Te Rangahau Aroturuki i ngā Rākau Rangatira o Te Wao Nui ā Tiriwa

2021 Waitākere Ranges Kauri Population Health Monitoring Survey

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Chapter 2

Baseline prevalence study of *Phytophthora agathidicida* and kauri dieback in the Waitākere Ranges and frequency of potential risk factors using a cross-sectional study

Te whakarāpopoto e whakaahua ana i te puruheka patu kauri i Te Wao Nui ā Tiriwa me te auau o ngā take tūraru tērā pea ka puta

Authors

Karyn Froud[1], Yue Chin Chew[2], John Kean[3], Hugo Geddes[2], Georgia Edwards[2], Lee Hill[4], Fredrik Hjelm[4], Robin Taua-Gordon[5], Edward Ashby[5], Sabrina Greening[6], Chris Compton[6], James Shepherd[7], John Dymond[7], Jane Meiforth[7], Nari Williams[8], Ian Horner[8], Bruce Burns[9], Luitgard Schwendenmann[9], Lisa Tolich[2]

[1]Biosecurity Research

[2]Auckland Council

[3]AgResearch

[4]Biosense

[5]Te Kawerau ā Maki

[6]Massey University

[7]Manaaki Whenua – Landcare Research

[8]Plant and Food Research

[9]University of Auckland

2.1 Abstract

Te whakatūporotanga

A cross-sectional study was co-designed with mana whenua to set a baseline for monitoring kauri (*Agathis australis*) health and the prevalence of both kauri dieback (disease) and *Phytophthora agathidicida*, the pathogen that causes kauri dieback, in space and over time. This study had 5 objectives: i) operationalise new remote sensing methods to develop a kauri sample frame; ii) spatially describe the baseline prevalence of *P. agathidicida*; iii) spatially describe the baseline prevalence and severity of symptomatic kauri; iv) identify and collect data on key factors that could affect disease risk for hypothesis generation; and v) collect baseline data on ecological factors as indicators of ecosystem impacts from kauri dieback.

A sample frame was constructed using remote sensing to detect kauri trees >15 m tall within the forest canopy of Te Wao Nui ā Tiriwa / the Waitākere Ranges parkland identifying 68,420 trees. A total of 2140 randomly selected trees were surveyed from this sample frame and the soils beneath a subset of 761 of these trees were tested for *P. agathidicida* presence.

The spatial distribution of *P. agathidicida* showed the pathogen was distributed in a localised pattern around the periphery of the study area. In contrast, symptomatic kauri trees were more widespread and present in the centre of the Park. There was an elevated relative risk of symptomatic kauri in the north of the Park, which matched an elevated relative risk for *P. agathidicida,* and in the south-east area of the Park. The relative risk of symptomatic kauri was also elevated, but to a lesser degree, in the mid-west area of the Park where there was also a higher risk for *P. agathidicida*. Baseline disease severity was recorded so repeated surveys can inform disease progression over time. The prevalence of *P. agathidicida* detected in soil from soil-sampled trees was 10%. The baseline prevalence of symptomatic kauri trees was 16.5% (95% CI =14.1 to 18.9%).

Baseline data collected during the survey were focused on potential risk factors affecting kauri tree health, which were identified through two hui / meetings involving kauri ecosystem health experts from mana whenua and research organisations for data collection and analysis. Baseline measures of ecological impact factors were collected on a subset of trees for future comparisons. An interesting finding was that kauri seedlings and saplings were surviving in soils where *P. agathidicida* was confirmed. This study provides a consistent cohort of monitored trees that can be remeasured to understand change in disease and pathogen prevalence over time.

Results will be used to help inform the ongoing and adaptive management of kauri dieback in Te Wao Nui ā Tiriwa / the Waitākere Ranges and across New Zealand.

2.2 Introduction

Te whakataki

Kauri dieback, caused by *Phytophthora agathidicida* (Weir et al., 2015), was reported causing kauri (*Agathis australis*) stand decline in Te Wao Nui ā Tiriwa / the Waitākere Ranges in 2006 (Beever et al., 2009). Subsequent delimiting surveys detected *P. agathidicida* in several, but not all areas where symptomatic trees were observed within the Waitākere Ranges (Hill, 2016, Hill et al., 2017). However, the overall symptomatic tree prevalence and *P. agathidicida* prevalence in the Waitākere Ranges remains unknown.

Auckland Council therefore carried out a large cross-sectional survey of tree-level kauri dieback monitoring across the Waitākere Ranges during the summer and autumn of 2021. The monitoring design and methodology was co-developed with consultation and discussion between Auckland Council staff, mana whenua (the indigenous people that hold authority and guardianship over the land) representatives of the wider Tāmaki Makaurau (Auckland) region, a multi-disciplinary group of researchers, and partners of Tiakina Kauri. Further detailed and ongoing discussion was undertaken with Te Kawerau ā Maki first and foremost as mana whenua and kaitiaki (guardians) of Te Wao Nui ā Tiriwa, the forested area of the Waitākere Ranges.

As well as detailing distribution and prevalence, this study measures the health status of individual kauri trees so that an increase or reduction in the number of symptomatic trees in the population over time can be assessed. In addition, the study measures the presence of *P. agathidicida* in soils of both healthy and unhealthy trees, so a change in distribution of the pathogen can be assessed over time with repeated surveys.

A kauri dieback case definition was developed to record disease in the forest consistently over time (Stevenson and Froud, 2020). The symptomatic criteria of this case definition for kauri dieback include 'bleeding' (release of copious resin) lesions on the basal trunk, lesions on lateral roots, yellowing of the foliage, the presence of canopy thinning, and ultimately tree death (Stevenson and Froud, 2020). These disease symptoms alone or in combination may also occur in the absence of *P. agathidicida* because the physiological disorders can be caused by other biotic (different pathogens) or abiotic (physical, environmental or climate) factors. However, bleeding lesions in conjunction with one or more other symptoms is typical for infection with *P. agathidicida* (Beever et al., 2009).

Cross-sectional studies (also called prevalence studies) are a common epidemiological study design, that are especially useful in disease outbreak investigations. This type of study is suited to document the prevalence of disease (or pathogen) at a given point in time and to identify characteristics associated with relatively high or low prevalence of disease (Diehr et al., 1995, Dohoo et al., 2009).

This study had five key objectives:

1. Operationalise new remote-sensing methods to randomly select kauri for ground survey.
2. Spatially describe the baseline prevalence of *P. agathidicida.*
3. Spatially describe the baseline prevalence and severity of symptomatic kauri with suspected kauri dieback.
4. Identify and collect data on key factors that could affect disease risk for hypothesis generation.
5. Collect baseline data on ecological factors as indicators of ecosystem impacts from kauri dieback.

The study design used for this survey broadly followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (O'Connor et al., 2016). The results from this study will be used to inform the ongoing and adaptive management of kauri dieback in the Waitākere Ranges and across New Zealand. The collection of baseline data will provide a comparison dataset for repeated cross-sectional monitoring of the same cohort of trees.

2.3 Methods

Ngā tikanga

2.3.1 Study design

2.3.1.1 Unit of interest

The units of interest were individual kauri trees. This is consistent with the recommended unit of interest for the National Kauri Dieback Programme (NKDP) baseline surveillance (Stevenson and Froud, 2020). The classification of individual trees was further refined by size with a minimum diameter at breast height (DBH) of 10 cm. This is consistent with historical tree assessments in native New Zealand forests of mature trees (Ahmed and Ogden, 1987).

2.3.1.2 Population of interest and sampling frame

The population of interest for this study was kauri within the Waitākere Ranges that could be detected by an analysis of remote sensing data. From the population of interest, a sample frame was derived for Auckland Council-managed land that included the Waitākere Ranges Regional Park and a small number of local parks that were contiguous to the Regional Park and these made up the study area (Figure 2-1).

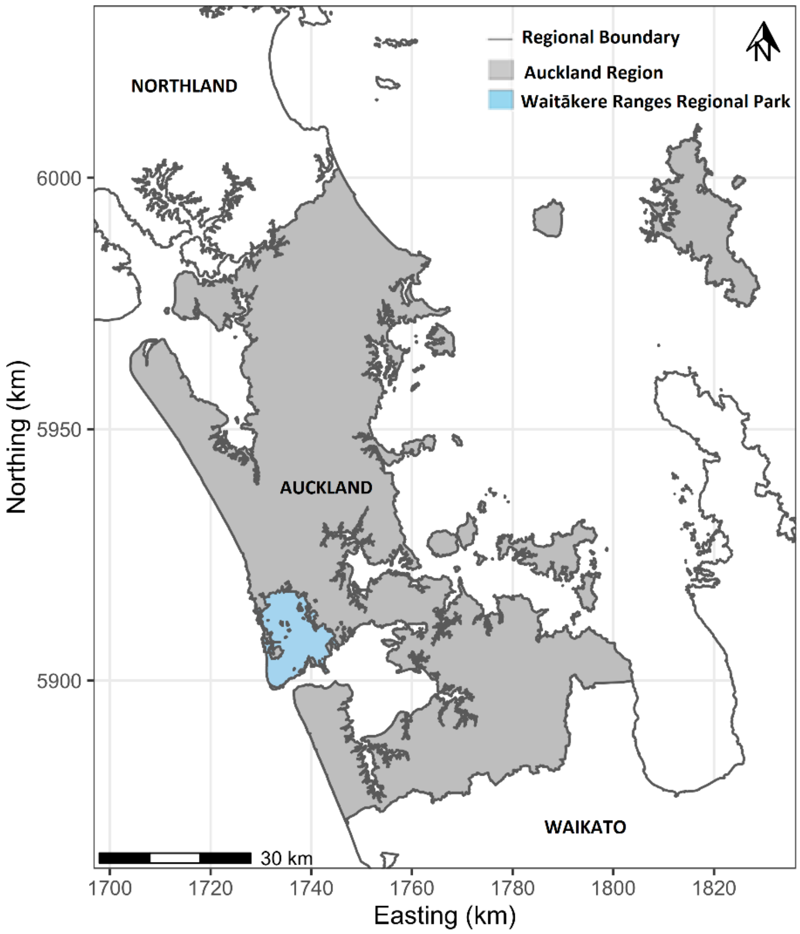


Figure 2-1. Geographical boundary for the study area (coloured in light blue) of the Waitākere Ranges Regional Park and adjacent local parks where this survey was undertaken.

The sample frame included all trees taller than 15 m that could be identified from LiDAR data and classified as kauri. It also included dead and dying trees from both kauri and other species that were indistinguishable from kauri.

Initially tree crowns in the entire Waitākere Ranges >15 m were identified from a canopy height model with 1 m pixel size following Zörner et al. (2018). This method identified 272,295 trees >15 m in the study area. Trees >15 m in the Waitākere Ranges were classified into either "kauri", "dead/dying", or "other" from HiRAMS aerial imagery and LiDAR data (Meiforth, 2020). Training reference data came from photo-interpretation of stereo HiRAMS imagery (systematic cluster sampling) (Meiforth et al., 2019). The method detected 62,998 kauri trees > 15 m and 2,765 dead/dying trees > 15 m in the Waitākere Ranges Regional and local parks where HiRAMS aerial imagery coverage was available. From those classified as "kauri", 1,300 kauri trees > 15 m were sampled randomly for manual validation and confirmed as "kauri" by photointerpretation of HiRAMS imagery (Figure 2-2).

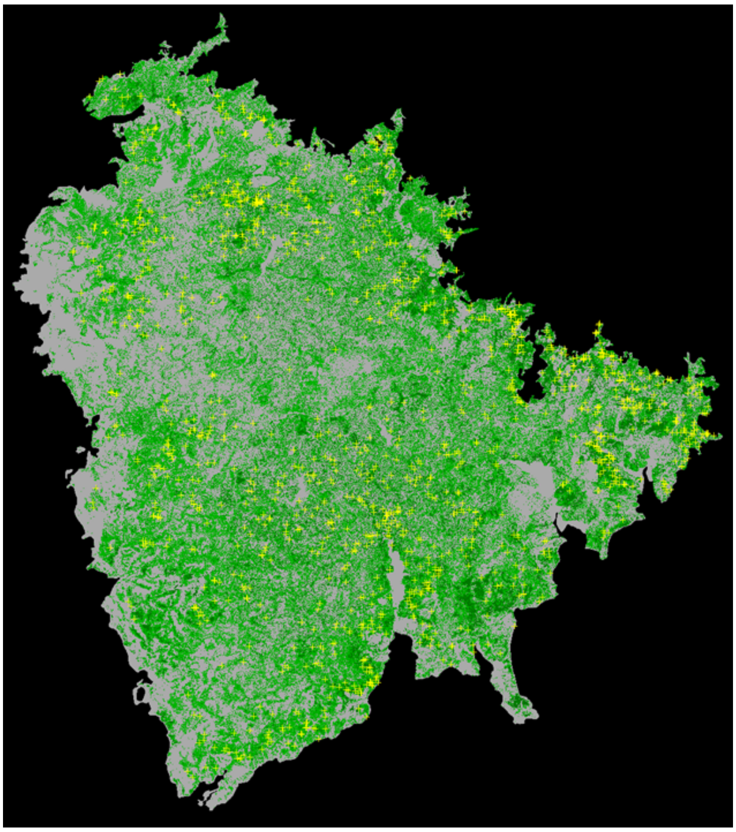


Figure 2-2. Random sample (yellow crosses) of 1300 kauri trees > 15 m tall in the Waitākere Ranges. Dark green is forest >15 m tall. Light green is forest 8-15 m in height. Grey is shrubland less than 8 m in height.

The sample frame GPS coordinates were extracted from a map, based on the central point of large trees (avg. height >20 m) that had been automatically canopy segmented and for the central point of smaller emergent canopy trees >15 m (or small ricker stands where canopy overlap prevents the automatic segmentation of individual crowns) (Figure 2-3).

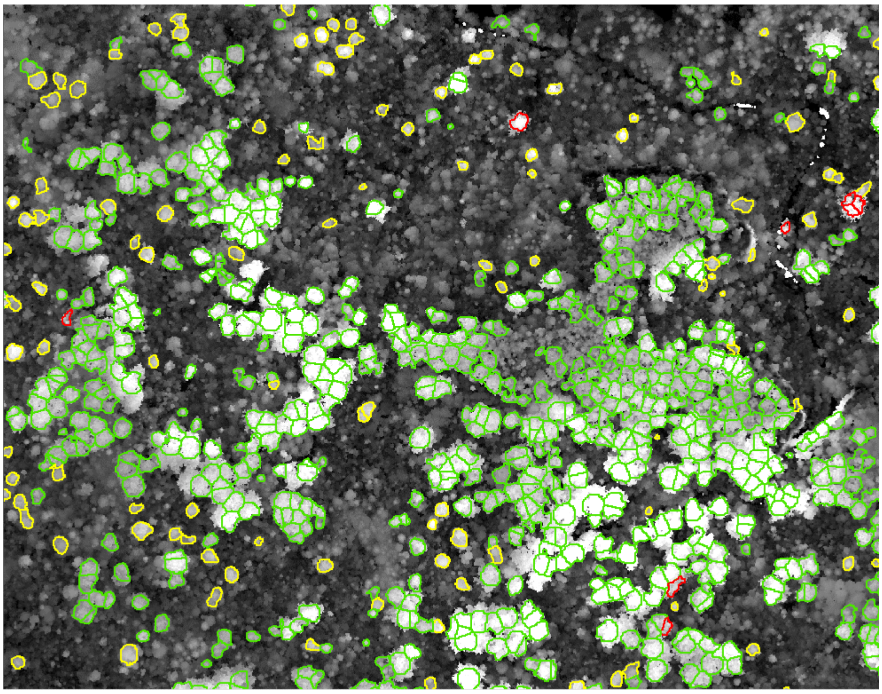


Figure 2-3. Example of large trees >20 m tall, identified by remote sensing in a map of the Cascades area of the Waitākere Ranges. The map has 3 classes: GREEN = kauri with healthy crowns or thinning canopy or thinning with some branch dieback (canopy score 1-3), RED = trees with severe dieback or dead trees (canopy score 4 & 5) and YELLOW = other tree species (canopy score 1-3).

Using aerial image interpretation on reference data of 807 trees >15 m across the Waitākere Ranges, the mapping accuracy (a measure commonly used in remote sensing, meaning the proportion of units correctly classified) was estimated to be 90.2%.

A predicted kauri extent layer for the whole of the Waitākere Ranges combining 68,420 GPS coordinates of predicted kauri trees formed the sampling frame within the study area (Figure 2-5).

The GPS coordinate sites for the sample trees were drawn at random from the sample frame and then confirmed as likely kauri by manual interpretation of imagery. Two separate classifications were performed using stereo image interpretation for training, an object-based LiDAR/HIRAMS combination and an object-based LiDAR/WorldView2 2019 (WV2) combination (where HiRAMS coverage was not complete). Both results had cross-validation scores around 91%. Where the random forests probability result from either classification process showed a strong likelihood of kauri, they were considered for potential sampling. Trees were chosen randomly from that population and checked on screen using a combination of three imagery sources side-by-side on screen, HiRAMS (25 cm) where available, HIRES (8 cm) and pan-sharpened WV2019 (50 cm). The trees in the Worldview2-only area (i.e., where HiRAMS was not available) were more difficult to confirm but were informed by what had been learned in the areas where all three image types were present. In addition, two distinct areas of the western coastal area had cloud obscuring both the HiRAMS and WV2 imagery. Kauri trees were manually identified from high resolution (7.5 cm)

RGB aerial imagery in these areas. There were 5228 trees above 15 m (identified via LiDAR) reviewed in these cloud areas, of which 1899 were classified as kauri. Some of the cloud covered areas did, however, overlap with AISA spectral imagery and therefore AISA methodology described above was used to identify 1244 kauri and 26 dead and dying trees in the overlap areas. The 1899 kauri from manual RGB classification plus the 1244 kauri and 26 dead and dying were combined and randomly sampled at the same rate as the LiDAR/HIRAMS detected trees (detailed numbers are provided in Figure 2-5).

While <15 m tall and non-canopy kauri rickers, saplings and seedlings were excluded from the sample frame due to the limitations of remote sensing, the field monitoring included a brief assessment of kauri in smaller size classes growing near sample trees.

Samples were drawn in a fully randomised process to ensure that all eligible trees had an equal chance of selection. As the remote sensing methodology does not differentiate groups of trees that fit within the kauri dieback canopy classes of dead (but still standing) and dying (canopy classes 5 and 4 respectively), both classes were included in the sample frame and were eligible for sample selection as they are an important component of the baseline prevalence. Canopy classes are defined in Figure 2-7. Dead trees were reported separately from the baseline prevalence estimate. However, as these dying trees may be lost to follow-up for repeat monitoring in the future, a sample size buffer was included to achieve robust sample numbers even in their absence.

Eligibility for inclusion of trees in the sample selection was considered with mana whenua to ensure appropriate cultural consideration was given to trees or areas of cultural significance. Mana whenua were offered the opportunity to review the location of selected trees to exclude any on cultural grounds, if necessary. No selected trees were excluded by mana whenua. Trees inaccessible due to health and safety risks were identified by field survey teams and these were replaced wherever possible with the kauri tree of > 10 cm DBH closest to the original selected tree, regardless of disease status.

The sample size calculation was adjusted to account for potential future loss of trees from the monitoring population. Loss of trees could occur through misclassification as kauri by remote sensing, incomplete field data, tree death, failure to locate tree from the ground survey, landslips, felling for works, accessibility issues or other reasons that may occur over time.

2.3.1.3 Sample size calculations

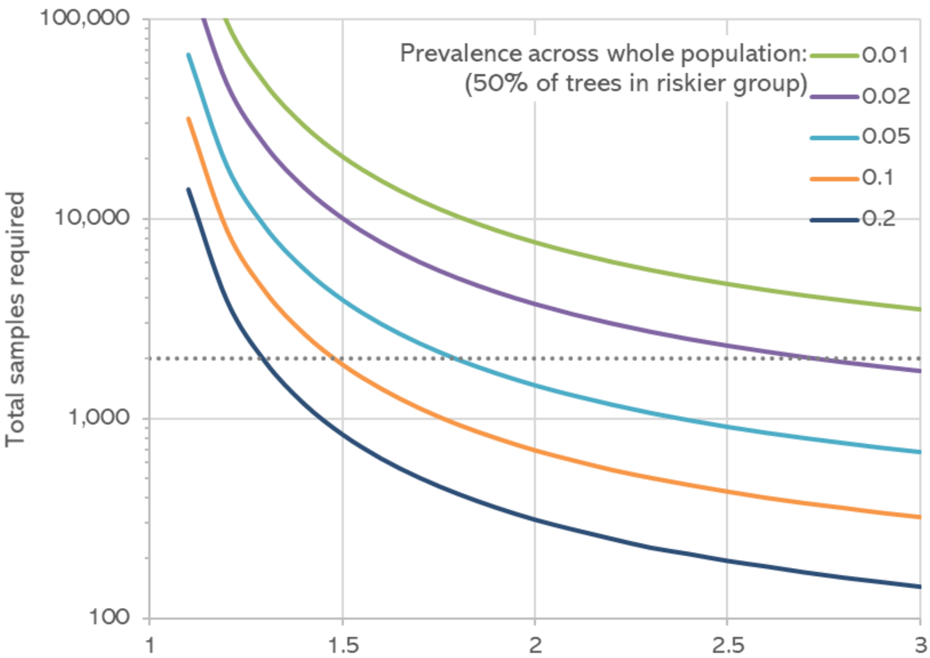
The number of mature kauri over 15 m tall in the study area was estimated by remote sensing to exceed 68,000 trees. Another aim of the study was to collect enough data to estimate the frequency of potential factors associated with the development of kauri dieback to guide future research on understanding such risk factors. A prior conservative estimate of kauri dieback disease prevalence of at least 5-10% (A. Jamieson, Auckland Council, pers. comm.) was used to inform sample size calculations to obtain sufficient risk factor data to measure effects (Lázaro et al., 2020, Thrusfield, 2007). In addition, sufficient random samples needed to be taken to ensure that enough were sampled across the main risk categories of interest, such as: proximity to walking tracks; forest age (mature or regenerating); and tree size (emergent or ricker). Ideally, comparison would occur between equal numbers of trees from high and low risk groups, but this is rarely possible from a completely random sample of trees, so it is important that sufficient samples are taken to have enough statistical power to analyse potential risk factors where the probability of exposure was low (i.e., the risk factor is uncommon in the population).

A suitable sample size minimises Type 1 and Type 2 error rates. A type 1 error occurs when a study declares a factor which is not truly a risk factor as significant. This is primarily guarded against during the statistical analyses by setting the probability that a non-important factor will be identified as a risk factor by chance alone at a suitably low level (usually 5% – which means the results have 95% “confidence”) (Kasiulevičius et al., 2006). A type 2 error occurs when a study fails to detect a risk factor which is real, and large enough to be relevant. This is guarded against by setting the “power” of the study to a relatively high level (usually 80%) and this determines the minimum sample size (Dohoo et al., 2009, Kasiulevičius et al., 2006). This means that if a risk factor is sufficiently important to warrant detection, the study has an 80% chance of detecting it.

The final element needed to determine minimum sample size is the magnitude of the risk effect that we wish to detect. This can be characterised by the prevalence ratio, being the prevalence of kauri dieback in the presence of the risk factor relative to that in its absence. Factors that elevate the risk of disease by only a little will be much more difficult to detect (i.e., require a greater number of observations) compared with those where the strength of association is much stronger. A disease prevalence ratio of 2 (i.e., the risk of disease in trees exposed to a specific risk factor is 2 times higher than those that are not exposed to the risk factor) was considered a reasonable magnitude of risk effect for the study to detect.

Given the overall estimated prevalence of kauri dieback, the proportion exposed to a risk factor, the prevalence ratio and the desired Type 1 and Type 2 error levels, the minimum random sample size required was calculated (Fleiss et al., 1980) (Figure 2-4).

(a)



(b)

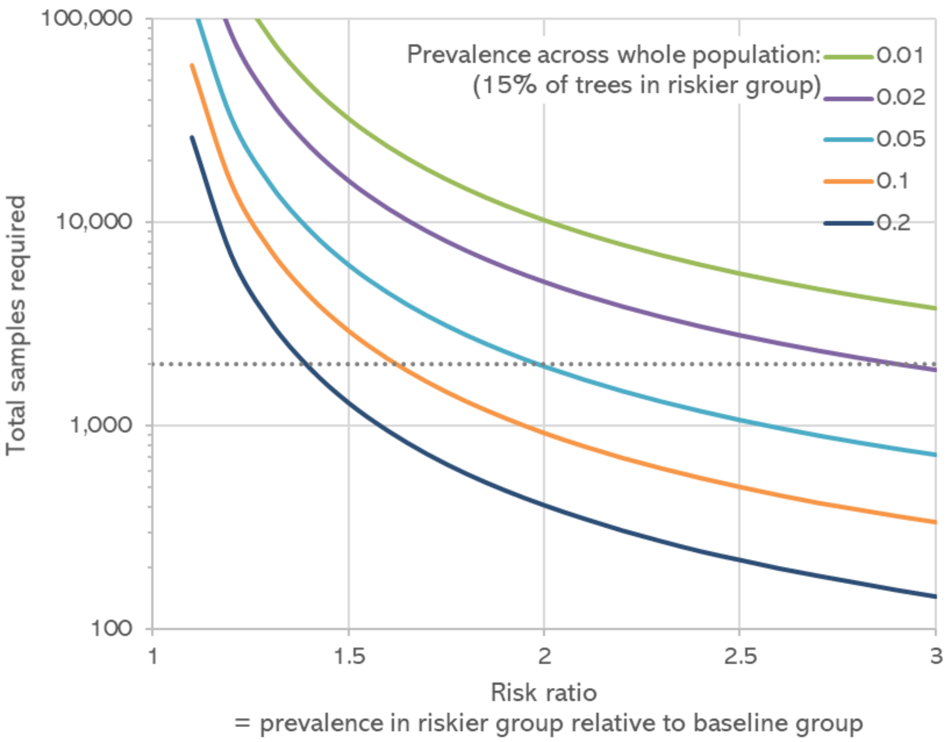


Figure 2-4. Total random samples required to detect a risk factor for kauri dieback disease with 80% power and 95% confidence, depending on the prevalence ratio (strength of the risk effect) and disease prevalence (different lines). In (a) half of all samples are exposed to the risk; in (b) only 15% of samples are exposed. The dotted line shows a proposed sample size of 2000 trees.

Given prior estimates ranging from 5-20% disease prevalence, provided by experts familiar with kauri dieback expression across the study area, a sample of 2000 trees provides a suitable minimum sample size according to the sample size calculations. However, consideration was given to ensuring the proposed sample size accounted for the possibility of misclassification bias arising from imperfect testing (visual assessment of kauri dieback). Misclassification of the disease status of trees means that targeting a prevalence ratio of 2 would require sampling for a prevalence ratio of 1.5 (I. Dohoo, University of Prince Edward Island, Canada, pers. comm.). Based on prior minimum estimates of 5% overall disease prevalence and 15% of trees exposed to risk, this would increase the number of samples needed from around 2000 to around 6000 but reduces to 3000 if the overall disease prevalence is closer to 10%, which is estimated to be more likely by our field experts. Given more sampling provides greater statistical support to assess factors contributing to development of disease in kauri consistent with kauri dieback and accounting for potential missing data, an initial target of 3500 trees was set and protocols to minimise misclassification by having standardised field observations performed by experienced and trained observers were established. As this was at the high end of sample size estimates, a review of sample sizes was undertaken 6 weeks into the survey to determine if it could be reduced. The sample size was subsequently adjusted to a target of 2500, based on our estimated disease prevalence being closer to 10% and predictions of how many samples could be completed before winter to avoid wet and muddy conditions that may risk further pathogen spread (Figure 2-5).

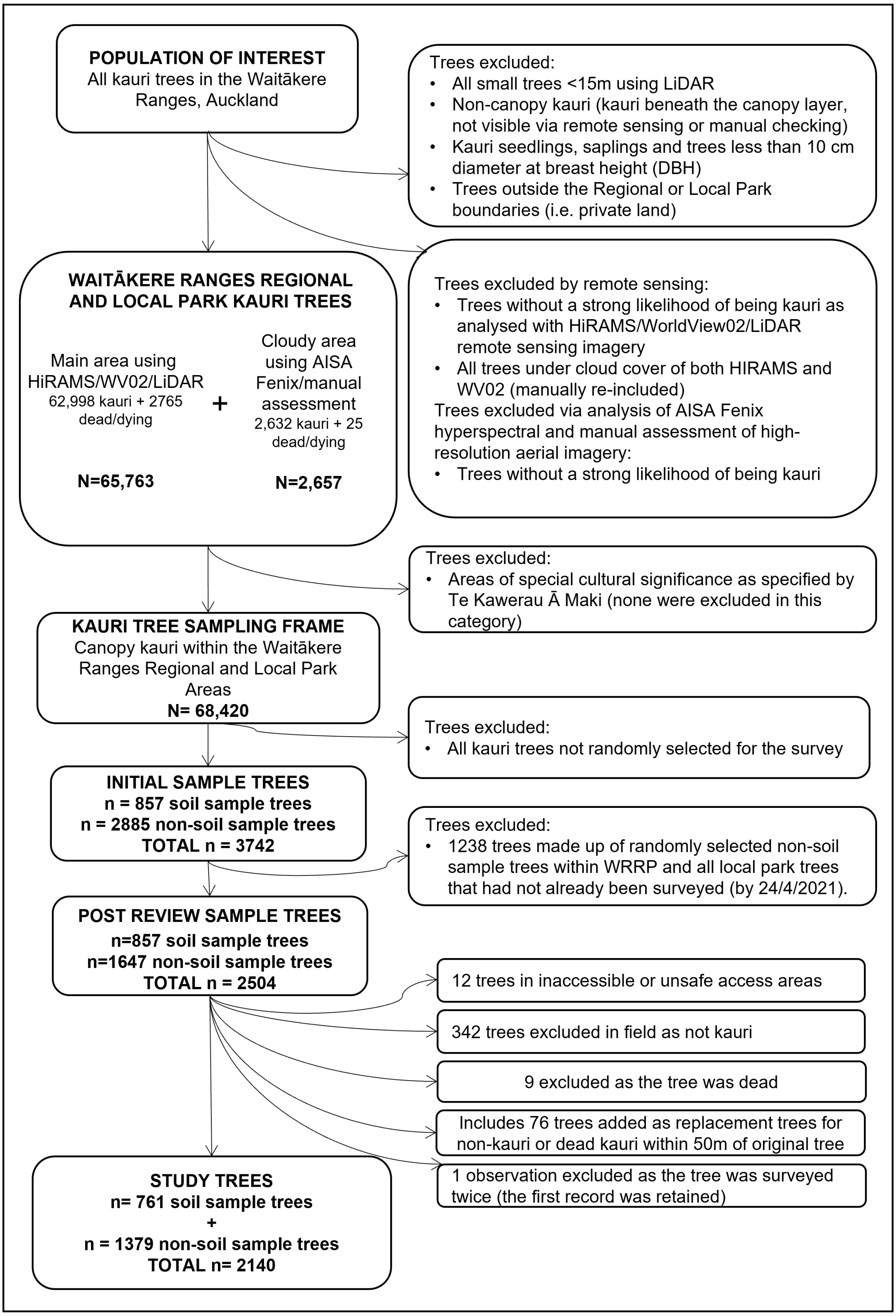


Figure 2-5. Sampling frame diagram showing how trees from the full population of interest were reduced to a sample frame for random selection of trees. It also shows the steps to reduce the sample size halfway through the survey and the final group of trees in the study. WRRP refers to the Waitākere Ranges Regional Park.

2.3.1.4 Identification of risk and impact factors

Variables of interest for ground monitoring were identified through a desktop review of existing ground surveillance variables and special hui (culturally informed workshops), with mana whenua, Auckland Council subject matter experts and a range of external experts in plant pathology and kauri ecosystem health.

The desktop review considered variables from the 2014/15 Auckland Council kauri dieback monitoring form, the National Kauri Dieback Programme (NKDP) monitoring form (unpublished report), Auckland Council kauri dieback objectives, recommended variables for the NKDP phosphite standard operating protocol for field monitoring (unpublished SOP), the Myrtle Rust monitoring form (Sutherland et al., 2019) and a draft kauri dieback causal diagram from Cogger et al. (2016).

Consideration was given to all ecosystem variables that were considered possible for ground monitoring and then a set of representative variables were developed for testing in the monitoring form. These measurements were refined during co-development, pre-testing and peer review by kauri dieback and plant pathology experts.

The final variables are in Appendix A.

2.3.1.5 Pre-testing the monitoring form

The data capture and in-field methodology were further refined during pre-testing prior to the commencement of the survey by a representative from Te Kawerau ā Maki, experienced field team members and ecologists. During pre-testing, each variable was measured, discussed and adjusted if required. The field monitoring form was estimated to take between 15-30 minutes per tree, depending on whether a soil sample was required. Through this process, some variables were identified as being more suitable for detailed plot-based ecological studies than routine surveillance and were not included on the monitoring form.

Adjustments included changes to the standard units of measurement, distance from tree for impact measurements, and changes to levels or options in categorical variables were made to ensure that they covered the range of each variable being observed. The detailed measurement instructions for each variable were updated to ensure clear language and consistency of interpretation of how to undertake the measurement by field survey teams (Appendix A). Hygiene requirements for each measurement were developed (e.g., cleaning of rods used to measure the organic soil layer depth after each tree) and the tikanga (culturally correct way) of undertaking the survey was shared by Te Kawerau ā Maki.

2.3.2 Data collection

Surveys were undertaken by a 16-person team of trained surveyors working in small teams for consistency of assessments and health and safety reasons. Areas estimated to have higher disease prevalence were initially prioritised to increase the exposure to a range of kauri dieback symptoms to allow the methodologies, data capture and consistency across the surveillance team to be tested. Thereafter target areas were scheduled to target different geographical sectors (NW, NE, SW, SE) of the Park each week to minimise the spatial and temporal bias in field assessment and soil collection over the duration of the surveillance programme. Field work was suspended during periods of rainy weather as part of the hygiene precautions.

The survey measurements were collected using a monitoring form loaded into ArcGIS Survey123 on waterproof hand-held tablets. Minor adjustments continued to be made to the electronic survey form to improve functionality during field team training at the start of the survey. Final adjustments were made 6 weeks after the start of the ground monitoring.

The survey was carried out between 8 March 2021 and 8 July 2021. An assessment of progress 6 weeks into the surveillance programme identified the need to rationalise the sample size based on field navigation, observational inputs and logistical challenges. At this point in the programme, 771 trees had been surveyed based on the original design. The revised design retained all previously selected soil sampling trees (857 trees), excluded all local park trees not already sampled and then sub-sampled a further 1647 trees from the originally pre-selected trees for required visual assessment only within the Waitākere Ranges Regional Park (Figure 2-5). Collection of ecological impact variables was reduced to soil-sampled trees only. Because of early site prioritisation for training, statistical advice was sought from expert reviewers and an adjustment to the weighting of samples contributing to the calculation of overall symptomatic kauri prevalence was advised. This was to avoid a bias towards an over-estimate of disease.

Teams were provided with the GPS coordinates of selected trees and used accurate hand-held field GPS units to locate trees. Where multiple kauri trees were present at GPS points, the closest kauri of >10 cm DBH to the GPS coordinate was selected by the ground survey team. Selection of the kauri was based purely on proximity and not on health status.

All monitored trees were tagged with robust aluminium tree tag identifiers to enable future identification and monitoring of the same tree. Tree tags were attached using nails at the uphill point of the tree, or north facing on non-sloping land 1.4 m above the ground as shown in Figure 2-6.

Figure 2-6. Tree tags used for permanent marking of monitored trees.

Measurement guidelines and additional details of all variables collected during the ground survey are detailed in Appendix A.

2.3.2.1 *P. agathidicida* sites

A ***P. agathidicida* site** was defined as a point location where the presence of *P. agathidicida* has been confirmed (from a tree, soil or other substrate), using an approved test at an approved laboratory. This includes historical *P. agathidicida* detections.

A ***P. agathidicida* not detected <u>site</u>** was defined as a point location where the presence of *P. agathidicida* was not detected (from a tree, soil or other substrate), using an approved test at an approved laboratory.

For samples tested in this study, the approved test was soil sampling and bioassay, and the approved laboratory was Plant and Food Research Ltd, Havelock North.

2.3.2.1.1 Soil sampling

Soil samples were collected from all trees that had been randomly pre-selected for soil sampling. The surveyors collected a composite sample comprising four c. 180 g sub-samples from within the root zone of the selected kauri. Soil sub-samples were taken at 90° intervals at 1-2 m from the trunk starting either below the tree tag, or if the tree had a basal or lateral root bleed, below the most active bleed. Soil was taken to a depth of 10-15 cm after scraping away the loose litter layer and contained a mixture of organic material, mineral soil and kauri feeder roots (wherever possible). Surveyors were instructed to optimise the recovery of *P. agathidicida* from the soil, by ensuring that kauri root material, distinguished by its characteristic colour and root nodules, was included in the soil sample. If surface-level roots were absent, surveyors retrieved samples from slightly further than 90°, based on topography of the site and knowledge of where roots are most likely to be located (e.g., away from rocky outcrops or wet depressions) and if there were still no roots at a second point, to collect the root-free sample so as not to disturb the soil any more than necessary. The total volume of the composite sample per tree was required to fill at least ¾ of a medium (220 mm by 250 mm) zip-lock bag and weigh approximately 650-750 g. Trowels were cleared of organic material and soil, washed with methylated spirits and left to dry for a few seconds after each sample before being stored to minimise cross-contamination among trees and meet hygiene requirements.

Samples were stored in backpacks during field collection and taken into storage at the end of each day. The soil samples were stored in a cool (10-25°C), dark place until dispatch. The samples were double-bagged and couriered in boxes overnight to the Plant and Food Research Pathology Laboratory in Havelock North for processing. To ensure they were not left in courier depots over the weekend, they were only sent Monday-Wednesday. Samples were stored at room temperature.

2.3.2.1.2 Soil bioassay

Samples were tested using the standard operating protocol for soil-baiting bioassay which has been optimised to preferentially obtain *P. agathidicida* (Beever et al., 2010). This was followed by morphological identification of resulting cultures following standard laboratory hygiene, isolation and surface sterilisation techniques and specific methods detailed by Beever et al. (2010, Section 7.3, Pg 42.) with a few minor alterations. Approximately 200 mL of soil was dried and then baited in 680 mL circular plastic pottles. Any clods of soil were crumbled with sterile spoons. The soils were moist incubated by spraying with a fine mist of Reverse Osmosis (RO) water until no dry areas were observed, sealed, and incubated for 3 days before being slowly flooded with RO water and baited. Lupin baits were germinated by soaking lupin seed in RO water for 1 h, sowing on moist paper towels sealed in a zip-lock bag and incubated at room temperature for 2 days before use. Himalayan cedar (*Cedrus deodara)* needles were harvested directly from nearby trees targeting dark green mature needles. Four lupin baits were suspended through parafilm on the water surface of the flooded soil samples, while six whole cedar needles were floated directly on the water surface. Samples were further incubated in the light at 20 °C for 2 days at which point bait tissues were removed, rinsed in sterile RO water, soaked in 70% ethanol for 30 s, rinsed again in sterile RO water, blotted dry on paper towels and placed onto P_5ARPH agar plates, sealed and incubated in the dark at 18-20 °C. Plates were inspected at 2-day intervals for *Phytophthora*-like cultures and sub-cultured onto V8 juice agar for 4-10 days and observed periodically for characteristic morphological features under a compound microscope for primary identification of expected species or to genus-level for more cryptic species. Species identities based on morphological features (Scott et al., 2009, Weir et al., 2015) were provided for *P. agathidicida, P.*

cinnamomi and P. multivora, otherwise *Phytophthora* cultures were recorded as *Phytophthora* spp. Where no *Phytophthora* cultures were obtained, samples were given a not-detected result.

Trees with positive *P. agathidicida* soil samples were classified as *P. agathidicida* detected vs *P. agathidicida* not detected and were also classified as *P. agathidicida* sites in accordance with the case definition of Stevenson and Froud (2020) for the calculation of GIS variables.

2.3.2.2 Disease severity variables

Basal or lateral root bleeds consistent with kauri dieback were measured as present, not sure, or absent. Bleed activity was measured following the Horner methodology of whether the gum is sticky (active), soft but not sticky (semi-active) or hard (not active) and relates to whether the tree is still exuding gum.

Basal bleed height was measured to indicate disease severity, in that it indicates how long a tree may have been infected as the pathogen infects via the roots and then travels up the trunk over time, remaining at the leading edge (outer/upper edge) of the lesion. This enables future monitoring to determine how fast lesions develop over time. Where more than one bleed was present on the trunk, then the highest one was assessed.

Percentage of trunk with basal bleeds was measured as an estimate (in deciles) of the base of the trunk that was affected by the basal bleed. This gives a crude indication of the diameter of girdling that has occurred through pathogen infection.

Canopy dieback was quantified based on the Dick and Bellgard (2012) 5-scale canopy health score, with an adjustment to include half-points. This was to provide more differentiation particularly between 2-3 and 3-4 canopy scores which is consistent with more recent disease scoring by Horner et al. (2019b) (Figure 2-7).

Figure 2-7. 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 2012) versus the modified half-point scale.

Kauri canopy and bleed symptoms could be caused by other biotic or abiotic factors and therefore the opinion of a trained observer/surveyor is required to determine if the recorded symptoms are consistent with kauri dieback. The kauri dieback field status was assessed by trained surveyors observing all symptoms, the surroundings of the tree and any other potential causes of symptoms. Field status considers whether the observed symptoms were consistent with kauri dieback (to meet the final symptomatic criteria of the case definition). Options were non-symptomatic kauri; kauri with ill-thrift (probably not kauri dieback); kauri with possible kauri dieback symptoms; and kauri with severe kauri dieback symptoms. The field status variable was updated during the sample size review and details of changes are provided in Appendix A.

Canopy colour was assessed from the ground based on all visible canopy and selection was based on what colour the majority of leaves were, rounding down to the healthiest colour if the result was uncertain to enable a change to be detected over time.

Detailed descriptions of disease severity variable measurement are in Appendix A.

2.3.2.3 Symptomatic kauri

The symptomatic kauri prevalence was reported against the Stevenson and Froud (2020) recommended case definition for kauri dieback disease which is updated and summarised in Appendix A. In brief, the case definition for symptomatic vs non-symptomatic trees was met if the symptomatic criteria for kauri dieback (bleeding lesions on the basal trunk, lesions on roots, the presence of canopy thinning, yellowing of the foliage, tree death) were recorded on a kauri tree AND the trained surveyor recorded that these were consistent with possible/probable or severe kauri dieback using the field status assessment variable in the monitoring form (Appendix A).

The surveyors were trained in the variety of basal and lateral root lesion presentations that have been associated with kauri dieback caused by *P. agathidicida.* Trained surveyors only wrote 'Yes' if the bleed was typical of kauri dieback bleeds. Further, they were instructed to select 'Unsure' when they could not determine whether a basal or lateral root bleed was due to kauri dieback or due to other causes (e.g., physical damage). Both 'Yes' and 'Unsure'; were included in the symptomatic criteria component of the algorithm to classify symptomatic kauri. If the field observer stated that symptoms were not consistent with kauri dieback, they were classified as non-symptomatic kauri trees - ill-thrift.

As canopy dieback and colour of foliage were categorical variables, a cut point was selected for each. The level of canopy health score required to be included in the symptomatic criteria was set to a canopy score of 3 or higher after discussion with the field team and I. Horner. This is consistent with being considered symptomatic by Bellgard et al. (2013). Scores from 1-2.5 relate to healthy canopy or some foliage or canopy thinning, whereas scores from 3-5 show signs of branch dieback through to canopy loss and death of the tree. To calculate symptomatic kauri prevalence, trees that scored 5 and were considered dead were excluded. The small number of dead trees are reported separately from the baseline prevalence estimate, as these trees cannot change their disease state in future monitoring, and it is difficult to estimate how long the tree has been dead. The canopy colour score required to be included in the symptomatic kauri group was set to a canopy colour that is more yellow than green and includes yellow-green, copper brown and dead leaves. Trees with a canopy score below 3 or with a canopy colour score below yellow-green were classified as non-symptomatic – healthy or non-symptomatic ill-thrift depending on score and field status. A binary symptomatic kauri and non-symptomatic kauri variable was calculated based on meeting the symptomatic criteria of the case definition, with both symptoms and field status assessed as described in the algorithm in Table 2-1.

In addition, classes within symptomatic kauri were defined by an epidemiological criteria that incorporated soil sample results, where kauri dieback was 'confirmed' for trees at a *P. agathidicida* site (defined in 2.3.2.3), 'probable' for trees within 50 m of a *P. agathidicida* site, and 'suspect' for trees > 50 m away from a *P. agathidicida* site (Stevenson and Froud, 2020).

Table 2-1. Decision algorithm for calculating if the symptomatic criteria were met for the symptomatic kauri trees kauri dieback case definition.

The symptomatic criteria were met if: Basal bleed = 'Yes' or 'Unsure' OR Lateral root bleed = ' Yes' or 'Unsure' OR Canopy score ≥3 OR Canopy colour = 'Yellow-Green' or 'Copper Brown' **AND** Kauri dieback field status (approved observer considers symptoms are consistent with kauri dieback) = 'Kauri with possible kauri dieback symptoms' or 'Kauri with severe kauri dieback symptoms'

2.3.2.4 Risk factors

Risk factors (both causative and protective) that could be measured at the individual tree level, either during ground survey or from existing data sources, were considered for inclusion. They covered host-related variables (e.g., diameter at breast height (DBH)), environmental variables (e.g., aspect, elevation, pig damage) and anthropogenic (human modified) variables (e.g., phosphite treatment, track proximity). The full list of variables and the instructions for data collection are included in Appendix A. Risk maps of GIS collected data are in Appendix G.

2.3.2.5 Ecological impact variables

Several long-term ecosystem outcomes were considered for baseline monitoring and future analysis. Due to the large sample size and relatively short monitoring time available for each sample, plot-based sampling was not considered feasible. However, several ecosystem function variables were included. These variables were measured for all trees selected for soil sampling and were also measured for all trees assessed during the first 6 weeks of the survey. Full details of measurement are provided in Appendix A.

Host-based impact variables included a count of kauri seedlings, saplings, and observations of reproductive structures. Kauri seedling and sapling counts within 5 m radius of monitored trees were assessed and size classes were based on standard plot measures in New Zealand indigenous forests (Hurst and Allen, 2007) of small seedlings (<15 cm); established seedlings (15 cm – 1.35 m) and saplings (>1.35 m tall and <10 cm DBH).

A closest neighbour measure (distance to and DBH) to inform density dependence and succession variables was measured by comparing the DBH of each monitored kauri tree to the nearest neighbouring tree species that had a DBH greater than 10 cm. If the monitored kauri was larger, it was classified as the dominant tree and if it was smaller, it was classified as subdominant.

Forest floor depth (the depth of the soil organic layer) was measured to indicate soil quality and provide a baseline for future potential ecosystem function changes (e.g., forest productivity, nutrient cycle) as described in Appendix A, following the methods of Silvester and Orchard (1999).

A common kauri tree community species checklist (based mostly on tree species) was developed using the University of Auckland Waitākere kauri plot data (unpublished data) and tree species that had the highest mean association with kauri from Wyse et al. (2014) (Table 2-2). These were used to come up with a list of 15 most common tree species within Auckland kauri forests. Presence of trees from this checklist were recorded within 10 m of the monitored tree to provide an indication of species diversity.

Table 2-2. Common kauri forest-associated plant species (scientific and common names) selected for observation during the 2021 Waitākere Ranges survey

Scientific name	Common name
Astelia trinervia	kauri grass
Brachyglottis kirkii	Kirk's tree daisy
Coprosma arborea	māmāngi
Coprosma lucida	shining karamū
Dacrydium cupressinum	rimu
Knightia excelsa	rewarewa
Kunzea robusta	kānuka
Leucopogon fasciculatus	mingimingi
Pseudopanax crassifolius	lancewood
Melicytus macrophyllus	large-leaved māhoe
Myrsine australis	māpou
Nestegis lanceolata	white maire
Olearia rani	heketara
Pectinopitys ferruginea	miro
Phyllocladus trichomanoides	tanekaha
Toronia toru	toru

2.3.3 Data analysis

All data analysis was carried out using R Statistical Software (R Core Team, 2020).

2.3.3.1 Descriptive statistics

A descriptive summary of each variable for the monitored trees was calculated to set a baseline for future monitoring. For variables that were similar, such as disturbance categories of fallen tree and windthrow, these data were combined into new variables for reporting.

Histograms and boxplots were used to visualise data distributions and frequencies. Univariable analyses using two by two tables and the Fisher exact test in the epiR package or separate, unmatched, logistic regression procedures were used to determine associations between ecological impact variables and disease. The level of statistical significance was set at $P \leq 0.05$ and was assessed using the log-likelihood ratio test statistic. Linear regression was used to determine associations between continuous variables and correlations were tested with the Pearson correlation coefficient.

2.3.3.2 Survey design adjustment

A weighted survey design adjustment procedure was used to calculate an adjusted symptomatic tree prevalence following the methodology of Kneipp et al. (2021) based on (Lumley, 2011). The weighting adjustment calculated the estimated symptomatic tree prevalence within stream sub-catchments where monitoring of one or more trees had occurred (n=162, with 59 stream sub-catchments excluded as they did not contain any surveyed trees). The total estimated number of kauri in each stream sub-catchment area was divided by the number of kauri sampled in each stream sub-catchment to return a sampling weight for each sub-catchment. The number of diseased kauri consistent with kauri dieback in each sub-catchment was then multiplied by the sub-catchment weight to return the estimated number of diseased kauri in the sub-catchment. The estimated number of diseased kauri in each sub-catchment area were then summed and divided by the total estimated number of kauri across all sub-catchment areas to return a survey adjusted prevalence estimate.

The adjusted prevalence and confidence intervals of diseased trees were calculated using the contributed epiR (Stevenson et al., 2012) and survey (Lumley, 2012) packages in R. This only applied to the symptomatic kauri prevalence calculation as the *P. agathidicida* prevalence was based on the soil sample trees where no sample size reduction was made.

2.3.3.3 Point pattern maps

Point pattern maps were generated using the geographical boundary for the Waitākere Ranges survey study area to plot two point pattern maps using the R package ggplot2 (Wickham et al., 2016). The first map plotted the point location of all the surveyed kauri trees with points coloured according to their disease status (i.e., symptomatic kauri trees and non-symptomatic (healthy and ill-thrift)) using the case definition. The second map plotted the point location of all the kauri trees from which a soil sample was taken with points coloured according to their *P. agathidicida* detection status.

2.3.3.4 Choropleth maps

The prevalence of symptomatic kauri trees was calculated as the proportion of surveyed trees that were classified as symptomatic while the prevalence of *P. agathidicida* was calculated as the proportion of soil samples in which the pathogen was detected. Crude prevalence estimates were calculated for different natural water drainage sub-catchments or stream sub-catchments and plotted as choropleth maps using the R package ggplot2 (Wickham, 2016). GIS data for sub-catchments were provided by Auckland Council and imported into R using the sf package

(Pebesma, 2018) and plotted using the same projection coordinate system as the point pattern plots (i.e., NZGD2000).

These maps, while useful, are limited by their sensitivity to sub-catchments with a small underlying population at risk. Therefore, to account for the heterogeneous density of kauri trees, a local empirical Bayes (EB) smoothing approach was used to compare the prevalence in each sub-catchment to a local estimate of the mean using the "EBlocal" function in the spdep package (Bivand and Wong, 2018) and plotted as choropleth maps.

2.3.3.5 Relative risk surfaces

In addition to the choropleth maps, four univariate kernel density maps were plotted to show the density of (i) symptomatic kauri trees, (ii) non-symptomatic kauri trees, (iii) *P. agathidicida* detected soil samples and (iv) *P. agathidicida* not detected soil samples using the spatstat package (Baddeley, 2015). The effect of the sample size reduction (after 6 weeks of sampling) on these analyses is to have a slightly higher precision in areas that were sampled early compared to those sampled later, so no adjustment was required. The spatial relative risks for both symptomatic kauri and the presence of *P. agathidicida* after accounting for the varying density of the sampled population were then estimated and plotted. The spatial relative risk represents the ratio of two kernel-estimated densities (i.e., symptomatic vs non-symptomatic and *P. agathidicida* detected vs not detected) after accounting for variability of the underlying population. These can be used to identify regions with significant elevated spatial risk (Davies et al., 2018). The relative risk is estimated on the natural log scale, such that values > 0 depict areas of elevated risk (log(0) = 1, and therefore log relative risk values > 0 equate to relative risks > 1, that is, increased risk). For these plots, an adaptive smoothing technique was used for the density estimates to provide the flexibility of reduced smoothing in densely occupied areas without compromising the stability of the estimate elsewhere. Where detected, tolerance contours delineating statistically significant risk elevations were drawn at a significance level of 0.1 and 0.05. The plots were created using the R package sparr (Davies and Marshall, 2018) using a pilot bandwidth of 609.1, a Gaussian kernel distribution, and an evaluation grid with dimensions of 128 raster cells in the east-west (150 m) and 128 raster cells in the north-south (166 m) directions.

2.4 Results

Ngā hua

2.4.1 Collection of samples

Approximately 4,450 field team hours were spent collecting data for our final dataset which contained 2140 completed observations, including 761 soil sampled trees. This equates to an average of 2 person-hours per observation (1 h per two-person team). This time included training, travel time to the forest, navigating to the tree, sites that were visited but not monitored, 10-30 min of direct observation, hygiene procedures and soil collection (where required).

2.4.2 Host detection

The initial estimate of kauri trees that were >15 m and present in the canopy layer of the study area was 68,420 kauri trees. It is unknown how many kauri shorter than 15 m are within the Waitākere Ranges as they were not easily detectable with remote sensing technologies available in 2020/21.

2.4.2.1 Misclassification of kauri

The positive predictive value for host detection was 86% based on the field data (in that 86% of trees classified as kauri by remote sensing were kauri), which is lower than the estimated mapping accuracy of 90.2%. Not all misclassifications had a record of what the tree species was at the point of interest (n=132), however where these were recorded rimu (n=80) was the most misclassified species, followed by northern rata, rewarewa, kahikatea and exotic pine (detailed results are in Appendix B). Based on an estimated population of 68,420 kauri trees in the study area from remote sensing, and a positive predictive value of 86%, we can estimate that the lower limit of kauri >15 m in height in this population is approximately 58,800 trees. As the diagnostic sensitivity of detecting kauri using the remote sensing methods applied is unknown, it is assumed that the method misses some kauri and therefore the upper population estimate limit is unknown. A cross reference with field data (Meiforth et al., 2019) indicates that the crown segmentation method had difficulties in segmenting crowns with small diameters and declining crowns. Another source of errors was manually distinguishing kauri (especially declining kauri) from other tree species on aerial imagery.

2.4.2.2 Dead or inaccessible kauri

On 21 occasions the tree was located as a kauri, however the survey could not be completed due to accessibility reasons (mostly wasp nests or steep terrain) (n=11), because the tree was dead (n=9) or no recorded reason (n=1). Of the 9 trees that were dead, 5 had been pre-selected for soil sampling, which was instructed to be collected for dead trees, and *P. agathidicida* was isolated from 3 of these 5 samples (consistent with the detection rate reported in the pathogen isolation section).

Where the randomly selected tree was not located or was not suitable for survey, a replacement kauri tree was selected for survey. Only 20% of the sites had a suitable replacement tree present (76 of the 363 sites) and typically this was because there were no kauri trees present within sight of the original point of interest.

2.4.3 Pathogen prevalence

Detection of *P. agathidicida* was assessed at 761 kauri tree sites where soil samples were collected. The baseline pathogen prevalence of *P. agathidicida* detection was 76/761 (10%).

The spatial distribution of *P. agathidicida* from the 761 soil sampled trees showed a greater density of *P. agathidicida* detections in the northern, central-western and southern borders of the study area. There was no detection of *P. agathidicida* in the central interior areas of the Park (Figure 2-8).

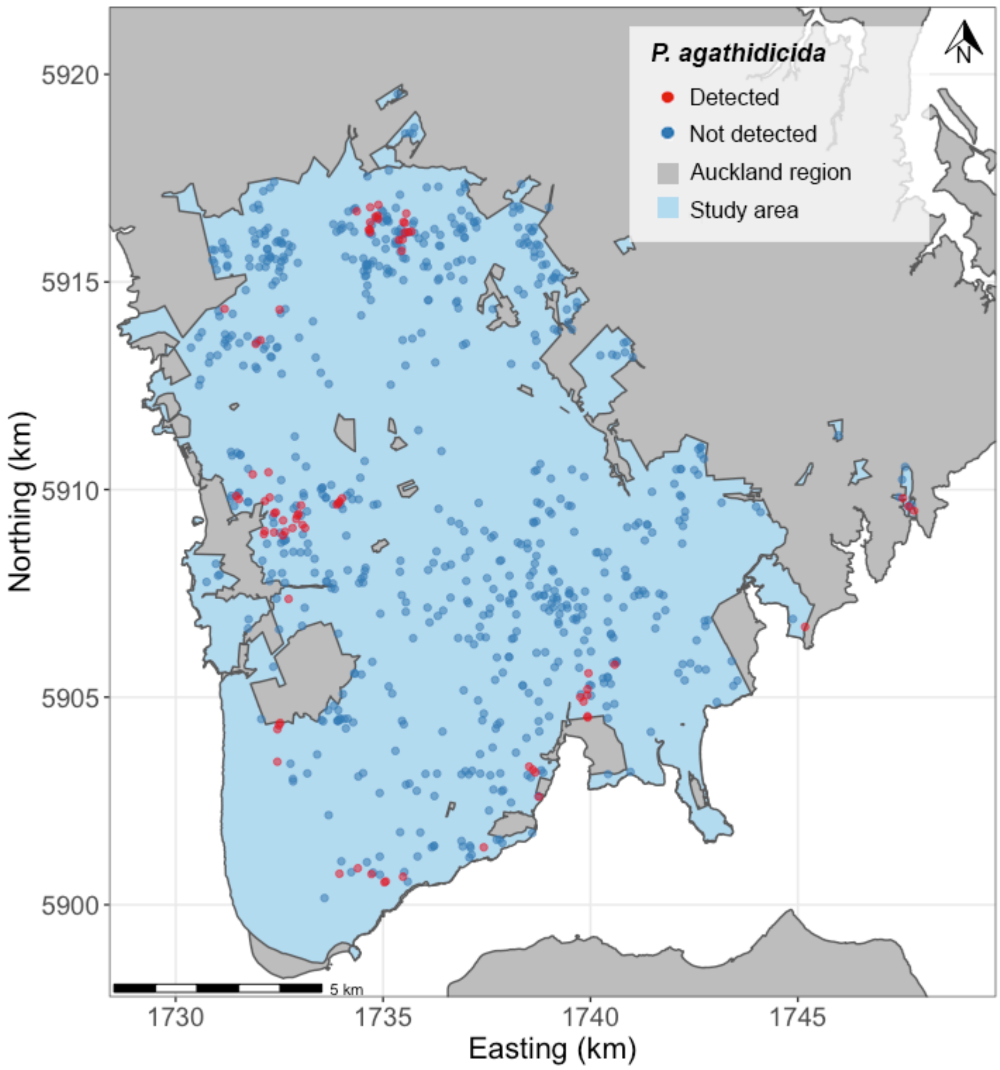


Figure 2-8. Spatial point map showing the location of kauri trees in the study area that had soil samples taken for diagnostic testing (n = 761) with red circles indicating the detection of *P. agathidicida* (n = 76) and blue circles indicating that *P. agathidicida* was not detected (n = 685).

The spatial relative risk surface for *P. agathidicida* detection (i.e., the ratio of positive soil samples to not detected soil samples) shows two regions of elevated detection risk at a significance level of 0.05 in the northern and mid-west areas of the Park (Figure 2-9).

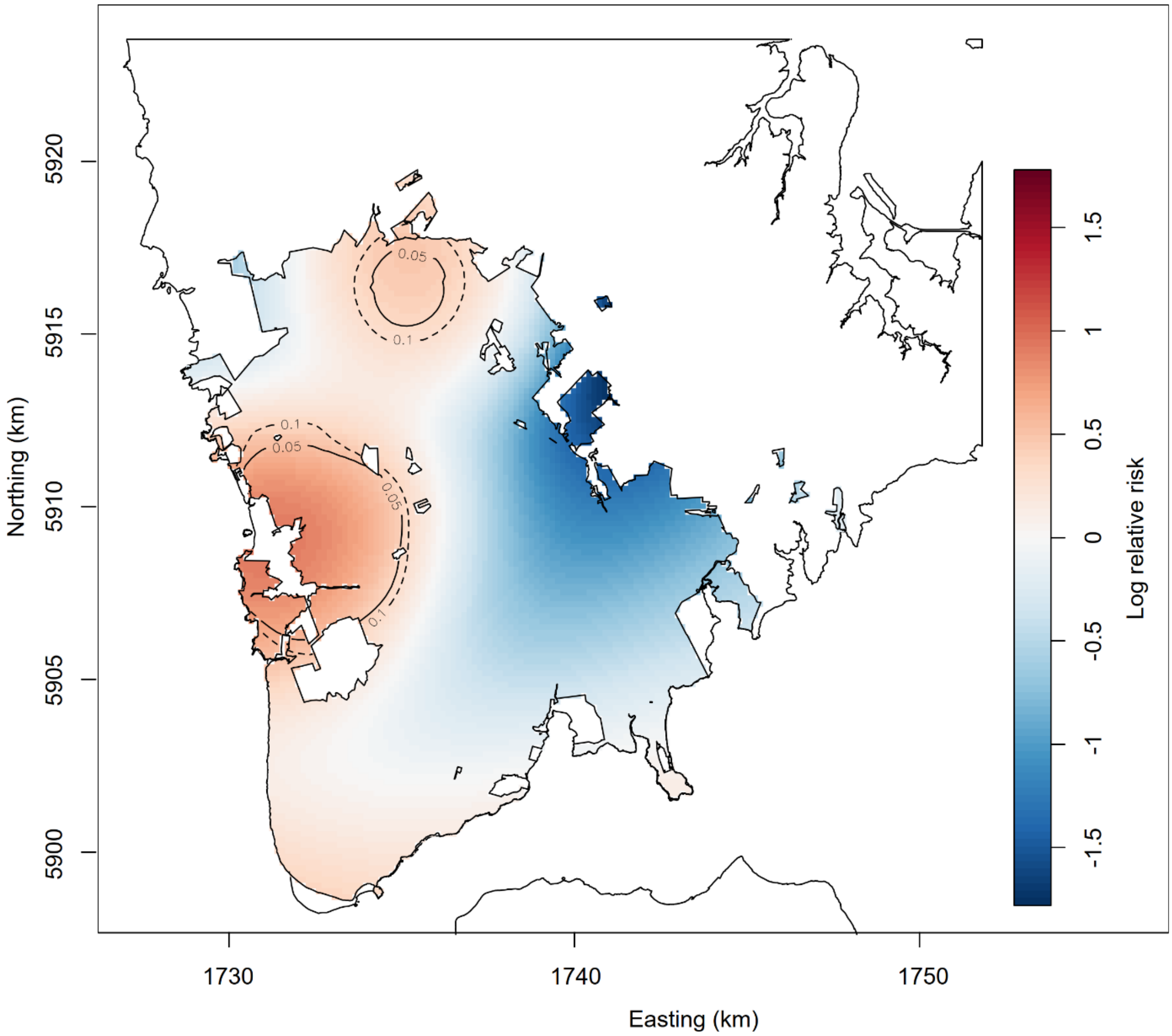


Figure 2-9. A symmetric adaptive bandwidth spatial log-relative risk surfaces map of *P. agathidicida* detection, estimated using kauri trees that had soil samples taken for diagnostic testing (n = 761). The relative risk is estimated on the natural log scale, such that values > 0 depict areas of elevated risk (log(0) = 1, and therefore log relative risk values > 0 equate to relative risks > 1, that is, increased risk). Where detected, tolerance contours delineating statistically significant risk elevations are drawn at significance levels of 0.1 (dashed line) and 0.05 (solid line). White inland spaces indicate areas outside the study area (e.g., Piha village in the central west of the map).

There were 150 small stream sub-catchments included in the study of 761 soil sampled trees. The median number of trees assessed per sub-catchment was 4 trees (25th percentile 2; 75th percentile 7; min. 1 tree; max. 23 trees). A total of 80 stream sub-catchments had 0% *P. agathidicida* prevalence (Figure 2-10).

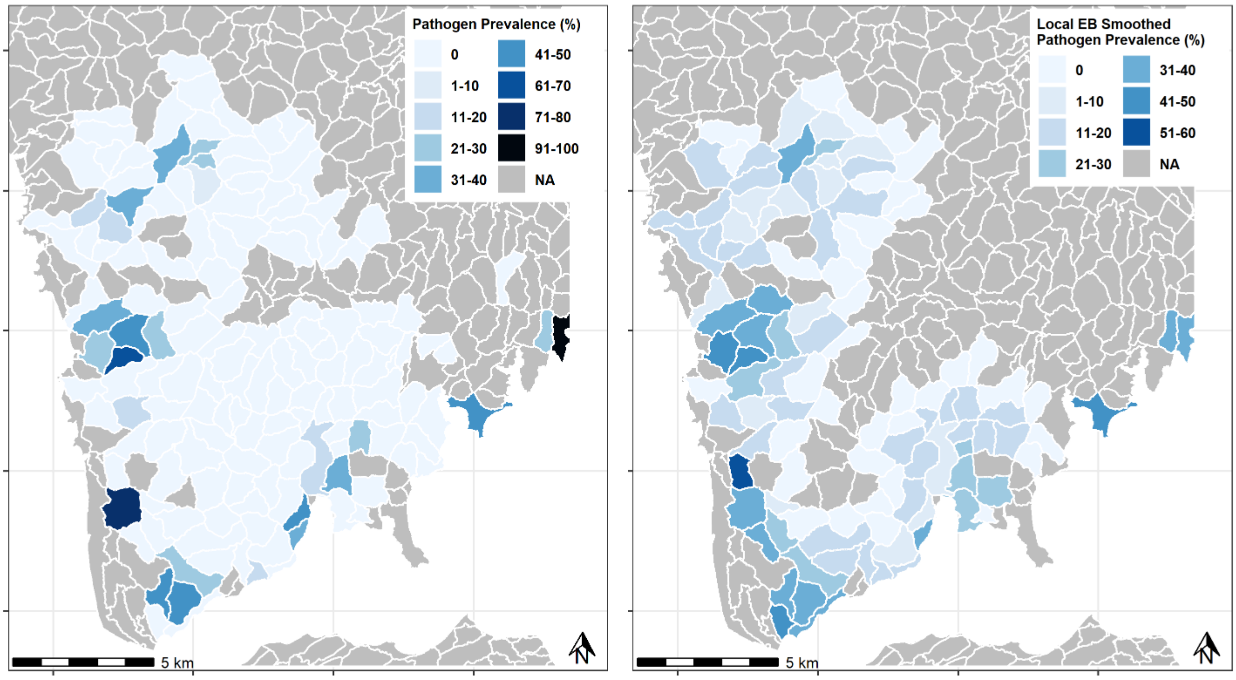


Figure 2-10. Choropleth map showing *P. agathidicida* prevalence (left) and a Bayesian smoothed *P. agathidicida* prevalence (right) calculated using 761 monitored kauri trees in stream sub-catchments. Cells with NA did not have any randomly selected kauri trees within the stream sub-catchment.

Stream sub-catchments provided a useful unit of interest for land management. Empirical local Bayesian smoothing was unable to estimate *P. agathidicida* prevalence in several sub-catchments because of low or zero surrounding sub-catchment prevalence, indicated by the increased number of sub-catchments with missing (NA) values. However, the map still showed a useful visualisation of higher and lower prevalence areas after accounting for differences in the density of kauri trees in each stream sub-catchment.

2.4.4 Symptomatic kauri prevalence

The survey-adjusted symptomatic kauri prevalence across all sites was 16.5% (95% CI: 14.1 to 18.9). This was lower than the overall symptomatic kauri prevalence across all surveyed sites of 19.3% (413/2140 trees) without the weighting adjustment. The symptomatic kauri prevalence within the randomly selected subset of soil sample trees was 17.0% (129/761 trees) which was similar to the adjusted overall symptomatic kauri prevalence of 16.5%. The distribution of symptomatic kauri was wider than the distribution of *P. agathidicida,* which is consistent with a disease which has symptoms that can also be caused by other biotic or abiotic factors. The greatest density of both the symptomatic trees and *P. agathidicida* detections overlap within the northern, central-western and southern coastal borders of the study area (Figure 2-11).

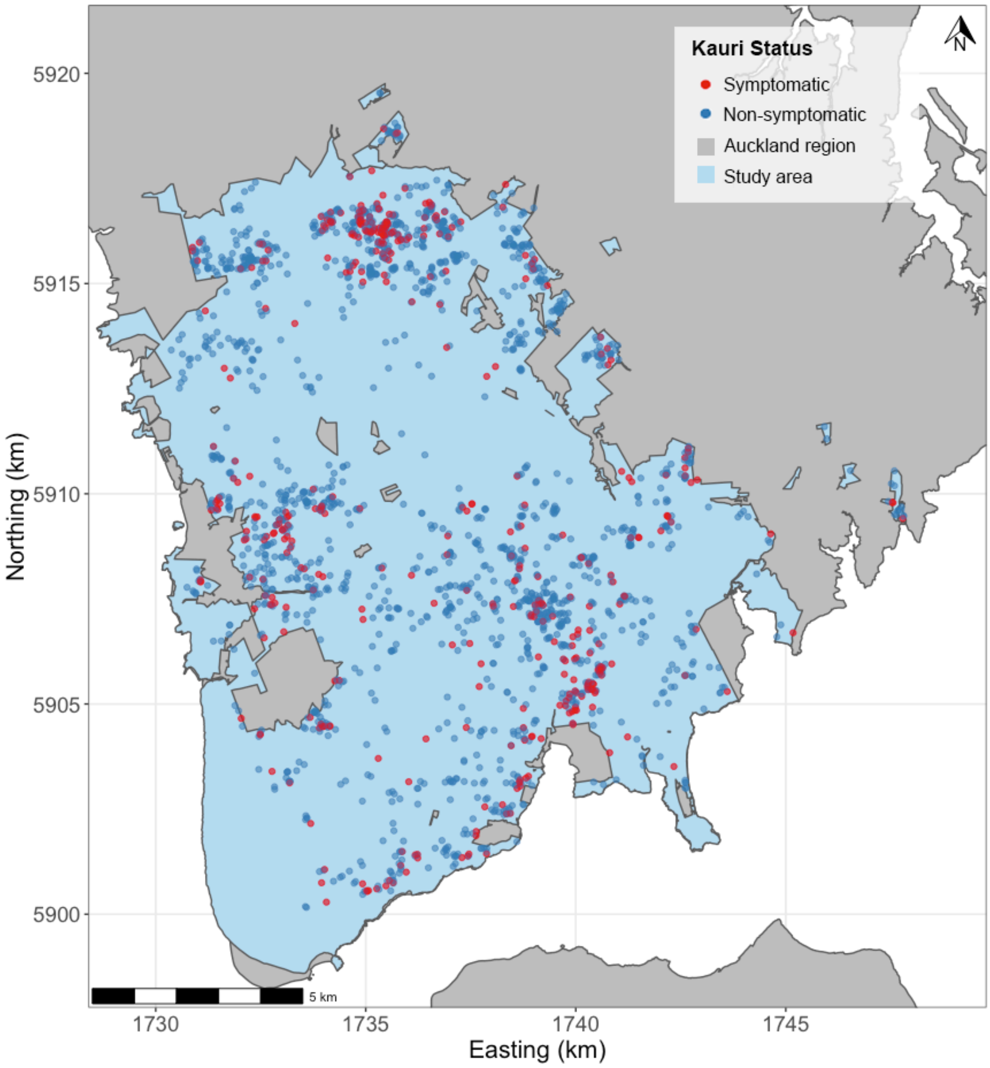


Figure 2-11. Spatial point map showing the location of surveyed kauri trees (n = 2140) with red circles indicating symptomatic kauri (n = 413) and blue circles indicating non-symptomatic kauri (n = 1727) based on the case definition.

The spatial relative risk surface for symptomatic kauri trees consistent with kauri dieback (i.e., the ratio of the density of symptomatic kauri to the density of non-symptomatic trees) shows two regions of significantly elevated symptomatic kauri risk, one in the north which is in the same area as the elevated *P. agathidicida* detection risk and in the south-east of the study area (at a significance level of 0.05) (Figure 2-12). There is an overlap along the western edge of the Park between a trend towards elevated *P. agathidicida* risk and elevated symptomatic kauri risk along with a similar reduced *P. agathidicida* and symptomatic kauri risk trend in the centre of the Park as illustrated by Figure 2-9 and Figure 2-12.

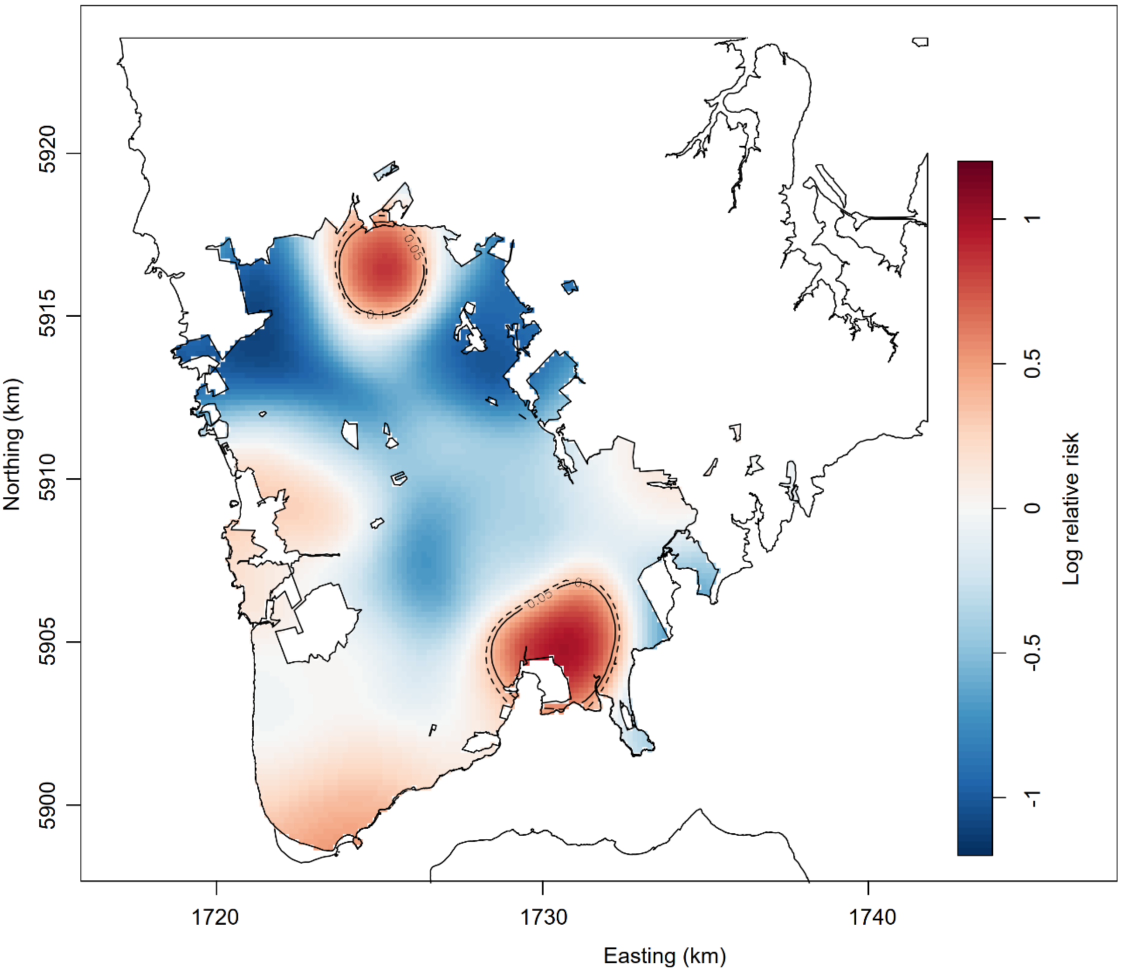


Figure 2-12. Symmetric adaptive relative risk surfaces (Davies et al., 2016) estimated using all the kauri trees included in the study (n = 2140; symptomatic = 413; non-symptomatic = 1727) within the study area. The relative risk is estimated on the natural log scale, such that values > 0 depict areas of elevated risk (log(0) = 1, and therefore log relative risk values > 0 equate to relative risks > 1, that is, increased risk). Where detected, tolerance contours delineating statistically significant risk elevations are drawn at significance levels of 0.05 and 0.1. White inland spaces indicate areas outside the study area (e.g., Piha village in the central west of the map).

We used stream sub-catchment boundaries to assess the proportion of monitored trees within them that were symptomatic. This fine-grained assessment looked at 162 stream sub-catchments. The median number of trees assessed per stream sub-catchment was 7 trees (25th percentile 3; 75th percentile 15; min. 1 tree; max. 82 trees). The median symptomatic kauri prevalence of the stream sub-catchments was 12.5% (25th percentile 0%; 75th percentile 25%). A total of 60 stream sub-catchments had 0% prevalence of symptomatic kauri. The local empirical Bayesian smoothed prevalence was estimated to address unstable raw prevalence estimates because of the small number of trees in some sub-catchments, and these were plotted, alongside the raw prevalence estimates. These plots indicate that symptomatic kauri prevalence was higher in the outer extent of the Park as shown in Figure 2-13. Note that stream sub-catchment areas outside the Waitākere

Ranges Regional Park and those without surveyed kauri are indicated by missing (NA) values in the figure. Additionally, urban areas outside the study boundary, e.g., Piha, which were not surveyed may have higher prevalence. Stream sub-catchments are useful as a way of visualising the data and could be considered as a practical management unit for land managers.

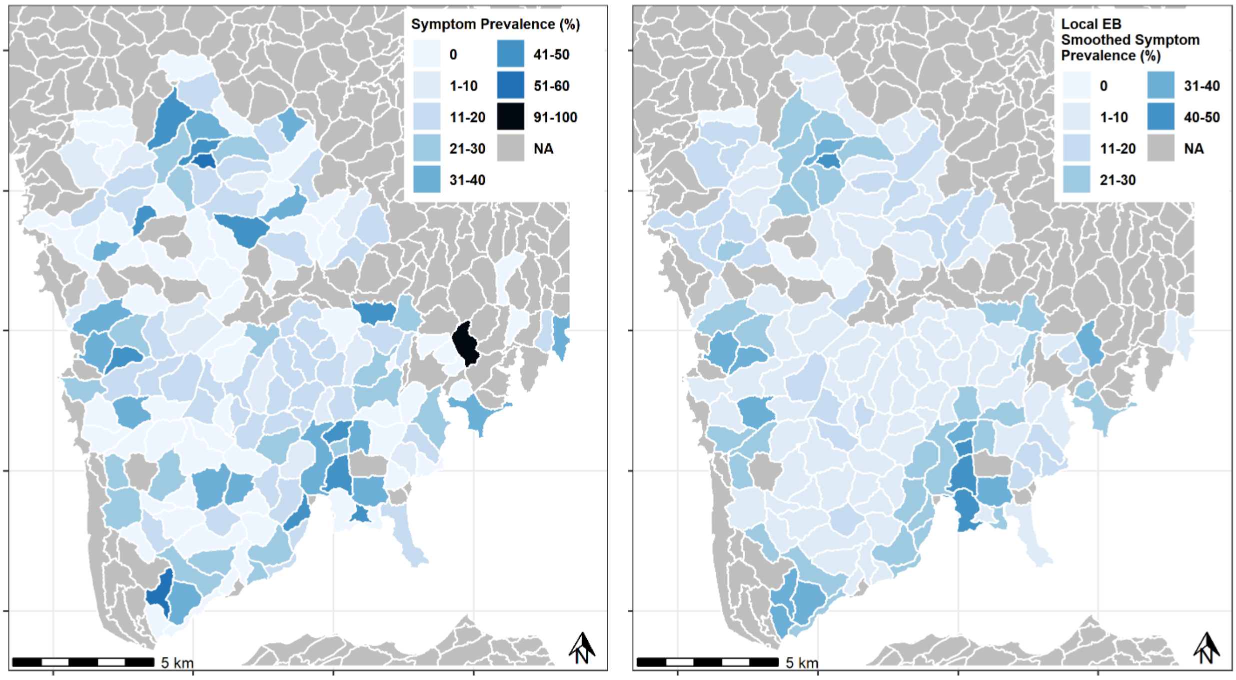


Figure 2-13. Choropleth map showing the spatial distribution of symptomatic kauri prevalence (left) and Bayesian smoothed symptomatic kauri prevalence (right) within discrete stream sub-catchments in the Waitakere Ranges Regional Park. Cells with NA did not have any randomly selected kauri trees within the stream sub-catchment. Note that stream sub-catchment areas include urban areas outside the study boundary e.g., Piha which were not surveyed and may have higher prevalence.

The classification of symptomatic kauri against the different classes of the Stevenson and Froud (2020) case definition (using the modified cut-points for classification) with an epidemiological criteria of 50 m from a *P. agathidicida* detection site (point location of a *P. agathidicida* detected test) is provided in Table 2-3. The large number of suspect cases are likely to contain both trees with kauri dieback and trees with other causes of symptoms. Likewise, the non-symptomatic ill-thrift group will contain a mix of trees with early-stage kauri dieback and trees with other causes of ill-thrift.

Table 2-3. Number of trees that meet the kauri dieback case definition stratified by the different classes within symptomatic kauri and non-symptomatic kauri. Where confirmed is on a *P. agathidicida* site, probable is within 50 m and suspect is >50 m of a *P. agathidicida* site. Note this is the total prevalence of symptomatic kauri, which is higher than the survey adjusted prevalence.

Symptomatic criteria status	**Epidemiological criteria class**	**Number of trees**	**Prevalence**
Symptomatic kauri	Confirmed	30	1.4%
	Probable	52	2.4%
	Suspect	331	15.5%
Non-symptomatic kauri	Ill-thrift	588	27.5%
	Healthy	1139	53.2%

2.4.5 Pathogen isolations

Phytophthora agathidicida was detected in 10% of soil samples (76 sites) (Table 2-4). In contrast, *P. cinnamomi* was detected more widely in 53% (401) of soil sample sites, which were much more spatially distributed across the study area (Figure 2-14). *Phytophthora multivora* was tentatively identified in only two soil samples and is reported, along with all other *Phytophthora* not identified to species level, as *P.* spp. These other *P.* spp. were detected in 10% (79) of soil samples. No *Phytophthora* were detected in 38% of sites (291). In just under half of the *P. agathidicida* detections (49%; 37/76), *P. cinnamomi* was also detected (5% of all sites), and a further 8% (6) of the *P. agathidicida* sites also had *P.* spp. present (0.8% of all sites).

Table 2-4. Detection of *P. agathidicida*, *P. cinnamomi* and *P.* spp. alone or in combination in the culture bioassay tests from 761 sites where soil samples were collected.

***Phytophthora* species detection**	**Percent of sites**	**Number of sites**
P. agathidicida only detected	5%	36
P. cinnamomi only detected	43%	324
P. spp*.* only detected	4%	30
P. agathidicida and *P. cinnamomi*	4%	31
P. agathidicida and *P.* spp.	0.4%	3
P. cinnamomi and *P.* spp.	5%	40
P. agathidicida and *P. cinnamomi* and *P.* spp.	0.8%	6
No *Phytophthora* detected	38%	291
Total sites		**761**

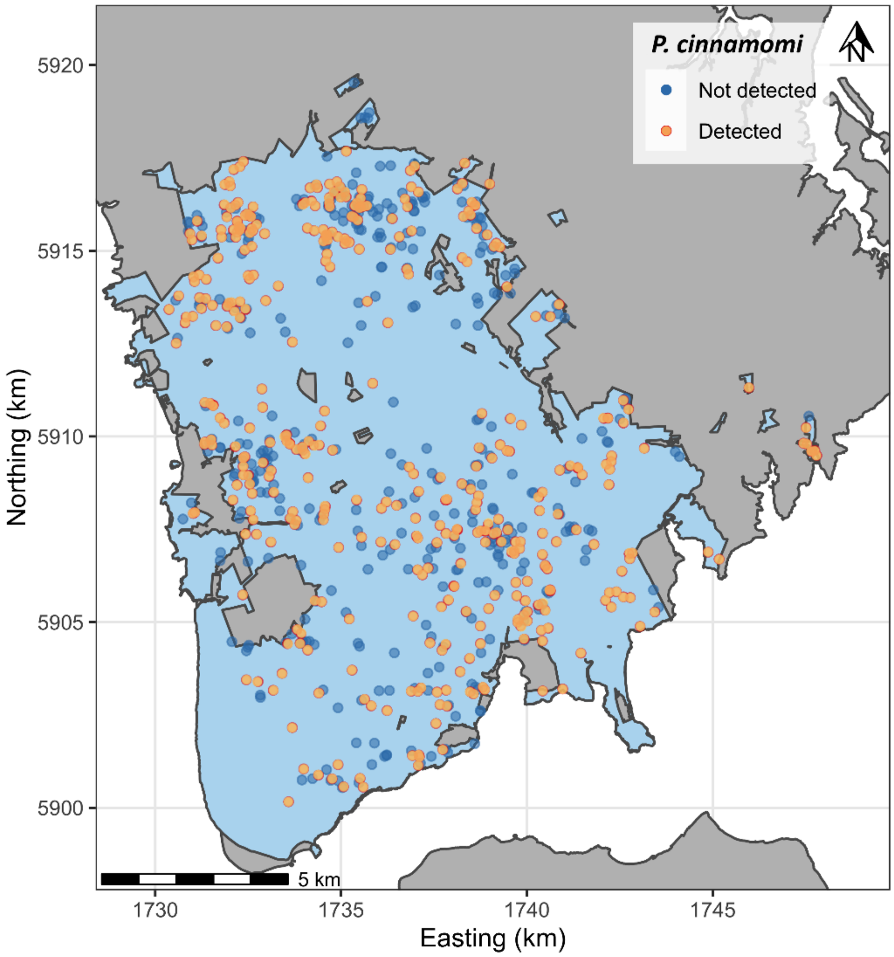


Figure 2-14. Spatial point pattern plot showing the location of kauri trees in the study area that had soil samples taken for diagnostic testing (n = 761) with orange circles indicating the detection of *P. cinnamomi* (n = 401) and blue circles indicating that *P. cinnamomi* was not detected (n =360).

P. agathidicida was detected by the culture bioassay in 23% (30/129) of the soil sampled trees that were assessed as being symptomatic kauri (consistent with kauri dieback), which was significantly ($p<0.001$) more than the 7% of non-symptomatic trees (46/632). Detection of *P. agathidicida* in the non-symptomatic trees were split between 10% in non-symptomatic – unhealthy kauri and 6% in non-symptomatic – healthy kauri (Table 2-5). In contrast, there was no significant difference ($p=0.63$) between *P. cinnamomi* detection in symptomatic tree soil samples 50% (65/129) and non-symptomatic samples 53% (336/632), nor between *P.* spp. detection in symptomatic versus non-symptomatic tree soil samples ($p=0.75$ with 12 versus 67 detections, respectively).

Table 2-5. Detection status of *P. agathidicida* within soil samples taken from 761 trees stratified by whether the trees were symptomatic or non-symptomatic under the case definition for kauri dieback.

Disease classification	*P. agathidicida* detected	*P. agathidicida* not detected	Total	Proportion with *P. agathidicida* detected
Symptomatic kauri trees	30	99	129	23%
Non-symptomatic – ill-thrift	22	198	220	10%
Non-symptomatic – healthy	24	388	412	6%

There were 20 symptomatic kauri trees that were soil sampled and were greater than 2 km from the nearest *P. agathidicida* detection. Of these 20 symptomatic trees, 8 had *P. cinnamomi* detected. In addition, two symptomatic trees that were not selected to be soil sampled with severe basal bleeds and an approved observer assessment of severe kauri dieback were over 3.5 km from the nearest *P. agathidicida* detection.

2.4.6 Severity of symptoms

Every monitored kauri tree (n=2140) was assessed for disease severity symptoms, which included canopy health scores and presence or absence of lesions, along with lesion activity, height and percent of the base affected. These will be used as a baseline for repeated monitoring assessments. Brief results are presented, and detailed results are in Appendix B.

2.4.6.1 Basal lesions

A total of 22% (463) of trees had either basal or lateral root lesions (including those where the observer was unsure). Basal lesions were observed on 19% (n=412) of trees and an additional 2% of trees (n=43) may have had basal bleeds, but the surveyor was unsure. In contrast lateral root bleeds were rare and observed on only 1% of trees (30) with an extra 4 trees where the surveyor was unsure. Of the 34 lateral root bleed trees, 26 were recorded on trees that also had a basal lesion. Basal or lateral root lesions can be caused by *P. agathidicida* or other biophysical injuries.

2.4.6.2 Disease lesion activity

Bleed activity was assessed for all 453 basal bleeds (including unsure bleeds) and 12% (254) had an active or semi-active basal bleed and 9% of trees (199) had an inactive basal bleed. Within the trees with basal bleeds (n=453), there was a higher rate of inactive bleeds in the unsure bleeds group with 56% not active, compared to the basal bleed 'Yes' group with 43% not active, indicating that inactive bleeds were harder to assess. Of the 34 lateral root bleeds, 5 were active, 5 were semi-active and the remaining 24 were not active (including all the unsure bleeds).

Within the trees with basal lesions (including the unsure ones), the height up the tree trunk to the apex of the lesion was measured for 453 trees. Height of lesions were left-skewed with a median of 40 cm (inter-quartile range of 17-103 cm) with a minimum of 0.4 cm and maximum of 600 cm high.

Of the 453 trees with basal lesions, the percent of the basal circumference that was affected by a basal bleed was measured for 449 trees and was strongly left skewed with most within 1-10% of the basal circumference affected. This indicates that the severity of basal bleed symptoms was towards the lower range in most affected trees.

2.4.6.3 Canopy health

The most common canopy score was 1.5 (between healthy crown and foliage thinning) which was observed for 39% of trees (845), followed by a score of 2 (foliage thinning) from 30% of trees (652), and 8% of trees had canopy scores of 3 or higher which was the cut-point for meeting canopy dieback for the symptomatic kauri case definition.

2.4.6.4 Canopy colour

There was a strong relationship between canopy colour and canopy scores with the majority of monitored trees having green canopy 72% (1544), or green-yellow 26% (559); few trees had yellow-green 1.5% (33) or copper-brown canopies 0.09% (2), (Figure 2-15).

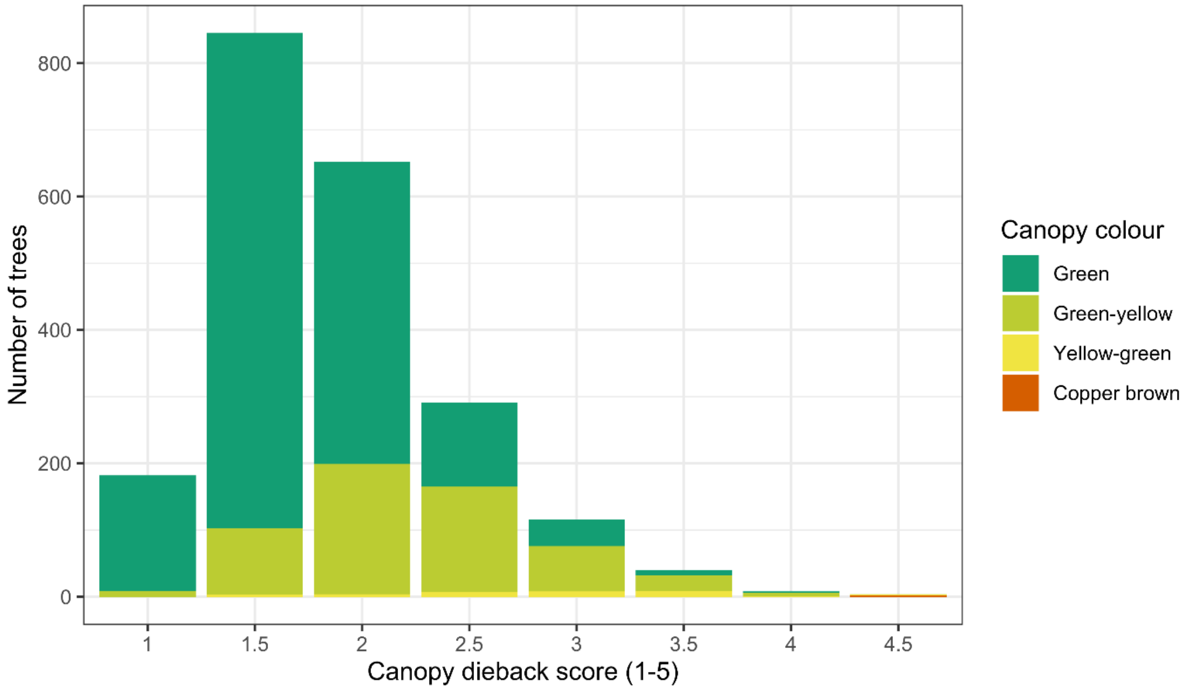


Figure 2-15. Bar chart showing the number of monitored trees within each canopy score class with a score of 1 being healthy and 4 significant dieback, stratified by canopy colour. Dead trees (canopy score of 5) were reported separately.

2.4.7 Host-related factors

The smallest tree that was surveyed had a DBH of 11 cm and the largest was 317 cm DBH. DBH was left skewed with a median DBH of 66 cm (25th percentile 48 cm; 75th percentile 99 cm). Most trees were in the intermediate size class (1388 (150-450 cm)), followed by rickers (527 (<150 cm)) and mature trees (218, (>450 cm)), 7 trees with missing circumference values were excluded. Our results reflect the use of remote sensing to detect our sample frame with taller (larger) canopy trees more likely to be included.

The presence of small (<15 cm) and established (15 cm – 1.35 m) kauri seedlings and saplings (>1.35 m tall and <10 cm DBH) was assessed at 1452 of the kauri monitoring sites. Seedlings and saplings were detected at 55% (794) sites. A total of 14% (199) of sites had all three size classes present along with the surveyed kauri tree. Immature kauri seedlings and saplings' presence or absence was not significantly associated with sites where *P. agathidicida* was detected (p=0.224, Fisher's exact test) (Table 2-6). Likewise immature kauri presence or absence was not significantly associated with sites where either *P. cinnamomi* or *P.* spp. were detected (p=0.380 and p=0.231 respectively) (Table 2-6).

Table 2-6. Counts and percent of sites where kauri seedlings and saplings were present or absent stratified by *Phytophthora* species detection status from 761 soil sampled sites.

***Phytophthora* status**	**Kauri seedlings and saplings**	
	Present	**Absent**
***P. agathidicida* detected**	48 (63%)	28 (37%)
***P. agathidicida* not detected**	380 (55%)	305 (45%)
***P. cinnamomi* detected**	232 (58%)	169 (42%)
***P. cinnamomi* not detected**	196 (54%)	164 (46%)
***P.* spp. detected**	39 (49%)	40 (51%)
***P.* spp. not detected**	389 (57%)	293 (43%)

Further results are in Appendix B.

2.4.8 Anthropogenic risk factors

Detailed results are available in Appendix B.

A total of 65% of the trees in the Waitākere Ranges survey were located within old logging areas with regenerating kauri forest, with just over a fifth (21%) in mature forest stands.

The distance to the nearest track was recorded for all 2140 trees and showed that the median distance from a track was 155 m (25th percentile 64 m; 75th percentile 299 m) and the most remote tree was 1.2 km from a track in any direction. The nearest tree was 0.1 m from a track.

Uphill distance to track is subtly different to the closest track which is based on an "as the crow flies" measurement. This variable is dependent on whether there is a track uphill of the monitored tree within the same sub-catchment of the tree, therefore 245 trees without a track uphill from them had no measurement leaving 1895 observations. Of these the median distance uphill to the closest track was 213 m (25th percentile 100 m; 75th percentile 375 m; min 0.6 m; max 1420 m).

2.4.9 Distance to closest *P. agathidicida* site

The distance to the closest current or historic confirmed *P. agathidicida* site (a point location of a positive *P. agathidicida* test), was recorded for all 2140 trees and showed that the median distance from a *P. agathidicida* site was 842 m (25th percentile 228 m; 75th percentile 1596 m) and the most remote tree was 4.07 km from a confirmed *P. agathidicida* site (Figure 2-16). A total of 1216 of the monitored trees (57%) were within 1 km of a confirmed *P. agathidicida* site.

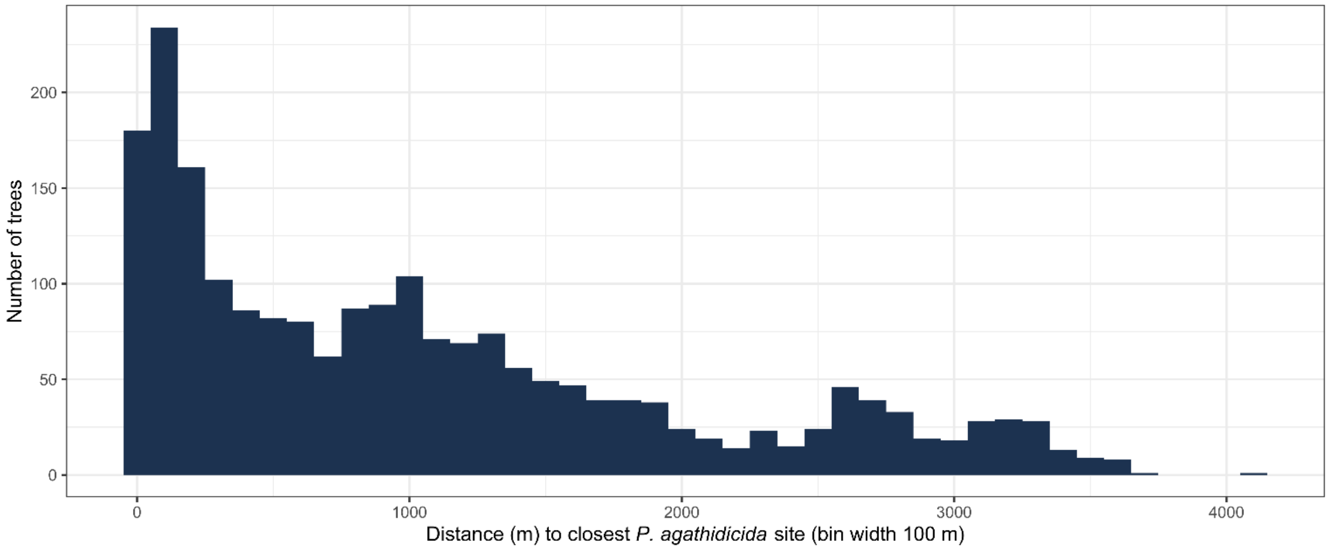


Figure 2-16. Frequency histogram showing the distribution of distance to the closest confirmed *P. agathidicida* site for 2140 monitored trees with a bin width set at 100 m.

2.4.10 Baseline ecological impact factors

2.4.10.1 Closest neighbour species

The closest neighbour tree species and DBH were recorded at 2080 monitoring sites. The DBH of each monitored kauri was compared to the nearest neighbouring tree species to calculate which was the larger and dominant tree. In most sites, the monitored kauri tree was the dominant tree at 91% (1945) of sites with only 9% (182) of the monitored kauri trees being smaller than the neighbouring tree and classified as subdominant. Kauri was both the most common dominant and subdominant neighbouring species at 62% (110/117) and 18% (334/1903) respectively. This is consistent with the remote sensing method used detecting the larger canopy occupying kauri trees. Full details and species are given in Appendix B.

2.4.10.2 Common species

A survey of the presence of kauri-associated plant species was conducted at 1406 sites, including all soil sampling sites and provides a detailed baseline dataset for repeated monitoring (data in Appendix B). Nine species (rewarewa, lancewood, mapou, kauri grass, shining karamu, rimu, mamangi, kanuka, and mingimingi) occurred near to 50% of these monitored kauri.

2.4.10.3 Forest floor depth (soil organic layer)

The forest floor depth was measured for 1452 of the monitored kauri. A mean from the left and right-side forest floor depth measurements per tree was calculated and used as the individual tree forest floor depth value. The population median forest floor depth was 16.5 cm (25th percentile 11.5 cm; 75th percentile 23.5 cm), with a minimum of 1.5 cm and maximum of 69.3 cm. Forest floor depth was positively correlated with DBH ($p<0.001$, Pearson correlation coefficient), with mature trees having much deeper organic layers than smaller ricker trees (Appendix 3). Change in forest floor depth is classified as a potential impact from kauri dieback, rather than a risk factor for kauri dieback, so the associations between symptomatic trees and forest floor depth and *P. agathidicida* and forest floor depth were tested. There was no significant association between forest floor depth and symptomatic kauri trees (p=0.80, Mann – Whitney test), however there was a significant association between *P. agathidicida* and forest floor depth (p<0.001, Mann – Whitney test) with much shallower depths under trees where *P. agathidicida* was detected with a median of 11.5 cm (25th percentile 7 cm; 75th percentile 15.5 cm) than not detected with a median of 16.5 cm (25th percentile 11 cm; 75th percentile 23.5 cm). This relationship was stratified against size class and shows an interesting pattern of lower organic layer depth where *P. agathidicida* was detected, regardless of kauri size class (Figure 2-17). However, the temporal and therefore causal nature of this relationship cannot be determined from these cross-sectional data.

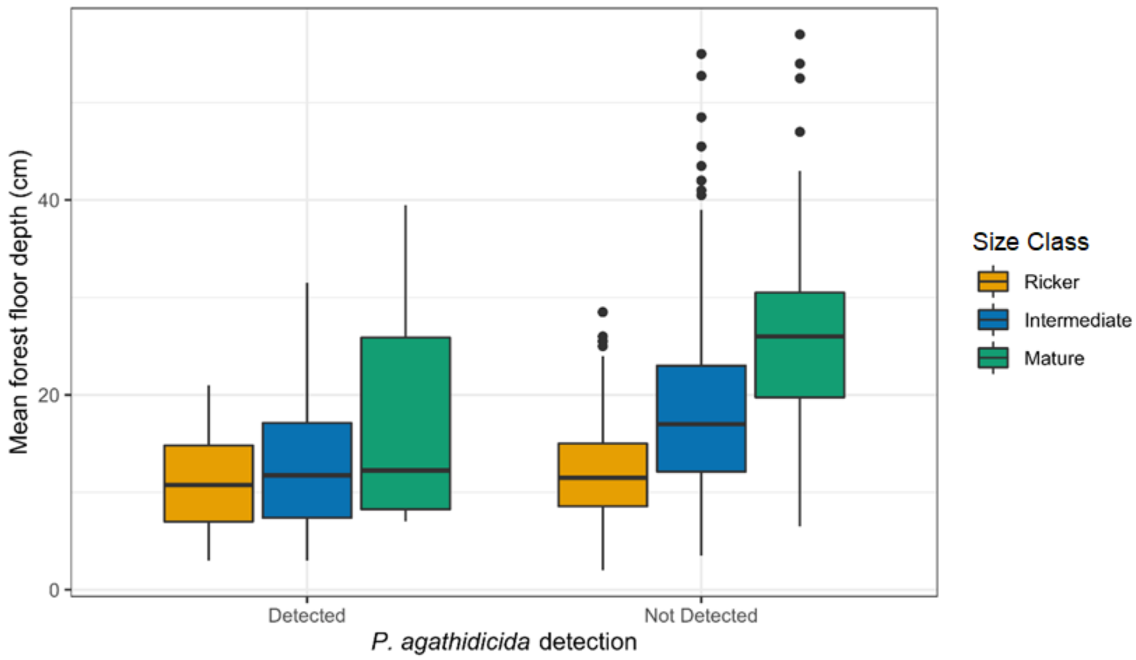


Figure 2-17. Box and whisker plots of mean forest floor depth (cm) per tree where *P. agathidicida* was detected or not detected, stratified by kauri tree size class from 759 monitored trees that were soil sampled and where the size class value was recorded (2 observations missing). Showing the median value (horizontal line), interquartile range (within box), maximum and minimum values (excluding outliers, vertical bars) and outliers (dots) for the population.

2.5 Discussion

Te matapaki

This study had 5 objectives: i) operationalise new remote sensing methods to develop a kauri sample frame; ii) spatially describe the baseline (in 2021) prevalence of *P. agathidicida*; iii) spatially describe the baseline (in 2021) prevalence and severity of symptomatic kauri in the Waitākere Ranges; iv) identify and collect data on key factors that could affect disease risk for hypothesis generation; and v) collect baseline (in 2021) data on ecological factors as possible indicators of ecosystem impacts from kauri dieback. These aims and findings are discussed in order of importance for understanding kauri dieback and kauri health in the Waitākere Ranges.

2.5.1 Prevalence and distribution of *P. agathidicida* and symptomatic kauri

The most important finding of this study was that *P. agathidicida* is currently (2021) in localised areas around the periphery of the Waitākere Ranges parkland, and this is consistent with historical *P. agathidicida* detections (Jamieson et al., 2014, Hill et al., 2017). This distribution is consistent with that of a slow-moving invasive soil-borne pathogen, which aligns with the hypothesis of the likely introduction of *P. agathidicida* from the Asia/Pacific region (Weir et al., 2015). It shows a pattern of point source introduction with initial long-distance (presumably human-assisted) spread into distinct foci, and natural spread (including via short distance vectoring) around those foci. The pattern of spread also indicates that *P. agathidicida* has not yet achieved its full potential range. This contrasts with the observed widespread distribution of *P. cinnamomi,* which is also an introduced pathogen into New Zealand. With a centre of origin in Taiwan, *P. cinnamomi* has spread widely worldwide (Shakya et al., 2021). An important difference of *P. cinnamomi* is its extensive host range, with more than 3000 susceptible hosts worldwide (Socorro Serrano et al., 2019) and at least 25 native species in New Zealand forests (Podger and Newhook, 1971), which likely has contributed to its extensive spread.

Spatially, the relative risk surface showed two regions of elevated *P. agathidicida* detection risk, one in the northern area and one in the mid-west area of the Park. It is possible that *P. agathidicida* has been present and spreading longer, or more efficiently, in these two elevated risk areas, and additional genomic analysis (Winkworth et al., 2021) of these *P. agathidicida* isolates may provide evidence for this observation. *Phytophthora agathidicida* is an Unwanted Organism and any areas where it is present are important for operational management. This study provides evidence to support the continuation of strategies to slow the spread of *P. agathidicida.*

The baseline pathogen prevalence of *P. agathidicida* detection in soils across the forest was 10% of sampled trees. In comparison the symptomatic kauri prevalence was higher at 16.5%. The majority (80.7%) of trees surveyed were either healthy (53.2%) or ill-thrift (27.5%) which is encouraging. The prevalence of symptomatic kauri in this study is not directly comparable to historical surveys as they used different methods.

In contrast to *P. agathidicida* distribution, symptomatic kauri showed a broader spatial distribution. Symptomatic kauri overlapped the same outer extent of the Park where *P. agathidicida* was present, but were also observed across the south-east region, where no *P. agathidicida* detections were made. This disease distribution was consistent with aerial detection of suspected kauri dieback symptoms in 2011 by Jamieson et al. (2014) and with the findings in Hill et al. (2017). The relative risk surface showed an elevated relative risk of disease in the north, which matched that for *P. agathidicida,* and in the south area of the Park, which, while not matching an area of higher relative risk for *P. agathidicida*, did overlap with *P. agathidicida* detection. In addition, the relative risk of disease was elevated, but not significantly, in the mid-west area where there was an elevated relative risk for *P. agathidicida*.

The observation of symptomatic kauri trees consistent with kauri dieback, including some trees with severe symptoms, in the south-east area of the Park in the absence of *P. agathidicida* detection indicates that these symptoms are caused by other abiotic factors such as drought, disturbance or another pathogen such as *P. cinnamomi.* With the number of samples taken in this area it is most likely that *P. agathidicida* is absent. This indicates that the symptomatic criteria of the case definition are over-estimating presence of kauri dieback and detecting symptoms caused by other factors.

It was also interesting that elevated disease risk (in conjunction with *P. agathidicida* detection without an elevated risk) was also present on the southern border of the Park. These trees may have contributing factors that are making them more vulnerable. Beever et al. (2010) state that similar canopy symptoms are observed with natural stand thinning on drought-prone sites and the Waitākere Ranges have recently (2019-2021) experienced a prolonged drought (NIWA, 2022).

Phytophthora cinnamomi has been reported widely in native forest, as it was in this study, and has been associated with ill-thrift of trees, particularly in regenerating stands (Beever et al., 2009, Podger and Newhook, 1971). However, no association between symptomatic kauri and *P. cinnamomi* was found in this study, in that, *P. cinnamomi* was just as common under non-symptomatic trees as symptomatic ones. Johnston et al. (2003) also found no such association in a study in Waipoua forest in Northland. Future research on these monitored trees using DNA-based tests (McDougal et al., 2014, Winkworth et al., 2020) or lesion samples of those with basal bleeds (Beever et al., 2010) may provide evidence to explain what is causing these symptoms away from *P. agathidicida* areas. More detailed examination of specific disease severity symptoms (data collected in this study) in relation to detection of *P. agathidicida* in soils below symptomatic, ill-thrift and healthy trees is also warranted.

Chapters 3 and 4 of this report provide further insight into the other factors that may be contributing to these symptoms and the limitations of the visual assessment and soil test to estimate *P. agathidicida* distribution.

This survey was focused on kauri health and understanding the other factors that could be contributing to driving kauri dieback symptoms in the forest in addition to *P. agathidicida* will be important to inform how best to manage unhealthy kauri trees in conjunction with managing the spread of *P. agathidicida.*

The results in this study showed that while over half of all monitored trees were within 1 km of a current (2021) or historic *P. agathidicida* site, 43% were more than 1 km away, some of which were classified as symptomatic kauri, and this risk factor will be explored further in Chapter 3. Historical detections of *P. agathidicida* follow a similar spatial distribution to those detected during this study (Jamieson et al., 2014, Hill et al., 2017). *Phytophthora* species are known to persist for years in the environment using dormant resting stages (Jung et al., 2018). *Phytophthora agathidicida* has persistent oospores with thickened walls that have been found to remain viable in stored soil for 10 years (Bradshaw et al., 2020) so it would be reasonable to assume that viable *P. agathidicida* remains in areas where it has been previously detected. Pathogen testing to confirm the cause of symptoms when kauri dieback is suspected will be important in the future, particularly in areas where *P. agathidicida* has not been detected.

There was a significant association between observation of symptoms and *P. agathidicida* detections, with 23% of the symptomatic kauri trees that were soil sampled detecting *P. agathidicida.* This relationship is explored further in Chapter 3. In contrast, within the non-symptomatic group there were more detections in the ill-thrift group (10%) than the healthy group (6%), both significantly lower than the symptomatic group. The relatively low recovery rate of *P. agathidicida* from symptomatic trees is consistent with earlier investigations such as McDougal et al. (2014) which found only 31% of soil samples detected *P. agathidicida* from known infected trees and this is investigated further in Chapter 4. It is recommended that DNA-based detection methods are implemented alongside the soil bioassay to improve detection, however they will require diagnostic sensitivity and specificity parameters to be assessed too.

Phytophthora agathidicida detection in the healthy and ill-thrift groups indicates firstly that *P. agathidicida* is present where we cannot visually detect disease, and secondly that the cut-point for canopy score and yellowing may need to be reassessed, particularly within different size classes as there are indications that smaller trees are more likely to show canopy symptoms than lesions (Beever et al., 2010). This is further supported by the discussion among experts when the symptomatic criteria were agreed that there are some unusual developing symptoms that may be associated with *P. agathidicida* infection (Stevenson and Froud, 2020). Future research into the cut-points for the symptomatic criteria will be useful. Repeated cross-sectional monitoring of the same cohort of kauri to observe the development of symptoms over time will provide information that could improve early visual disease detection. Any modifications to the cut-points for the symptomatic criteria, informed by repeated monitoring, would require re-scoring of the baseline trees so that they can be compared using a consistent definition.

The baseline prevalence and spatial distribution results for *P. agathidicida* and for disease in the forest are valuable to help inform which intervention strategy or combined strategies could be applied to different areas of the Waitākere Ranges. To date several kauri dieback interventions have been developed, firstly to control vectoring aimed to stop spread of the pathogen (pest control, hygiene stations, track closures, track upgrades and rāhui (cultural restrictions)), to restrict access to the forest to rebuild forest health (rāhui, weed and pest control and track closure and upgrades) and to treat symptomatic trees to stop decline and tree death (phosphite and rongoā (cultural health measures)). These strategies are applied within a wider decision-making framework which includes consideration of tikanga, natural values, biosecurity risks and impacts, geological and landscape values, historic and cultural heritage values, cumulative effects on any values, recreational values and accessibility, visitor safety, climate change risk and the feasibility and whole-of-life cost. Areas with *P. agathidicida* may require proactive management alongside a continued strategy to stop or slow the spread of *P. agathidicida*. In contrast, where *P. agathidicida* has not been detected, there is now additional evidence to support a protective management strategy to maintain absence through stopping the spread of *P. agathidicida* particularly in areas where *P. agathidicida* was not detected but trees are showing signs of disease, such as the south-east section of the Waitakere Ranges parkland, as these trees may be even more vulnerable.

This study showed that stream sub-catchments are a useful way of visualising data and have potential as a practical land management unit for assigning areas for different kauri health management strategies. These could be used with buffers around stream sub-catchments with high *P. agathidicida* prevalence or no *P. agathidicida*. It is also important to note that estimated prevalence in some stream sub-catchments near urban areas is based only on a small part of the sub-catchment, e.g., around Piha village in the central west. The bush blocks of private land in these areas are known to have kauri dieback disease and positive *P. agathidicida* detections that may be at a higher prevalence than that observed within the Park boundary.

It will be useful to apply the classes within the kauri dieback case definition in the future for operational management. Particularly the distinction between probable (symptomatic and close to known *P. agathidicida* detection sites) and suspect (symptomatic and away from known *P. agathidicida* sites). A probable kauri dieback classification is useful for land managers to effectively fill in the gaps between tested and untested trees that are symptomatic and 'close' to each other. The definition of 'close' is currently 50 m, but this may be too conservative to be practical for land management decisions. An example of use would be in a semi-urban environment where confirmed kauri dieback (*P. agathidicida* positive, symptomatic kauri trees) could confer a probable kauri dieback tree status to neighbouring symptomatic trees, enabling landowners to access treatments without the expense of testing. It also aids management decisions, where land managers may decide to manage suspect trees in a different way to confirmed or probable trees. An example of this would be to consider an area in which there are many suspect symptomatic kauri trees but no positive soil tests. This may indicate that trees have cryptic disease, and the use of specific *P. agathidicida* treatments like phosphite injections may not be beneficial or warranted in that area.

2.5.2 Host detection

The first operational use of new remote sensing methods to identify kauri trees for inclusion in the sample frame and cross-validation of randomly selected trees was successful. Most misclassifications were against other native tree species which were consistent with previous kauri detection research (Meiforth et al., 2019). Tree species that are commonly confused with kauri are species with conical growth forms (in younger stages) like rimu, tanekaha, rewarewa and kahikatea, as well as species with needle like leaves and rough foliage surfaces like tōtara and pine trees (Meiforth et al., 2019). Pine trees were the most common misclassified exotic tree species both within the monitored sample (1% of misclassifications) and during cross-validation of trees randomly selected for inclusion. These exotic trees have been misclassified as kauri due to no prior algorithm training, as exotic trees were absent from the Meiforth et al. (2019) research sites. Some exotic tree misclassifications were easily dealt with during the manual confirmation process that was applied. However, this was time-intensive and future research to train the classifier with more evenly spaced data across the forest area would improve the predicted kauri extent map, particularly for use in areas with higher density of exotic species such as parts of the Hunua Ranges.

The method used to detect the kauri extent map was constrained by tree height, presence in the canopy and remote sensing algorithms that may have biased our host detection estimates. The accuracy to detect kauri trees with remote sensing depends on the size of the crowns, the symptom stages and the type of other tree species present in the forest area. Previous on-ground validation on an independent field reference dataset in three study areas within the Waitākere Ranges showed the detection accuracy using the methods of Meiforth et al. (2019) was dependent on tree size and disease expression. Large non-symptomatic kauri were detected with a high accuracy of 93% while detection of smaller trees was far more limited. For the remote sensing methods applied to this study, host detection accuracy would have been highest for larger non-symptomatic kauri and lowest for small crowns and dead and dying trees (J. Meiforth unpublished results). These underlying host detection accuracy conditions may have biased our sample frame towards larger and healthier trees, which means we may have underestimated the baseline prevalence of symptomatic kauri in the population. Extrapolating the study results to smaller size classes needs to take account of this potential bias. The host detection methodology used in this study can be improved in the future with more manual crown segmentation, especially for dead and dying and small trees, a consistent cloud-free HiRAMS dataset with high sun elevation, and a balanced reference crown set that includes kauri and other tree species in all symptom stages and size classes. In addition, it would be valuable to undertake a diagnostic test performance evaluation on the sensitivity and specificity of the remote sensing method of kauri detection as they are the preferred measures of test validity (Vallee and Cogger, 2019). Unlike accuracy measures, diagnostic sensitivity and specificity do not vary with the prevalence of the hosts in the forest and will not vary between sites with differing densities of kauri trees (Vallee and Cogger, 2019).

The estimated kauri population map layer for trees above 15 m, along with the calculated positive predictive value of 86%, can be used as an estimate to plan management interventions across the forest and to estimate the lower limit of tree numbers within management areas. However, regenerating areas where trees are not yet above 15 m will have been missed from our sample frame and population estimates. Remote sensing improvements to detect smaller kauri could provide additional sample points for repeated monitoring and to assess if disease or pathogen prevalence differs in these populations.

2.5.3 Disease severity

The percent of the tree trunk base affected by a basal bleed is an indication of the extent of girdling of the trunk, which affects the transfer of water and nutrients to the canopy due to vascular dysfunction (Bellgard et al., 2016). The baseline of this severity measure showed that half the trees with basal bleeds covered less than 10% of the trunk and 80% of the trees with basal bleeds covered less than 30% of the trunk. This measure will be important to collect in repeated monitoring to determine if disease is progressing over time. This severity measure indicates that most trees will be good candidates for phosphite treatments, as mildly affected trees have a better response and survival than severely affected trees (Horner and Arnet, 2020, Horner et al., 2019a).

The basal bleed age results show in that 44% of bleeds were not active, which is a similar rate to the untreated controls in the Horner et al. (2015) phosphite trials. There was no apparent trend in lesion activity over the 4-month survey period, but they may change across seasons and more intensive studies such as those planned by the researchers within Ngā Rākau Taketake would be required to understand this. Repeated monitoring of these trees will show if inactive lesions remain inactive over time.

A correlation between baseline disease severity measures such as higher canopy health scores and basal bleed height, percent and activity scores with subsequent tree decline and death from repeated monitoring could be used to predict the extent of tree loss over time. These baseline disease severity measures provide evidence of areas where interventions are best targeted, in that discrete spatial areas with high prevalence of severe symptoms can be prioritised. The data can also be extrapolated (within the limitations described) to estimate the number of affected trees within areas to assist with intervention planning and costing. Ongoing monitoring of disease and severity measures will provide incidence rate data to quantify the efficacy of interventions. In addition, analysis of kauri dieback symptoms and severity classes to validate remote sensing stress detection methods for the future would assist in identifying stands of trees for management interventions.

2.5.4 Frequency of potential risk factors

Our study aimed to identify and collect data for factors that could contribute to or protect from disease for hypothesis generation (Chapter 3). Once associations between risk factors and disease are understood, the frequency and distribution of potential risk factors (detailed in Appendix B) will enable land managers to calculate a population estimate of trees with specific characteristics within the forest and to spatially apply risk maps based on their distribution. For example, if mature trees have a higher disease prevalence, a population estimate of the proportion of mature kauri in the population could be estimated, e.g., 10% of 68,000 trees would be approximately 6800 trees with 60% located within mature forest stands. These estimates can then be used to plan and budget for protection measures. Further research into host detection of smaller size class kauri using remote sensing will be important to accurately estimate trees at risk for planning.

2.5.5 Baseline ecological impacts

One of the key findings from the collection of baseline data was the observation of kauri seedlings and saplings at 55% of monitored sites. These seedlings and sapling observations were aimed at monitoring if recruitment was occurring, especially under symptomatic trees. It also set a baseline to measure if disease may be reducing reproduction even when it is not killing the trees. *Phytophthora agathidicida* is thought to be particularly lethal to seedlings from glasshouse trials (Gadgil, 1974, Horner and Hough, 2013, Horner and Hough, 2011), however, kauri seedling and sapling presence was not significantly associated with *P. agathidicida* (or with *P. cinnamomi*) detection. These observations provide evidence that kauri can germinate and grow in association with *P. agathidicida.* However, the survival rate of these seedlings is unknown and multiple factors will influence their survival, including different environmental conditions under diseased compared to healthy kauri stands. Future monitoring will be needed to see if that extends to kauri regeneration and replacement of lost trees at a rate sufficient to maintain a kauri dominant forest.

A consideration in interpretation of this measurement is the potentially confounding effects that i) *P. agathidicida* may be causing canopy loss, leading to increased light favouring seedling germination and growth; ii) seedling and sapling roots may not extend deep enough into the soil layer to encounter *P. agathidicida* zoospores; and iii) very healthy trees may not have any seedlings nearby due to the Janzen-Connell effect (Packer and Clay, 2000), which, in brief, implies that seedling survival is greatest further from the parent. However, how well the Janzen-Connell effect is supported in temperate species has been questioned (Hyatt et al., 2003). Further analysis of the presence of kauri seedlings and saplings with different soil characteristics and tree health/disease severity would be a valuable extension of this dataset. These monitored sites could also potentially be used to select sites to further investigate *P. agathidicida* virulence and host resistance under natural conditions to augment *in vitro* research where some variability in pathogen virulence and host susceptibility has been observed (Herewini et al., 2018).

The results showed that kauri was the dominant tree in 91% of sites surveyed, which is consistent with a kauri-dominated forest. However, our host population at risk detection method, where only trees greater than 15 m high and visible in the canopy were eligible for selection to be monitored, is likely to have biased us towards dominant trees as they were easier to detect using remote sensing.

P. agathidicida mostly infects the distal feeder and secondary roots of kauri within the upper 20 cm of soil layers (Bellgard et al., 2013). The difference in forest floor depth between sites with and without *P. agathidicida* detected was an interesting finding, especially as there was no relationship between symptomatic trees and forest floor depth. Because of the cross-sectional nature of this baseline study, it is not possible to determine the direction of a causal link between *P. agathidicida* presence and a reduced forest floor depth. In that is, a shallower organic layer may be more hospitable to *P. agathidicida* than deeper organic layers or that *P. agathidicida* may be causing shallower organic soil layers through slow or no tree growth causing a reduction in tree litter input (Wyse et al., 2014). Higher microbial populations may be present in deeper organic layers, which may be antagonistic to *P. agathidicida* (Bradshaw et al., 2020). It is possible that on sites with restricted forest floor depths there is a higher concentration of both kauri roots and *P. agathidicida* which would increase the probability of isolation from the soil bioassay. Future monitoring of this kauri population within the Waitākere Ranges Regional Park may explain a causal link between *P. agathidicida* and shallow organic layers. The potential impact of a reduction in forest floor depth, if that is proven to be caused by *P. agathidicida*, could lead to loss of kauri-associated species (Bradshaw et al., 2020), a change in the composition of the forest (Wyse et al., 2014) and will have implications for the carbon and nutrient cycling within the forest (Schwendenmann and Michalzik, 2019). The addition of plot surveys in combination with repeated monitoring would be valuable for understanding these ecological processes.

2.6 Conclusion

Te whakatau

This study found *P. agathidicida* in localised areas within the outer periphery of the Waitākere Ranges parkland, which suggests that *P. agathidicida* has not yet achieved its full potential range and provides evidence to support the continuation of strategies to slow or stop the spread of *P. agathidicida.*

Elevated disease risk overlapped areas where *P. agathidicida* was detected.

Kauri trees with visible symptoms similar to those of kauri dieback were found scattered throughout most areas of the Park, including in areas where *P. agathidicida* was not detected, indicating that other factors can cause poor health in kauri, which need to be identified.

The description of symptomatic kauri and of *P. agathidicida* prevalence in space and time can be used to inform different forest health strategies within a wider decision framework.

The study also showed that new remote sensing techniques to detect hosts could be operationalised and were a practical, accurate and efficient method to build a sample frame for a large-scale native forest survey of a canopy species.

The dataset collected during this study provides a taonga (valued treasure) for future study to explore different variables and develop capability and capacity in researching environmental biosecurity epidemics. The study was designed to provide robust data and a consistent cohort of monitored trees to be remeasured over time using a repeated cross-sectional study design.

It also provides the baseline for ongoing monitoring of a small sub-set of ecological impacts to detect changes in forest composition over time. These results will be used to inform the ongoing and adaptive management of kauri dieback in the Waitākere Ranges and across Tāmaki Makaurau. References are provided at the end of the report.

Find out more: kauri@aucklandcouncil.govt.nz
or visit knowledgeauckland.org.nz and
aucklandcouncil.govt.nz