrades. Social science research on compliance with signage, remaining on track, track closures and use of hygiene stations has been undertaken under the KDP, however, social science is outside the scope of this review. Research has also been undertaken by Auckland Council and the Department of Conservation to inform the optimal hygiene station design, to enable an effective and efficient cleaning process for the end user. The KDP has also funded the development of a national standard around track infrastructure and track mitigation in kauri forests. This is important to minimise the risk of soil transfer on track user footwear during track construction and usage.

Providing evidence of the efficacy of exclusion and track management is an extremely complex undertaking particularly because of the long latency period of kauri dieback. Interventions put in place since 2010 may not show any effect for a decade because the trees showing disease now may have been infected before the intervention.

It is recommended future research attempts to quantify the impact of track upgrades and realignments, installation of hygiene stations and exclusion strategies on the spread of *P. agathidicida.* This is a long-term question, and designing measures that can be implemented now and used in future research has been a focus of recent KDP research into defining a baseline monitoring programme, which is under consultation (Stevenson & Froud 2019). Work towards developing a baseline monitoring programme will help the future assessment of interventions, but because it was not implemented before the interventions the temporal relationship between cause and effect will need to be carefully interpreted (Rothman & Greenland 2005).

6.4 BIOLOGICAL CONTROL

The KDP has a limited history of looking at biological control agents. In the first TAG meeting, in 2008, it was noted there were “no known effective biological control agents despite claims of some manufacturers” (Kauri Dieback TAG1 2008 Pg 7) to date no evidence has been provided to support or refute this statement.

Biological control was raised in the KDP 2011 research priorities analysis but not made a priority due to limited research funds and was recommended for future funding in the medium term.

KDP partners were receiving enquires to test products, and a small-scale screening service was provided by Auckland Council. From this screening, Auckland Council identified ten products for laboratory testing. From the KDP-funded research, 3 of the 10 products showed potential and were recommended for field testing. Field testing was placed on hold by the KDP Planning and Intelligence Team, as further information was required into the feasibility of using biological control in a native forest ecosystem. In 2019, the KDP funded desktop review of biological control options, was completed (Bellgard et al. 2019).

An important result of the biological control desktop review was the indication that mycorrhizal fungi may have potential for kauri dieback management, based on preliminary results from several trials (Bellgard et al. 2019). However, the desktop review strongly recommended that research into potential implications of introducing biological control agents into native forest be addressed prior to further research. These implications were (Bellgard et al. 2019):

1. understanding the native endophyte ecology sufficiently to be able to assess non-target impacts;
2. understanding the potential for introduced endomycorrhizal fungi (which penetrate the root and exchange nutrients) and ectomycorrhizal fungi (which remain external to the root) to invade and displace native endophytes on both kauri and other podocarps;
3. investigating the side-effects associated with nutrient enrichment on kauri growth.

Questions about the cultural concerns of obtaining or introducing biological control agents need to be appropriately and fully addressed before field trials are started. Biological control research has been progressed by other researchers for managing *P. agathidicida* using both rongoā and Western science approaches. As at July 2020, no biological control agents had been shown to have field efficacy for *P. agathidicida*. It will be extremely difficult to find an effective, and ideally persistent, biological control agent for a soil-borne plant pathogen in natural ecosystems across large areas.

Interestingly, as early as 1967, scientists were investigating the use of mycorrhizal fungi to enhance kauri growth, and found that they stimulated absorption of phosphate (Morrison & English 1967). Ecroyd (1982) noted that, despite high accumulation of nitrogen in soils and litter, it may not be readily available to kauri so will be strongly dependent on mycorrhizal penetration. Peterson (1962) also found a widespread nitrogen deficiency and possible general deficiency in phosphate in nutritional analysis of kauri seedlings from 10 locations in New Zealand (noted from Ecroyd 1982). Enhanced uptake of nitrogen using mycorrhizal fungi may be worthy of further exploration, subject to appropriate cultural approvals as mentioned above.

6.5 ALTERNATIVE TREATMENTS

The KDP invested in assessing the efficacy of alternative treatments and decided in April 2015 that future alternative treatment enquiries needed to show some degree of efficacy before entering the KDP trial process. That led to the introduction in 2019 of a literature review process, through Scion, to determine if any potential efficacy could be anticipated, followed by assessment from a KDP Planning and Intelligence sub-committee that included representatives from the Tangata Whenua Roopu, MPI, Department of Conservation and Auckland Council. As at September 2019, 34 products had completed the literature review process and were under assessment. Sixteen products have literature that indicates they are potentially effective, however, six also have evidence of non-target impacts (results provided by KDP Planning and Intelligence Team – product review tracking sheet and individual product review reports).

External to the KDP, mātauranga Māori antimicrobial research against *P. agathidicida* has been funded by MBIE and through the Biological Heritage National Science Challenge, and researchers have found promising flavanones from *Kunzea* (kānuka) in laboratory studies (Lawrence et al. 2019). These results indicate action against zoospore motility and germination but not mycelial growth, and further research is required to determine mode of action and potential operational application of

this knowledge (Lawrence et al. 2019). In a separate project, also funded by the Biological Heritage National Science Challenge, Lawrence et al. (2019) screened over 100 compounds to establish if any had activity against *P. agathidicida*. They identified one compound, benzethonium chloride, with activity against *P. agathidicida* mycelium and zoospore life stages in laboratory trials, however, the resistant oospore life stage was not included in the assay (Lawrence et al. 2019). The research by Lawrence et al. (2017); Lawrence et al. (2019) is preliminary and could take years to operationalise if *in planta* studies show efficacy. As a point of reference, it took eight years from laboratory trials to field use for phosphite, a known anti-*Phytophthora* compound.

The use of rongoā, or traditional Māori medicines has been explored by the KDP, however, the review of the work done to date is outside the scope of this review.

6.6 GENETIC RESISTANCE AND/OR TOLERANCE

Scientists at the first TAG, in 2008, recommended that screening of seedling populations for resistance was a "good first step" towards resistance breeding (Kauri Dieback TAG1 2008). Early research by Horner and Hough (2014a) showed that leaf and seedling assays were useful to investigate pathogenicity of *P. agathidicida* on kauri. They used two trees in the leaf assay and six seedlings all from the same source tree in the seedling assays. As Horner and Hough (2014a) did not present the standard deviation of the mean so it is not possible to interpret the variability of lesion development between the replicates or between the seedlings in the results.

At the time of writing, the KDP investment in genetic resistance had been through co-funding the MBIE Healthy Trees, Healthy Future programme. The programme developed an *in vitro* detached-leaf-based resistance screening assay (Herewini et al. 2018) that was piloted on 10 leaves each from six kauri trees using three isolates of *P. agathidicida* (two from Coromandel and one from the Waitākere Ranges).

Of interest is the origin of the saplings, they were grown from seed collected from plantation kauri in the Hawke's Bay that originated from seed taken from the Waipoua grafted nursery in the 1980s (Herewini et al. 2018). The authors found that colonisation of wounded leaf tissue occurred in all trees both with and without visible lesions (Herewini et al. 2018). They also observed variability in isolate virulence. One isolate was less virulent than the other two and all trees were susceptible, however, variation in susceptibility was evident, with one tree significantly more susceptible to disease development compared with the others (Herewini et al. (2018). The less virulent isolate was notably more aggressive on the most susceptible tree, and the results table is interesting reading, given the single origin of the trees (Herewini et al. 2018).

This variability is interesting and may be due to the inter-tree variability or intra-tree variability in leaves, and results of leaf assays for a root pathogen that has no record of infecting foliage should be treated with caution (C Green, pers. comm., 2020). Additional work by Herewini that is currently unpublished is on screening a much larger collection of kauri clones that also showed significant variability (www.appsnet.org/Publications/Fremantle_Presentations/herewini_echo.pdf).

Collection of seed from within infected and non-infected areas was completed in 2019, following the establishment of partnerships with mana whenua (Williams & Hodder 2019). Seed was collected from 650 trees and produced 20,000 seedlings for screening (Williams & Hodder 2019). Unpublished

results of root-flooding inoculation of families of kauri in glasshouse trials have also shown promising variability in susceptibility, with 5 percent to 80 percent of individuals from each family surviving after 106 days (Bradshaw et al. 2020). This kauri taonga is currently being maintained by Scion, but the research funding has ended. However, continuation of genetic resistance research is extremely important, given the encouraging results from initial screening and the cultural expectation that collection of these seeds will lead to improved understanding and solutions for kauri dieback.

An interesting observation from UK Forest Research (S Green, UK Forest Research, pers. comm. 2019) was the suggestion that, post-logging, individual trees in a stand are likely to be closely related (from the same parent tree that was removed) and, if they share 50 percent of their genes from a common parent could this explain whole stand decline because of poor resistance within a specific population?

6.7 KNOWLEDGE GAPS FOR CONTROL

The following knowledge gaps need further research:

- investigate economic modelling of the long-term practicality and efficacy of phosphite at the forest level, as recommended by Black and Dickie (2016);
- investigate the opportunity to transfer from experimental trials to observational trials as phosphite treatments are rolled out across kauri lands, to fine-tune rates and frequency of treatment, and to record non-target effects. Land managers should be ready to stop use of phosphite if adverse ecological effects are observed;
- complete existing experimental large tree phosphite and trunk-spray research because it may give more certainty around rates for large trees, longevity of lesion suppression and canopy recovery;
- investigate the biology of and options to break dormancy, that is, how to force all oospores to germinate so chemical or bioactive agents are given the opportunity to control the resulting susceptible life stages;
- complete field testing of high temperature oospore deactivation and validation to see if all oospores are deactivated using temperature protocols (that is, are not retained in a dormant state);
- investigate the efficacy of methylated spirits against oospores;
- investigate the efficacy of chlorine disinfection in water against the pathogen;
- understand the role of stromata hyphal aggregations within kauri roots;
- determine the operational implementation potential of high temperature deactivation;
- investigate further oospore deactivation testing of high-temperature, short-duration combinations;
- quantify the impact of track upgrades and realignments, installation of hygiene stations and exclusion strategies on the spread of *P. agathidicida*;
- implement a baseline monitoring programme for future assessment of interventions;
- continue genetic resistance research.

7 DECISION SUPPORT

7.1 MĀTAURANGA MĀORI AND SCIENCE PLAN

The KDP knowledge-based systems include mātauranga Māori (traditional and contemporary Māori knowledge) and Western science. Robust processes are required to inform decision-making across both knowledge systems and to identify opportunities where they can be aligned and shared (Ashcroft, 2019a). This is to encourage rich and innovative outcomes and take advantage of the synergies that come from the richness of experiences, perspectives and worldviews across diverse knowledge systems and practices (Ashcroft 2019a).

The KDP Planning and Intelligence Team has been developing a decision-making process to let the KDP identify:

- alignment opportunities between Māori and non-Māori;
- how science and mātauranga Māori research, tools and monitoring will be implemented;
- priority knowledge gaps that need to be addressed;
- how advice from experts will be obtained and used;
- arrangements to provide assurance and show that scientific evidence and analysis are sought, obtained, interpreted, used and communicated appropriately within the KDP.

The benefits of this process are:

- increased confidence that KDP is harnessing the right advice and its decision-making is based on robust scientific and cultural knowledge;
- a standardised process for how KDP procures research;
- consistent criteria for the proposed activities, to enable robust and consensual decision-making;
- increased transparency of the rationale behind decisions and the drivers that underpin prioritisation;
- integration of mātauranga in KDP Planning and Intelligence decisions as well as supporting mātauranga in its own right;
- enhanced knowledge of how to manage kauri dieback;
- clarity around decision-making and clearly communicating how decisions are made;
- knowledge gained from, and used by, those who are kaitiaki of kauri and kauri forests as well as KDP partners and stakeholders;
- guidance for future decision-makers.

The Mātauranga Māori and Science Plan was being finalised by the KDP at the time of writing.

7.2 PRIORITISATION FRAMEWORK

In 2015, the KDP commissioned a decision framework to prioritise sites for management and optimise interventions at those sites (O'Connor & Sinclair 2015). The authors identified two types of priority sites: those considered high value and those considered high risk (O'Connor & Sinclair 2015). The high-value sites were deemed to have high ecological, cultural or social value and required protection. In contrast, the high-risk sites were those that posed a high risk of spread to other, particularly high value, sites (O'Connor & Sinclair 2015). In addition, the authors aimed to optimise decision-making on the best suite of interventions to avoid disease spread into high-value or out of high-risk sites.

The basic idea of the framework was to build consistency across partners of the KDP for operational decision-making. It is an Excel-based tool with 10 colour-coded tabs, covering:

1. use of the framework, which is a process flow-chart (Figure 7-1);
2. set-up and context, which is a pre-process step to encourage collaboration between KDP partners and consideration of policy and strategy (yellow diamond in Figure 7-1);
3. a site profile framework, which records the outputs from the other tabs and list of sites (green diamond in Figure 7-1);
4. a risk assessment framework, which lists risk factors associated with soil movement (red diamond in Figure 7-1);
5. a significance framework (optional), with a list of significance factors to prioritise resourcing across multiple sites covering social (community amenity value), spiritual (including a list of Māori cultural values), environmental and economic (purple diamond in Figure 7-1);
6. a prioritisation framework, which combines risk and significance and can be weighted (orange diamond in Figure 7-1);
7. KDP decision-making inputs, which provide links to useful best practice guidelines and research documents;
8. a site intervention choice, which lists all operational interventions (as of 2015) and the benefits or risks for each (blue diamond in Figure 7-1). This includes hygiene stations, signage, area closures, proximity planting to reduce root zone access, vector controls (fencing or culling) and track upgrades, closures or relocations;
9. a policy intervention choice, which lists the policy or strategy interventions KDP partners have agreed to for consideration before final prioritisation and selection of interventions (blue diamond in Figure 7-1). This includes surveillance, stock movement, awareness, nursery regional controls, hygiene guidelines and management of contaminated soil;
10. post-framework questions, which encourage KDP partners to test their priorities with other partners.

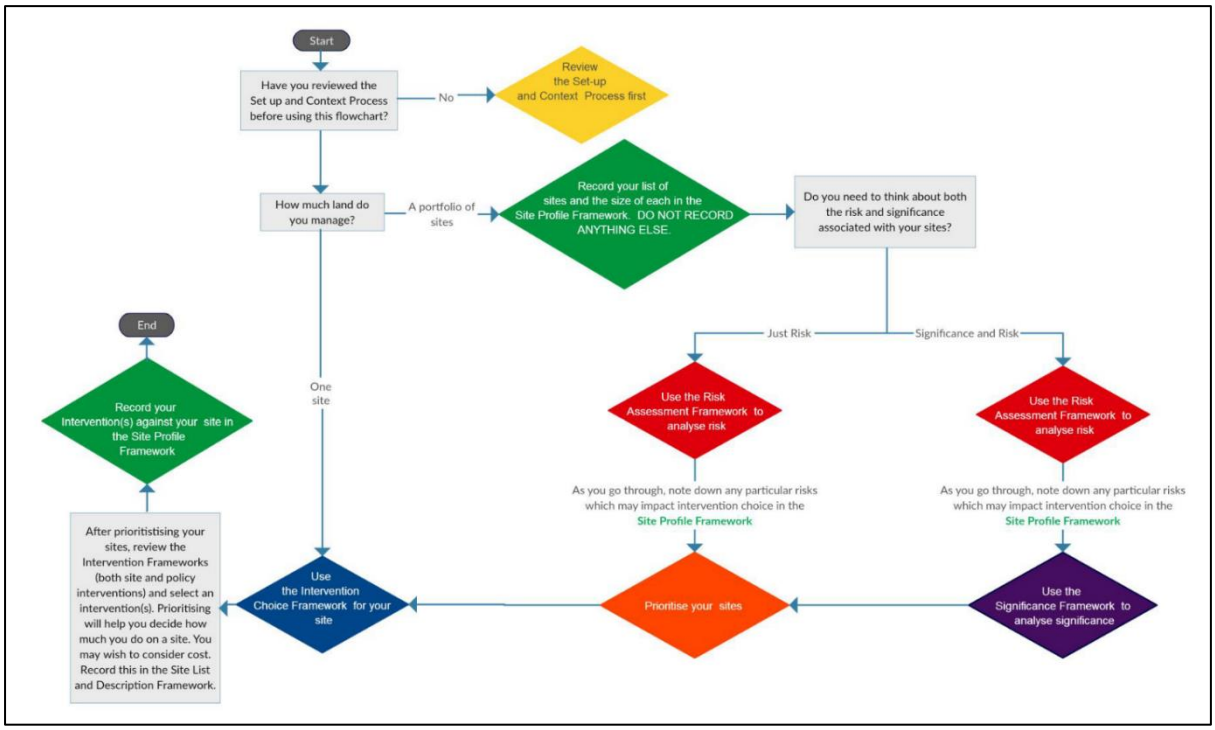

Figure 7-1: Decision support framework flow chart showing the process from the different framework tabs in the Excel spreadsheet tool

Source: O'Connor and Sinclair (2015)

Testing of the draft decision-support framework over a six-month pilot period by KDP partners before final revision and implementation, was scheduled. To allow thorough testing, the framework required a relevant operational project that needed to be implemented during the six-month testing timeframe. However, at the end of this period, no feedback was received, which ultimately led to the tool not being fully implemented as intended across the KDP (T Ashcroft, pers. comm., 2020). The perceived lack of active projects being implemented during the testing period likely contributed to this outcome (T Ashcroft, pers. comm., 2020).

7.3 KAURI GEODATABASE

A kauri geodatabase has been developed over time, by incorporating spatial information collected from surveillance activities and geo-based spatial mapping showing polygon-based information on several kauri attributes, such as historical pathways (nurseries, plantations) and anthropogenic profiles that may inform spread risk. The continual improvement of this baseline information will allow sound decision-making to inform value areas for protection and areas to actively manage due to the vector profile of an area. Further information is covered under Chapter 4, Surveillance, kauri mapping, detection, diagnostics.

7.4 BEST PRACTICE GUIDELINES

The KDP Planning and Intelligence Team has developed various best practice guidelines based on the latest available research (where applicable) and updated important guidelines as new information has been reviewed. The guidelines are not policy but should be considered by planners, land

managers and contractors when planning any operations. The sections below show the main best practice guidelines developed by the KDP Planning and Intelligence Team.

Each guideline has a purpose, background, and assumptions and constraints section before providing guidance on planning and on-site instructions and considerations. The assumption and constraints section outlines the research (referenced) supporting the guidelines and indicates where knowledge is lacking and therefore a more conservative risk-based approach was required. For example, the tree removal and pruning of kauri best practice guideline is comprehensive and clearly specifies what is known and unknown, with 13 assumptions listed (Beauchamp 2017b).

Several guidelines needed input from industry, to identify any barriers that may have prevented their successful uptake and usage.

The dripline diagram in

Figure 7-2 is used as a risk-based reference point for activities near kauri in most of the best practice guidelines. It shows the area where soil is most likely to be infected around a kauri tree and is based on the area defined by its root zone. This is defined as three times the radius of the dripline of a kauri tree or a stand of several trees, if they overlap (Beauchamp 2017b).

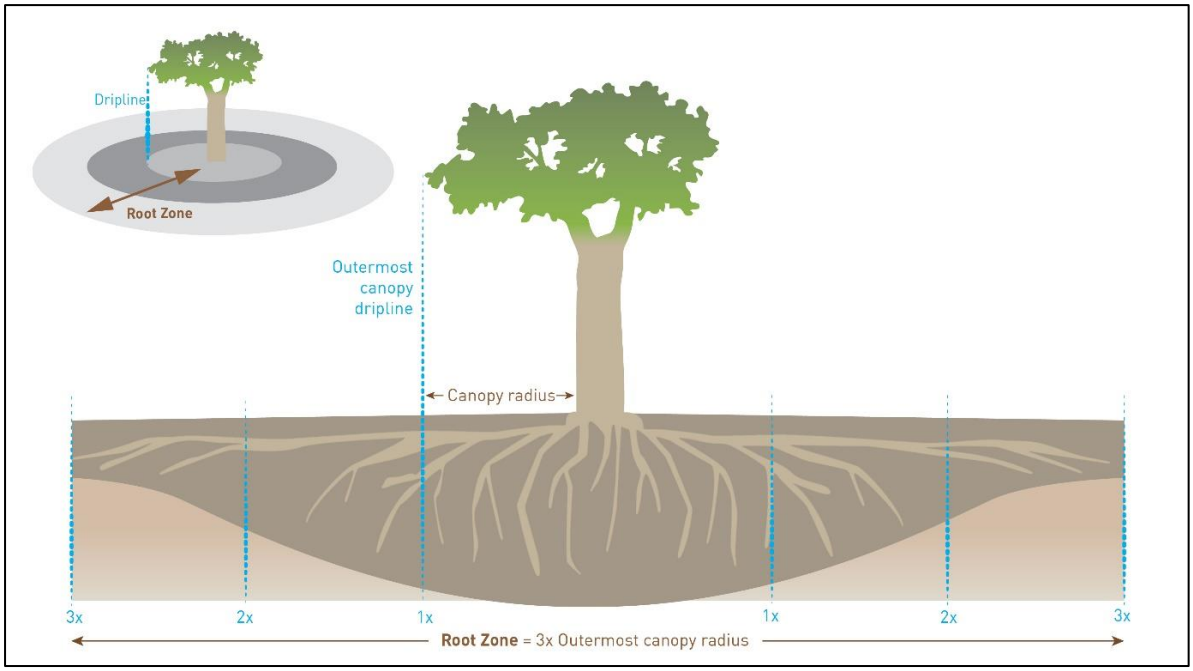

Figure 7-2: Three times the dripline risk zone for kauri dieback

Source: Beauchamp (2017a).

All the guidelines follow the same basic structure. Each document references the other guidelines that need to be read for further clarity. Methods from the “Surveillance and soil sampling” best practice guideline are described in Chapter 4, Surveillance, kauri mapping, detection, diagnostics.

7.4.1 LAND DISTURBANCE ACTIVITIES (INCLUDING EARTHWORKS) AROUND KAURI

This guideline was written in 2017 to address issues with controlling the spread of *P. agathidicida* by earthworks within and between sections of land, and between stands of kauri on the same land (Beauchamp 2017a). The main assumptions for this guideline are based on very early recognition within the KDP that any process that moved soil would move *P. agathidicida*, and research found that soil and root material over 2 metres deep could be contaminated (Bellgard et al. 2013). In addition, symptomology of kauri dieback was not detectable in the tree until relatively late in the infection cycle, so apparently healthy trees could be contaminated and therefore needed to be managed as infected. The guideline aims to define where disturbed material can be placed, that is, material from within three times the radius of the dripline needs to remain on site versus material outside that zone, which needs to be taken to an approved landfill (Beauchamp 2017a).

The guideline specifies the hygiene measures that need to be taken when entering and exiting a kauri zone and what to do when moving between zones (Beauchamp 2017a). Three times the radius of the drip line is also considered to be the location where people need to be cautious while undertaking more minor earthworks like gardening. Soil from the zone should retained in the zone, and seedlings should not be moved to other areas or parts of the same garden (Beauchamp 2017a).

7.4.2 LANDFILL DISPOSAL OF CONTAMINATED MATERIAL

The "Land disturbance activities (including earthworks)" best practice guideline requires material within three times the radius of the kauri canopy to be left in that defined region or removed to landfill (Beauchamp 2017a). *P. agathidicida* is classified as an unwanted organism, and the landfill disposal guideline was written to provides information on how management of this unwanted organism should take place.

The guideline outlines the criteria recommended for a land management agency to consider when assessing if a landfill is suitable to receive material for disposal. If certain criteria cannot be fulfilled by a landfill then other mitigation measures should be explored to reduce the risk before the landfill receives material (T Ashcroft, pers. comm., 2020).

The removal requires safe and controlled disposal of material that must be kept for over two decades (the potential lifespan of oospores (Bellgard et al. 2013)) and the ability to clean transporting vehicles. This guideline was initially drafted under urgency in December 2015, due to the increasing demand of finding safe areas to dispose of potentially infected material. The guidelines were updated and finalised in 2018, to provide clarity on where to dump potentially contaminated soil and plant material (Ashcroft 2019b). The characteristics of potential landfills were assessed to ensure *P. agathidicida* would not exit the site and that washing of transport vehicles was possible on site (Ashcroft 2019b).

The guideline defines the behaviour needed to transport and dispose of contaminated soil and timber and requires deep burial of contaminated material (more than 2 metres) (Ashcroft 2019b). Deep burial needs preparation at the site before material arrives and immediate coverage of that material (Ashcroft 2019b). Consequently, the guideline is specifically designed for contractors (not members of the public). This does not stop the disposal of small amounts of material, but that material needs to be collected by a contractor and disposed of by them. Although not an official

approval list, the guideline identifies five landfills that were assessed as being suitable to receive potentially contaminated material: one each in Northland, Auckland and Coromandel and two in Waikato. It also provides contacts for contractors and the landfills (Ashcroft 2019b).

7.4.3 VEHICLE AND HEAVY MACHINERY HYGIENE

Vehicles and heavy machinery have been implicated in the movement of *Phytophthora* in Australia during firefighting, and numerous studies have suggested they can carry seeds of many weeds. Vehicles are needed, however, to carry out many functions in the kauri dieback management space.

The "Vehicle and heavy machinery hygiene" best practice guideline was written in 2017 and provides information on the expected cleanliness of machinery entering a kauri site and the expected cleaning at that site after use (Ashcroft 2017). Material collected at a site can be left at that site if it meets the zone or distance requirements of the "Land disturbance activities (including earthworks)" guideline (Ashcroft 2017; Beauchamp 2017a). The "Vehicle and heavy machinery hygiene" guideline covers where machinery could be cleaned and provides information on the best construction and location of a site cleaning station (Ashcroft 2017). If cleaning cannot be undertaken on site, the guideline describes how machinery should initially be treated before being taken off site for cleaning. It also lists the types of off-site facilities that are appropriate, including links to a temporary bunding solution (Ashcroft 2017).

7.4.4 TREE REMOVAL AND PRUNING OF KAURI

Trees need to be removed for many reasons, but when killed by kauri dieback it needs to be done more carefully so the process of cutting and transporting the tree away does not spread *P. agathidicida*-contaminated material. Kauri dieback kills kauri of any size and usually leaves the trees standing, although rickers lack strong inner heartwood and tend to fall over in a few years. Often trees need to be removed if they are over a road, in an urban area or beside a track, to reduce both the health and safety risk and transfer risk of contaminated bark, phloem and xylem.

The "Tree removal and pruning of kauri" best practice guideline was written in 2017. It uses data from three studies to define where in the tree dieback oospores are likely to be found (Beauchamp 2017b). This helps identify where it is safer to cut trees, based on trunk size and the location and size of basal lesions (Beauchamp 2017b). The guideline covers the hygiene issues associated with pruning work and includes comments on the ability to clean various types of equipment (Beauchamp 2017b).

7.4.5 PROPAGATION AND PLANTING OF KAURI

Nurseries were identified as the potential source of some of the historic movement of kauri dieback (Beachman 2017). Kauri dieback kills kauri of all ages and is deadly when it affects seed trays of seedlings and closely spaced plants in a nursery. Even small nurseries can pose a substantial risk to the transfer of *P. agathidicida* (A Beauchamp, pers. comm., 2020). Kauri have been planted for many years for forestry and during restoration work, and dieback has been moved to sites by contaminated trees and equipment (A Beauchamp, pers. comm., 2020).

The "Propagation and planting of kauri" best practice guideline was written in 2018. It covers the safe propagation of kauri in nurseries, the types and cleanliness of materials, the way a nursery should be set up to reduce the issues with propagation, the way plants need to be held raised above

the ground, the issues with watering plants and the holding periods before planting (Waipara 2018). The guideline describes when fungicides and phosphite should not be used, and how dead and dying plants should be removed and disposed of (Waipara 2018). The guideline was developed with input from industry, that is, NZPPI(NZ Plant Producers Incorporated. Information from the guideline has been used to inform the NZPPI Plant Production Biosecurity Scheme, which is a science-based framework to help producers identify, manage and avoid biosecurity risk in nursery propagation (NZPPI 2020a). The scheme includes a *P. agathidicida* schedule, which identifies nursery measures (and audit criteria) for growers of kauri to manage risk of *P. agathidicida* being spread on nursery stock in New Zealand (NZPPI 2020b).

7.4.6 QUARRY HYGIENE: AGGREGATE HANDLING, TRANSPORTATION AND STORAGE

Quarries provide aggregate for roading and to maintain dry track surfaces. This material is almost always required in bulk and is piled in places for on-site use (Beauchamp & Ashcroft 2019). Quarry faces are stripped of soil and plant material (overburden) and, in northern New Zealand, may contain viable spores of *P. agathidicida* from historical kauri remnant vegetation (Beauchamp & Ashcroft 2019). Quarries also use a lot of water in the processing of aggregate and that water may come from watersheds in kauri ecosystems where soil may be contaminated (Beauchamp & Ashcroft 2019). Stripped soil and vegetation can be stored in special areas on site. The trucks and equipment used to supply aggregate can go into kauri dieback sites, so need to be washed before entering and exiting quarries (Beauchamp & Ashcroft 2019).

The "Quarry hygiene: Aggregate handling, transportation and storage" best practice guideline was written in 2019 with industry, to find ways to reduce the likelihood of any contaminated quarry product being transferred to a kauri-associated road or track site (Beauchamp & Ashcroft 2019). The guideline discusses the activities associated with overburden and aggregate, equipment, machinery, water and feral animals. It considers the issues around the risks and associated management actions that will reduce the potential for aggregate becoming a vector of *P. agathidicida* (Beauchamp & Ashcroft 2019).

7.4.7 PHOSPHITE CONTROL

Best practice guidelines have been drafted for phosphite control and, at the time of writing, are under review. They are due to be released before the end of 2020 (T Ashcroft, pers. comm., 2020).

7.4.8 EVENT MANAGEMENT

The "Event management" guideline was completed in 2020. It aims to provide hygiene guidance for managed events, such as running or trail biking events, in or near kauri forest to minimise the risk of participants spreading *P. agathidicida* in soil (Ashcroft 2020a).

The guideline makes several assumptions. The first is that asymptomatic trees may be infected so all kauri, regardless of infection status, are considered a risk. Second, long-lived oospores can be a reasonable distance from infected trees and can be spread to new areas through contaminated footwear and equipment (Ashcroft 2020a). Last, it was assumed the risk of spread is proportional to the volume of soil moved and the frequency and distance of such movement (Ashcroft 2020a). The guidelines are based on four main principles of:

1. arrive clean and leave clean, which includes trail-based footwear hygiene;

2. keep away from kauri;
3. “Scrub, Spray and Stay”, which involves appropriate cleaning procedures and staying on marked tracks at all times;
4. avoid wet or muddy conditions when hygiene is more difficult to achieve (Ashcroft 2020a).

7.4.9 OTHER BEST PRACTICE GUIDELINES IN DRAFT

Best practice guidelines for heat treatment; principles of hygiene when around kauri; and rural hygiene are under KDP Planning and Intelligence Team review (Y C Chew, Auckland Council, pers. comm., 2020; K Parker, Waikato Regional Council, pers. comm., 2020). Cultural harvesting protocols and track construction and mitigation standards are also under development.

7.5 DECISION SUPPORT KNOWLEDGE GAPS

It is important for operational research to have a clearly defined implementation pathway before starting or funding research. The best practice guidelines are the main knowledge transfer documents for operational research.

- It is recommended a review be done within each partner organisation to determine to what extent each best practice guideline is informing operational activities in the agency, tangata whenua or other landowners, to ensure investment in research achieves its full value.
- Significant investment went into the decision support tool that has had little to no uptake. Future research in this area should include social science, to ensure any future development in decision support frameworks (including best practice guidelines) results in strategies that will improve social licensing of these tools to make sure the outcome is valued and uptake is high.

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APPENDIX - KEY KNOWLEDGE GAPS A3 TABLE

Table 2 The key knowledge gaps identified in the five main research review areas, including synergies (ticked cells) and overlaps (green shaded cells) with other research areas.

Key Knowledge gaps	Biology and Impacts	Surveillance and detection	Pathways and vectors	Control	Decision Support
Understand the biological mechanisms that control oospore dormancy and options to break dormancy	✓	✓		✓	✓
Understand if P. agathidicida is present at levels that are below the level of detection, or if it is only present where disease is being detected.	✓	✓	✓	✓	✓
Understanding the long-term cultural and ecological impact of kauri dieback on forest health and the impacts of mitigation measures	✓	✓	✓	✓	✓
Understanding the role of alternative hosts in *P. agathidicida* distribution and spread	✓		✓	✓	✓
Review results from all forms of surveillance for *P. agathidicida,* kauri dieback (disease symptoms) and hosts (with cultural approval)		✓		✓	✓
Reclassification of where *P. agathidicida* positive sites are in comparison to where kauri dieback (disease) has been observed, using existing surveillance data consistent with the proposed case definitions.		✓		✓	✓
Undertake test performance research to obtain the sensitivity and specificity values from field testing and all new methods.	✓	✓	✓	✓	✓
Investigate the potential for DNA / metabarcoding as a rapid detection method for *P. agathidicida* in soil, root samples and water.		✓		✓	✓
Investigate an in-expensive remote disease detection methodology by integrating the implementation of aerial multispectral, satellite, vertical (helicopter) and oblique angle photography methods for canopy health assessment and impacts.	✓	✓		✓	✓
Identify healthy kauri forests with disease undetected and undertake research to clarify whether *P. agathidicida* is present even where disease is not observed and prioritise for protection.		✓			✓
Research biological factors that contribute to breaking dormancy of oospores and how this influences diagnostics.	✓	✓		✓	
Investigate the potential to use metabolite profiling as an early detection tool.	✓	✓			
Quantify the risk of human and invasive pig vectoring of *P. agathidicida* using proximity to track network and pig density counts against kauri dieback cases and non-cases.	✓		✓		✓

Key Knowledge gaps	Biology and Impacts	Surveillance and detection	Pathways and vectors	Control	Decision Support
Investigate the efficacy and uptake of vector mitigation measures (e.g. hygiene, track closures, stock exclusion, best practice guidelines and pest control) and the impact of these measures on the distribution and severity of disease.	✓	✓	✓		✓
Research into the social and cultural desirability of vertebrate control and stock exclusion in the context of possible kauri forest loss.	✓		✓		✓
Research into low-risk pest control options to reduce human mediated spread of *P. agathidicida* (e.g. via off-track activities such as bait-lines or pig hunting).			✓		✓
Economic modelling of the long-term practicality and efficacy of phosphite at the forest level, as recommended by Black and Dickie (2016).	✓			✓	
As phosphite treatments are being rolled out across kauri lands, there is an opportunity to transfer from experimental trials, to observational trials, to fine tune rates and frequency of treatment, and to record non-target effects. Land managers should be ready to cease use of phosphite if adverse ecological effects are observed	✓	✓		✓	✓
Completion of existing experimental large tree phosphite and trunk spray research is important and may give more certainty around rates for large trees, longevity of lesion suppression and canopy recovery.				✓	✓
Investigation into how to force all oospores to germinate so that chemical or bioactive agents are given the opportunity to control the resulting susceptible life stages.	✓	✓		✓	
Field testing of high temperature oospore deactivation and validation that all oospores are deactivated using temperature protocols (i.e. are not retained in a dormant state).				✓	✓
Investigate the efficacy of methylated spirits against oospores.				✓	✓
Understand the role of stromata hyphal aggregations within kauri roots.	✓			✓	
Quantify the impact of track upgrades and realignments, installation of hygiene stations and exclusion strategies on the spread of P. agathidicida	✓		✓	✓	✓
Implement a baseline monitoring programme for future assessment of interventions.	✓			✓	✓
Continue genetic resistance research.	✓	✓		✓	✓
Review KDP partner organisations to determine implementation and uptake of best practice guidelines within their agency, tangata whenua or with other landowners to ensure investment in research achieves its full value.		✓	✓	✓	✓
Future research into decision support tools should include Mātauranga Māori and social science to ensure cultural and social licence is obtained and uptake is high.		✓	✓	✓	✓