Surveillance and Monitoring Strategy for Kauri Protection

Foreword

Tiakina Kauri inherited a wealth of knowledge from the previous Kauri Dieback Programme partnership that, over many years, collected information on the state and health of Kauri. This approach, however, was often ad hoc and funding dependent. In recent times, studies of the Waitakere Forest (by Auckland Council and Te Kawerau ā Maki) and in Waipoua (by Te Roroa and DOC/BNZ) have provided a great deal of data and learnings, which has underpinned Tiakina Kauri's approach to surveillance.

The learnings from the Nga Rakau Taketake (NRT) platform [1]have also been incorporated into the Tiakina Kauri Surveillance Strategy when possible. It is timely to formally revisit the overall strategic approach towards surveillance and monitoring, to capitalise on lessons learned and to solidify actions emerging from Tiakina Kauri's co-designed processes.

Robust decision-making in the Kauri Protection Programme must take into account the risk and impact of *PA* spread. Foundational to this is an understanding of where kauri trees and stands are located, the presence and absence of *PA*, the presence and absence of risk factors (such as tracks, large scale earth disturbance, stock, etc) and the efficacy of risk mitigations. This information is reliant on a strong surveillance and monitoring regime which, once gathered, can be combined with the impact of spread– taking into account the many values held by kauri forests (ecological, biological, cultural and spiritual).

In fulfilling this intent, and meeting the requirements of the NPMP, the approach by Tiakina Kauri is to engage and work in partnership with Māori at all levels and stages – from information collecting to decision-making to implementing. While this may mean progress is initially slow, with emphasis placed on training and setting high and enduring standards, significant work has already been undertaken to date with 12 trained mana whenua kaimahi active.

Introduction

Phytophthora agathidicida (*PA*) is a destructive pathogen that causes a lethal disease in kauri trees growing in natural, urban and plantation forests. Affected trees display various symptoms, including gummosis, lesions, thinning canopies, bronzing of the crown leading to eventual death, and the presence of leafless and stag-headed individuals and groups of kauri trees. It causes an infectious disease that spreads between hosts via motile zoospores in soil water and is transported into new areas as dormant oospores in soil. Both *PA* and the disease are strongly associated with disturbance to the forest.

PA cannot be eradicated, and treatment options are only currently viable at the individual tree level. Therefore our main management options are to reduce the impact of *PA* on kauri forests. We can do this by containing *PA* within infected sites and protecting areas that are thought to be free from *PA* using a range of

[1] The NRT Platform is a suite of research projects funded through the Strategic Science Investment Fund, through MBIE. They have had funding of $34.5m over the past four years and terminate in March 2024.

management strategies and by using powers available to us under the NPMP. To inform these management options we need to know where the kauri trees are located in the landscape and where *PA* is present.

Currently, there is no systematic mapping of the host population at risk, the extent of *PA* presence, or of trees with disease. The current understanding of the distribution of *PA* is based on investigations conducted by various agencies, reports from concerned landowners, and several large-scale, systematic surveys conducted by Auckland Council in the Waitakere Ranges and other forests (e.g., Waipoua). In addition, we lack a centralised database and associated tools to accurately depict the known extent of *PA* or the presence of trees displaying possible disease symptoms.

Objectives

The surveillance and monitoring programme is guided by the principal measures of the NPMP for *PA*:

1) Mapping the distribution of kauri and kauri forests
2) Mapping the presence and absence of *PA*.

The purpose of this strategy is to provide tools and information to forest managers to support co-designed kauri protection plans, while simultaneously forming a national understanding of kauri and pathogen distribution, meeting the principal measures of the NPMP.

Collecting baseline information about the trees and the pathogen is fundamental to meeting the objectives of the NPMP, and will support the achievement of further principal measures, including:

- understanding the rate of spread of *PA*
- determining and establishing containment and exclusion areas
- understanding the impacts of *PA* on kauri forests
- understanding of the application and effectiveness of *PA* control tools, mātauranga Māori, and other management practices to manage the spread of *PA*.

Recommended Approach

Advanced signs of disease, such as canopy thinning and discoloration can be observed from the air during aerial surveillance. However, it is important to note that these canopy symptoms can also be caused by other factors unrelated to the disease, such as drought or root damage. Additionally, early-stage infections characterised by root loss and gummosis of the lower trunk are not visible from the air. Results from the 2021 Waitakere Survey indicated that aerial surveillance under-estimates the presence of *PA* by a factor of 4. Therefore, it is not possible to diagnose the disease or detect the presence of *PA* based solely on aerial imagery.

However, using remote sensing (satellite or aerial survey imagery) can be useful to identify the population, map known and predicted risk factors for *PA* introduction and spread (such as tracks, traplines, historic logging activity and disease symptoms) in major kauri forests. This, combined with previous *PA* detection data, will provide valuable information to land managers (e.g., mana whenua, DOC, Councils) about where kauri are and where *PA* has been detected or not detected and where historical and current risks are.

Landscape-level risk mapping over the host population layer will enable land managers to identify management units within forests where there is sufficient information (host, *PA* and risk) to undertake kauri

health management. It will also identify management units that can be selected from for small scale *PA* and disease prevalence ground surveys to provide more information to make evidence-based decisions on kauri health management.

A useful management unit to assess and manage kauri is at the stream sub-catchment (SSC) level as this provides a biologically meaningful spatial unit. Although the management unit can easily be redefined for certain landscapes such as farmland with remnant stands. Maps at the stand-level (>10 ha) can be used to define these areas across the landscape.

As discrete management units are ground surveyed, they will inform changes to kauri management both within them and to those management units with similar risk profiles. They will also contribute to a national dataset of risk factor and ecological impact data that can be used to further refine and inform management efficacy. Risk maps will improve over time, through ground survey validation and on-going knowledge gathering.

These host and risk maps will serve as a preliminary baseline to monitor three key aspects: 1) identify *PA* free areas for protection, 2) identify areas for containment of *PA*, and 3) assess the effectiveness of containment, protection and treatments implemented by management partners.

The surveillance strategy aims to provide the capability, tools and evidence needed to assess kauri health and assign management actions for protection from and containment of *PA*. This is a long-term plan that has been co-developed with mana whenua, researchers, contractors, technical experts, with shorter-term objectives that can be achieved over the next five years.

Recommendations

The strategy proposes:

1. Conducting aerial surveillance to gather imagery for use in Kauri population mapping and tree health monitoring. This imagery will also provide a baseline of Kauri forest health.

2. Deploying a training programme to build capacity in mana whenua to understand the principles of hygiene and perform kauri health and *PA* prevalence ground surveillance.

3. Mapping Kauri trees at different spatial scales. Mapping at the individual tree level will guide sampling designs for surveillance activities. Mapping stands of Kauri trees (tree clusters >10 ha) will help managers design risk management plans, which may include identifying areas where mitigations may be necessary (e.g., where to install cleaning stations, where to divert tracks and traplines).

4. Risk mapping across all of Kauri lands identifying areas where PA is most likely to have been introduced. Risk factors include potential historic and contemporary pathways of introduction, based off of observations and evidence from previous studies. Factors can be weighted based on confidence of risk.

5. Sampling plans across selected forests can be designed with the aim of baseline kauri health and prevalence monitoring for *PA* at the landscape-scale. The kauri health and *PA* baseline prevalence monitoring will follow a mixed randomised and risk-based tree selection and soil sampling, with population thinning algorithms tested in the Kaimai Study and aimed at reducing the number of trees requiring sampling. Repeated monitoring over the long-term can be done to assess change in *PA* status or kauri health over time.

6. Data collation and mapping be undertaken and assessed to identify management units that are known to have *PA*, appear to be *PA* free or are unknown with different risk profiles. This will be shared with land managers, through a national database[2] so that initial management actions can be informed as soon as possible.

7. Management units (i.e., sub-catchments) where PA has been detected can be sampled with higher intensity (compared with the landscape-scale), using a fully randomised sampling approach, to delimit pathogen distribution, inform management and validate risk maps.

Together, ground surveys designed using careful statistical designs that are informed by maps of the host population and risk factors associated with PA introduction and spread will provide a greater understanding of the distribution of both healthy kauri forest areas and those with *PA* and disease symptoms and the efficacy of long-term management and monitoring of *PA* on kauri ora.

Draft implementation plan

Objectives

Tiakina Kauri aspires to achieve the following measures by the end of 2025 calendar year:
1) Map the host population spatially
2) Develop risk maps using explanatory and predicted risk factors from previous studies and local knowledge holders to inform surveillance for the presence and absence of *PA* to

- Inform management units where sufficient information about *PA* risk is known to identify sites suitable for protection or containment.
- Inform areas for initial delimiting surveys to address immediate knowledge gaps for management
- Implementing risk-informed kauri health and baseline *PA* prevalence surveys to validate risk factors and inform long-term monitoring and management

3) Build capability and capacity in mana whenua using a consistent training approach
4) Optimise management and surveillance tools (including diagnostic testing methods)
5) Develop storage, communication and management plans for data
6) Provide tools and knowledge to develop a kauri protection plan template.

Detailed planning and progress to date

[2] LINZ have been contracted to build and maintain a database to hold all Kauri data – called Kete Aronui. It mirrors the wilding pine programme approach and enables Tiakina Kauri to hold all data securely with access controlled in partnership. Governance approved this approach.

1) <u>Map the host population spatially</u>:

Host population maps can be used to support and inform kauri protection plans and to guide location selection for longer-term prevalence monitoring.

There are multiple approaches and imagery types available for mapping kauri. Low resolution imagery, such as Sentinel (satellite) and oblique photography can be used to get a big-picture overview of general kauri locations, current health status and change over time but are too coarse to direct ground activities. High-resolution imagery is well geo-referenced, making it useful for directing ground surveillance work, but our capacity to analyse this imagery is limited. To prioritise high resolution analysis efforts, areas of interest should be identified using coarse imagery first, to target the areas to be mapped at a finer level. The image type chosen depends on the objective (Fig. 1).

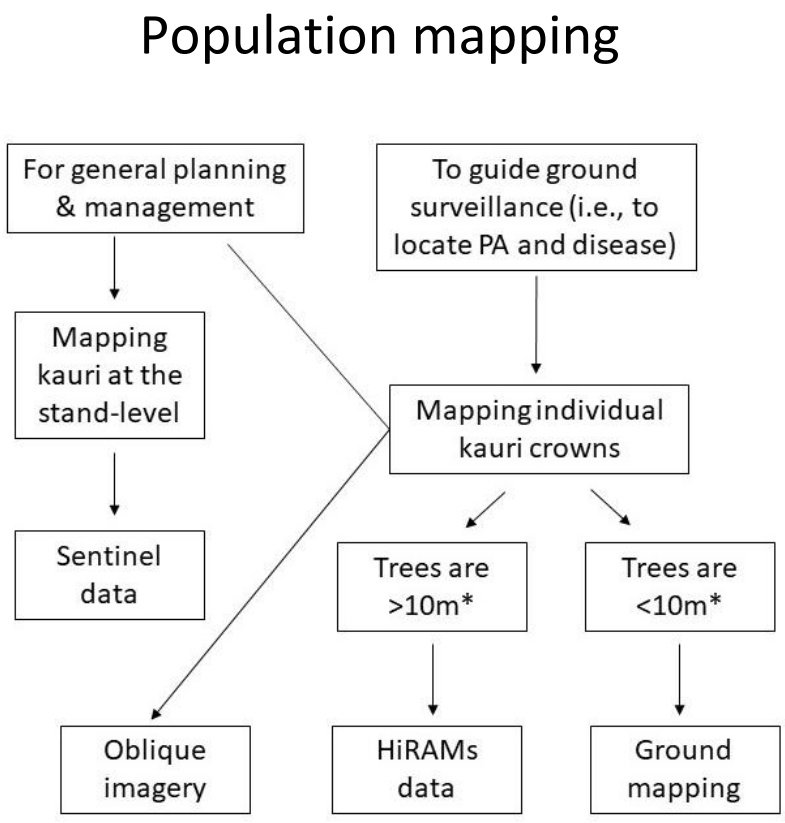


Fig 1. Decision tree for mapping kauri. *Note, methods are in development to optimise our ability to locate trees <10m, while 10m or higher is the current threshold.

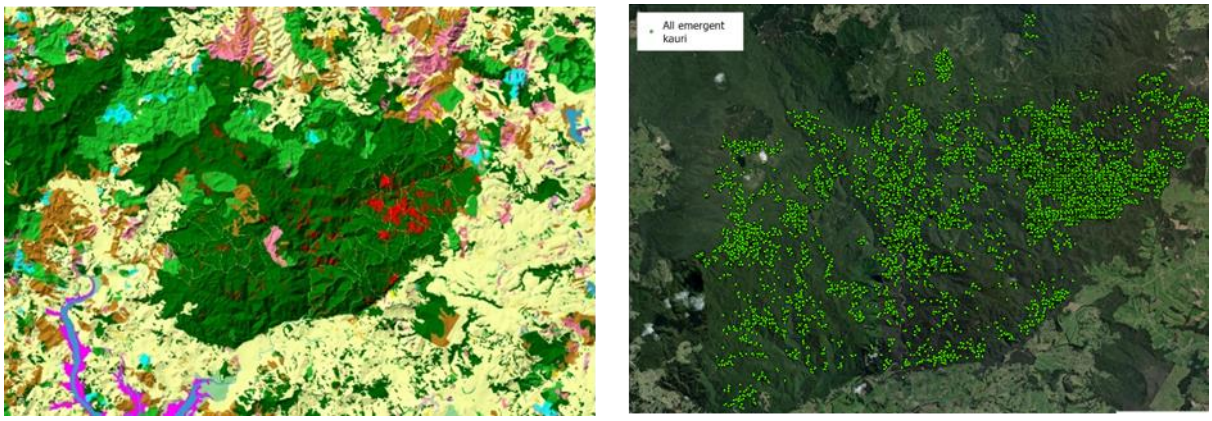
For general planning and management, stand-level mapping is sufficient, as kauri stand maps can guide managers performing desktop operations aimed at protecting kauri, such as moving tracks and traplines away from kauri stands. If a finer-scale map is needed, deep learning technology can be used on oblique imagery to identify locations of individual kauri, but oblique analyses are prone to over-estimating kauri and are difficult to locate on the ground.

For designing and guiding ground surveillance activities, maps of individual kauri can locate target trees for sampling and guide ground crews for surveying. These maps are built using imagery that is geo-referenced,

so trees can be randomly selected (based on the surveillance design) and field crews can locate targets on the ground. This imagery is biased to larger kauri (trees >10m tall) that are visible in the canopy, so smaller trees, especially those below the canopy are omitted when using this technology. Currently, the only way to geo-locate smaller trees is by using hand-held GPS devices.

a) Stand-level maps have been developed by Manaaki Whenua using publicly available Sentinel data (Fig. 2). These maps can delineate kauri from pine forests, which was problematic in existing stand-level maps.

b) Oblique imagery has been captured several times over the entirety of kauri lands (2017, 2020, 2023) and has been used to map to the crown-level across the majority of Kauri lands. This population data is being used in risk maps and ground surveillance activities, but tree data from oblique imagery is not well georeferenced, making it less suitable to guide ground surveillance activities. Thus, future investment should be allocated towards crown-level kauri mapping using previously acquired higher resolution imagery. Further, because of its affordability and capture frequency, oblique imagery is well-suited for understanding where additional higher resolution mapping should be prioritised and for change detection, making oblique imagery an excellent tool for monitoring..



a. b.

Fig. 2. a) Stand-level map and b) deep-learning kauri crown mapping from oblique imagery of Puketī and Omahuta forests

c) Hyperspectral and HiRAMs imagery has been captured across the majority of Tai Tokerau (Fig. 3) kauri lands and parts of Coromandel. This imagery is ideal for guiding ground surveillance activities because it is well geo-referenced. Image analyses are less accessible, however, due to the limited capacity available to analyse imagery. However, where areas have been prioritised based on need and risk, analyses of these areas can be contracted.

Fig. 3. HiRams capture over Tai Tokerau by Specterra. Captured areas are outlined in green. Orange and pink areas designate areas that could not be captured due to clouds.

2) Develop risk maps using explanatory and predicted risk factors from previous studies and local knowledge holders to inform surveillance for the presence and absence of *PA*

Understanding the full distribution of *PA* across kauri lands is an unobtainable goal, because of the resources required to accomplish such a large-scale and complex job. Mapping risk factors associated with disease and pathogen spread is more feasible; targeted, smaller-sized areas can be validated using randomised surveys of priority management units and where local knowledge can inform points of interest for targeted sampling. Risk-based surveillance plans, using explanatory and predicted risk factors from past studies, can be used to prioritise areas for surveillance and guide the development of long-term monitoring sites.

Manaaki Whenua researchers, working under Nga Rakau Taketake's Integrated Surveillance Theme, have developed risk maps across Kauri forests for several iwi, hapū and iwi-hapū collaborations in Northland (i.e., Puketī and Omahuta for the Eastern Collab and Warawara, Cape Reigna and Ngati Wai for the Iwi Collab). These maps are constructed using known and predicted risks, including kauri health scores derived from oblique imagery (i.e., indicators of disease), tracks and traplines, and environmental variables, such as hydrology and geology. Individual kauri crowns were identified and geo-located using oblique imagery and management areas (i.e., at the sub-catchment level) displaying the highest risk of *PA* (indicated by the additive risk scores from multiple factors) were chosen across each forest for ground surveillance.

Surveillance experts (Karyn Froud, John Kean and Jane Meiforth) with experience working on Auckland Council's large scale surveillance projects (e.g., Waitakere and Hunua) have developed tools for landscape-scale surveillance planning (i.e., at a broader spatial scale than the sub-catchment level). Using the Kaimai range as a pilot study, methods were developed to map risks predicting PA presence. Risk factors largely include known historic introduction pathways, but can also include pathways identified by mana whenua and other forest experts. Each risk factor is assigned a unique weight (i.e., importance) and a range (i.e., distance from the centerline of the risk factor), which may be adjusted following input from the land manager.

Risk factors included in the Kaimai pilot include:

- The 1942 topo map as a proxy for historical introduction pathways (land clearance, logging and regenerating shrubland) along with other relevant digitised historical maps, mātauranga and local knowledge.
- Historical and contemporary risks of *PA* introduction and localised spread (roads, tracks and bait lines).
- The current confirmed PA-sites geospatial data (with a buffer ~250m)
- The current symptomatic kauri geospatial data and potentially stress data
- Risk associations from the Waitākere Survey and risk mapping methods developed for the Hunua survey (when available)
- Other risk factor data as available (e.g. NRT data, visible signs of decline from aerial imagery)

Risk maps identify areas where PA is most likely to be or not to be. Because PA is most likely to be spread to trees in close proximity, the kauri population is "thinned" prior to choosing the sample frame. To thin the population, trees are grouped into clusters containing all trees within 50m of one another. Risk scores can then be calculated for each cluster (the tree with the highest risk score), indicating which clusters have the highest likelihood of being infected and should be surveyed on the ground.

a. Identify management units where sufficient information about *PA* risk is known, to identify sites suitable for protection or containment.

- Risk factors, both known and predicted, can be mapped and classified with specific risk profiles (to be co-developed, see draft below) then used to help identify management units for protection or containment across kauri lands. Previous work on strategic mapping by the KDP (2015, 2016) will also be used to inform risk profiles.

Suggested draft risk profiles (requiring further collaboration and revision) are:

Risk	**Description (not all criteria are required to be met)**
Low	No apparent introduction risks, no clear evidence of disease, previous testing with results of '*PA* not detected'
Uncertain low	No apparent introduction risks, no clear evidence of disease, no previous testing for *PA*
Moderate	Some introduction risk, some spread risk, some evidence of disease, previous testing with results of '*PA* not detected'
Uncertain moderate	Some introduction risk, some spread risk, some evidence of disease, no previous testing for *PA*
Uncertain high	Significant historical or contemporary introduction risk, strong evidence of disease, no previous testing for *PA* or inconclusive results
High	Significant historical or contemporary introduction risk, strong evidence of disease, and/or confirmed *PA* tests

As an example, using the draft risk profiles above, low and uncertain low would potentially be suitable for protection whereas high and uncertain high could be containment areas. Under a precautionary approach, uncertain moderate and moderate could also be considered for containment areas. Decisions on the management of these risk profile areas remain with land managers. These areas may be revised over time as new *PA* data and kauri health data are obtained through ongoing monitoring and other surveillance activities.

b. Implementing risk-informed kauri health and *PA* prevalence surveys to validate risk factors and inform long-term monitoring and management

A sample frame calculator has been developed to determine how many trees should be sampled to provide 95% confidence that the pathogen is absent from an area of interest. The sample frame can contain a mix of risk-based and random trees. Sampling trees from the risk-based sample will increase the probability of detecting the pathogen if it is there. The random sample is better suited for long-term monitoring.

The Kaimai map is complete with 17 risk layers and the Kauri host layer. Maps were shared among local DOC staff, botanists and mana whenua partners for feedback, which was incorporated. An estimated 295 sample points were chosen, where 80% were risk-based and 20% were random. Sampling is planned to commence in summer of 2024-25. Improved risk maps are now being developed for Northland forests where trees have already been mapped.

Then baseline surveillance in the field can begin, including tree health assessments and soil sampling, using standardised data collection forms and optimised diagnostic tests. Trees selected in this process will be tagged for repeated long-term monitoring.

Monitoring data will then be used to validate the risk profiles and improve identification of management units suitable for protection or containment across kauri lands.

c. Inform areas for initial delimiting surveys to address immediate knowledge gaps for management

In many cases, mana whenua already know where they want to perform kauri health surveys to address imminent management questions. However, for large forests, it may be difficult to know where to begin. Risk profiling can aid managers in prioritising ground surveillance efforts in those forests. Landscape-scale prevalence studies, as described above, can focus survey areas to the catchments requiring more intense surveying. Once positive detections have been made, catchment-level surveillance can be done to delimit the pathogen's spread, if that is desired by managers. Alternatively, positive detections can signal closure of sub-catchments and/or management diversions out of the areas (e.g., moving tracks and traplines away from infected sub-catchments).

3) <u>Build capability and capacity in mana whenua using a consistent training approach</u>

To facilitate collaboration among managers working across kauri lands, and to build a national picture of pathogen distribution and tree health, ground surveillance data should be collected in a safe, consistent and

methodical way. To optimise and accelerate surveillance efforts, different iwi and hapū can be trained using the same approach, building work crews with shared goals and methods across all of kauri lands. This will result in a better picture across all of Kauri lands, however there will be gaps, which is why a risk-based approach is necessary.

Progress and planning to date

BioSense Ltd, a consulting company with extensive experience designing and implementing *PA* surveillance strategies for government agencies, councils, researchers, iwi and hapū, has been contracted to deliver a training programme aimed at building capability and capacity in mana whenua and ensuring consistent data capture. The training includes disease diagnosis, data collection, soil sampling and hygiene and survey kits including digital data platforms, GPS units, tablets, sampling gear and hygiene kits to use in the field.

Limited targeted surveillance will be done during the training, at known trees of concern. Once training is complete, surveyors will be better placed to perform validation of larger areas of interest, using the same data collection forms as others working in the programme.

Tiakina Kauri has engaged external contractors to develop a Best Practices Guide for Hygiene for Surveillance and to produce a video demonstrating tree health surveys and soil sampling, to support the training. Training goals and methodology were peer reviewed by Tiakina Kauri's Technical Advisory Group for Ground Surveillance. Ground surveillance training will undergo a review in the field season of 2024

4) Optimise management and surveillance tools

Diagnostic testing tools for improved *PA* detection

Current diagnostic tests for *PA* range widely in their sensitivity, limiting our ability to detect *PA* from field soils. The tests most commonly used to detect *PA* are two-step: first, in a "soil baiting" step, soils are flooded with water and baited with a specific type of plant material that the pathogen can grow on. Second, the bait from step one is used to detect *PA* using a morphological or molecular test. For morphological identification, the baits from step one are placed on a petri dish so the pathogen can grow out, followed by expert identification of whatever organism grows on the plate. Although the media used to isolate *PA* can be very effective, the pathogen is not guaranteed to grow out on the plate. Molecular tests can improve the detection rates from baits. For molecular testing, the loop-mediated isothermal amplification (LAMP) test can detect *PA* DNA extracted from the baits from step one. This makes the likelihood of detecting the pathogen much higher, since the LAMP test is highly sensitive and can detect even minute amounts of DNA in the baits. LAMP can be performed by general lab technicians and results are delivered more quickly than morphological tests.

The primary limitation of both tests is that oospores, the long-term storage and reproductive structures produced by PA, may not be present in the sample and/or have low germination rates in the soil baiting step. This is a significant issue, since oospores may often be the only life stage found in the samples that go to the lab (zoospores, which germinate easily, are very short-lived). Thus, a fully molecular test with a method that allows technicians to use LAMP (or PCR) tests on DNA extracts from large volumes of soil containing oospores is needed. Methods are available, but they are cumbersome and require large machinery, such as a paint shaker, and can only process limited numbers of samples.

Tiakina Kauri has partnered with MPI's Plant Health and Environment Laboratory (PHEL) to optimise and accredit the tests and the labs performing the tests. The optimised protocol for soil baiting can be found here [Soil baiting SOP]. In addition, Tiakina Kauri has funded a PhD student, Jade Palmer, who has developed a DNA extraction method from large volumes of soil that can detect oospores as well as other life stages. This method is currently being optimised and expected to be operationally ready by spring 2023 for the summer field season.

An investigation template has been developed to validate questionable results from screening tests (Tiakina Kauri Surveillance Communication and Investigation Template and in **Appendix 2**).

Aerial and ground surveillance tools

The aerial surveillance tools available to survey kauri lands are at the cutting edge of technology. However, our methods for using these tools in kauri forests for the specific objectives outlined are in their early days. Remote sensing can be used to indicate areas of decline, but tree health assessments and soil sampling must be done to determine whether decline is due to *PA* or another factor. Once decline has been identified with remote sensing and validated on the ground, Tiakina Kauri's goal is to shift to using aerial surveillance to monitor disease spread of known infections in management areas. However, a comparative evaluation of the available platforms is needed to determine how to use them effectively and facilitate future decision making. Further, new methods for tree and health identification are being developed in collaboration with national and international partners.

Diagnostic testing of large soil volumes is currently our only tool for confirming *PA* positive trees, but soil and root sampling methods are inconsistent across the various practitioners who take these samples. Tiakina Kauri convened a group of experts who regularly collect soil and roots for sampling to optimise the method. The updated soil and root sampling method can be found here..

5) Develop communication, storage and management plans for data

A document has been developed to plan how new detections of *PA* will be communicated and investigated [Tiakina Kauri Surveillance Communication and Investigation Template and in Appendix 3]. It is recommended that this communication plan template is completed in partnership with mana whenua and land management agencies when designing surveillance activities (i.e. prior to undertaking surveillance). It will be particularly useful in areas believed to be free or partially free of *PA*.

Depending on the reason for undertaking surveillance, all or only parts of this Plan may be required. Data sharing agreements that have been signed previously may already cover all or parts of the communication plan. These details should be discussed prior to initiating any surveillance activity.

6) Provide tools and knowledge to develop a kauri protection plan template

Kauri protection plans are developed in place, by mana whenua, in partnership with agencies. The ultimate goal of the surveillance and monitoring strategy is to provide mana whenua with the tools, skills and baseline

knowledge needed to co-develop their kauri protection plans. Wānanga will be underway in the coming months to begin the co-design process on a template for kauri protection plans.

APPENDIX 1

INVESTIGATION PLAN: VALIDATION OF SCREENING TEST RESULTS FOR A CONFIRMED DETECTION

This plan notes the difference between a screening test result and a confirmed detection of PA. A positive screening test (indicating presence of PA) provides a piece of evidence towards validating whether a detection of the organism is confirmed.

- *Describe the process (e.g. phone calls, email, urgent online hui) and timeframes for the lead agency to notify partners and key stakeholders of the progress of validation for a positive screening test.*
- *Describe expectations for validating results, including timeframes to complete validation.*
- *Ensure confidentiality is maintained when validating results.*
- *Describe the funding mechanism for validation of diagnostic results.*

SCREENING TEST BACKGROUND

Observing symptoms of disease on kauri trees gives an indication of the presence of PA, the causal pathogen of kauri dieback, however the symptoms are not unique and can be caused by other biotic and abiotic factors. It is also possible to have *PA* present in the soil or kauri roots prior to the development of symptoms. To confirm the detection of *PA* we currently have two MPI approved screening tests, a DNA-based LAMP bioassay and culture-based Morphological bioassay.

Screening tests are used to give an indication if the pathogen is present and the two tests have different characteristics that together can help to confirm presence of the pathogen, alongside epidemiological criteria.

When screening for a pathogen, it is useful to have a test with high sensitivity, which will find most of the sites where the pathogen is present (true positives) but may also identify sites where the pathogen is not present (false positives). In contrast, a test with high specificity will correctly identify sites as true positives but may miss sites where the pathogen is present (false quantified the diagnostic sensitivity or specificity of the LAMP test, so can't accurately estimate how many false negatives it will have, but we can guess that it will be less than the morphological test.

The LAMP test performs well and there are no known issues with cross-reactions or misidentification (confusion with other *Phytophthora* species in NZ), however, due to the very high analytical sensitivity of the test to detect the DNA of *PA*, there is a risk of cross-contamination. This means there is a (low) risk that a positive result may be from a different sample. MPI's PHEL (Plant Health and Environment Laboratory) has worked with test providers to identify cross-contamination risk points during the diagnostic test process (Table 3) and approved test providers have implemented measures to minimise this risk. In addition, extra steps can be taken during surveillance and sample collection to address cross-contamination (Table 3). A recent review of soil sampling procedures has identified a change that may improve the sensitivity of the morphological test by modifying where soil is collected around the tree (4 x cardinal points and 4 x risk-based points around the tree base). This is now the standard soil sampling procedure (SOP).

DNA based LAMP test

		Detected	Questionable	Not detected
True Pathogen status	Present	Detected	Suspect positive Result is uncertain due to low titre of pathogen in sample.	FALSE negative Sample did not collect the pathogen.
	Absent	FALSE positive Result is positive due to possible cross-contamination.	FALSE suspect positive Result is uncertain due to possible cross-contamination.	Not detected

Table 1 Table showing the result options for the DNA-based LAMP test compared to the true status of the pathogen in the field. The main risk with this test is a False Positive result

Morphological test

		Present	Not detected
True Pathogen status	Present	Detected	**Possible** False negative Sample did not collect enough pathogen

Absent	**Unlikely** False positive Cultured pathogen is misidentified.	Not detected

Table 2 Table showing the result options for the morphological test compared to the true status of the pathogen in the field. The main risk with this test is a False Negative result.

GEOGRAPHICAL CRITERIA FOR VALIDATION

Proximity to previous *PA* detections (many of which were via the morphological bioassay test) and an estimate of the prevalence of *PA* in an area that is to be surveyed can inform the effort required for screening test validation. For example, the consequence of a false positive may be very low in an area where *PA* is widely known and distributed, compared to an area where it is unknown.

Several decisions are required for setting the requirement for screening test validation (Table 3). A decision is required between partners to identify specific survey sites or areas that require screening test validation (see validation process below). In addition, a geographical distance beyond which screening test validation is required needs to be agreed. This may be in the form of a set distance (e.g. 300m) or between spatially based management units (e.g. water catchments or stream sub-catchments). An indication on the conditions for validation is also required, in that, is validation only required for the first instance, for instances or will this be reviewed at a certain point during surveillance (e.g. if more than 3 stream sub-catchments have confirmed *PA* detections, the validation requirement for the survey will be reviewed).

Screening test validation required	**Geographic details**	**Validation conditions**
Specific sites/areas	· *Describe the geographical locations or areas that require validation of positive or questionable screening test results (e.g., areas perceived as high consequence, where a PA detection is unexpected).*	

Geographical distance or spatial management units	· *Describe the geographical distance or spatial management areas beyond which validation of positive or questionable screening test results are required (e.g. beyond a 250m radius from a known PA site, or detections in stream sub-catchments that are not contiguous with stream sub-catchments that contain a known PA site).*	

Table 3 Screening test validation requirements and conditions.

VALIDATION PROCESS

· *Describe the process for validating positive screening test results for* P. agathidicida *with the diagnostics service provider.* * Completed below.

· *Describe the process for validating questionable, positive or negative screening test results when DNA and morphological tests return different results. *Completed below.*

It is recommended that the DNA-based LAMP test is used to screen samples and the morphological test is used as part of the process to validate samples. Some surveys may choose to use both tests in parallel.

For the LAMP test three results are possible, positive screening test, questionable screening test and not detected screening test. A questionable screening test is where the test result value lies within the measurement of uncertainty (MU) of a test. The measurement of uncertainty should be incorporated into assessing the results. For example, if the cut off value is Cq 36 and MU is 0.5, test results with Cq values between 35.5 and 36.5 should be interpreted as questionable and need to be further determined (e.g. run a gel to confirm product size is expected) if this is consistent with the expected size, the result can be validated in the same way as a positive screening test for LAMP.

Note: MU = Square root of [(Average of standard deviation of reproducibility)^2 + (Average of standard deviation of repeatability)^2].

On the receipt of a positive or questionable screening result in an area where validation is required the following actions are required:

· Request the diagnostic service provider checks sample reception records to ascertain if samples from other areas were being processed at the same time and request processing dates and diagnostic results for those records (anonymous).

· Request the diagnostic service provider checks records to rule out any potential mix up of samples, e.g. similar sample submission code.

· Check time to detection for LAMP results to inform questionable results threshold values (i.e., low target concentration in the sample)

· Validation of screening tests can be undertaken using several options (Table 4):

- start with re-testing any remaining or peeled frozen baits (useful to determine if cross-contamination occurred after baiting),
- then re-test remaining soil (useful if cross-contamination occurred during sample splitting and baiting).
- If these are inconclusive or the point of cross-contamination is possibly prior to soil splitting, the next step is to collect new samples from the same location and test using morphological testing followed by LAMP testing of peeled baits.

- Collection of new samples:
  - undertake a field investigation of the site to collect standard soil samples (8-point protocol) around the original tree and up to 9 other Kauri (to account for poor test sensitivity) within 50-100 m of the test positive site for additional testing.
  - the field investigation team should include team members from the partner organisations that are very experienced in *PA* field sampling.
  - Store any unused soil until the investigation is completed.

- If suspected *PA* is confirmed detected in a new region or special area (e.g. Bay of Plenty or Hunua):
  - Send the isolate to MPI Plant Health and Environment Laboratory for confirmation. Confirmation technique involves morphological examination and multi-locus sequence typing. The latter includes sequencing at least two of the taxonomic informative genes (e.g. COX-1, COX-2, HSP90, ND1) from the newly detected isolate and compare with reference sequences from taxonomic ex-holotype isolate (ICMP 17027) to confirm species identification.
  - Send the isolate to the International Collection of Microorganisms from Plants (ICMP) for long term preservation and storage.

Table 4 Table of points in the soil sample and DNA-based LAMP test process where cross-contamination may occur, procedures for risk mitigation and recommended retest options. Where the options are LAMP (retesting using LAMP for remaining frozen LAMP baits and frozen peeled baits from morphological testing) and Morph + LAMP (morphological tests undertaken in series with LAMP test by peeling baits from the morphological test substrate and sending frozen baits for LAMP testing).

Point of cross-contamination	**Mitigation**	**Retest options for validation**			
		Remaining baits (LAMP)	**Peeled baits (LAMP)**	**Remaining soil (Morph + LAMP)**	**New soil sample (Morph + LAMP)**

Field collection					
Trowel used to collect soil	Follow the soil sampling SOP for trowel hygiene.	X	X	X	√
Sample labelling	Carefully label bags and include label photo in data entry form.	X	X	X	√
Sample transport in backpack	Double bag individual samples.	X	X	X	√
Sample transport to lab	Separate batches of samples (from the same location) into separate bags.	X	X	X	√
Sample storage in lab	Check for holes in bags (re-bag). Separate batches of samples (from the same location) into separate bags or bins and ensure storage bins are decontaminated with bleach between batches. Change gloves between batches for all steps.	X	X	X	√
Baiting lab					
Sample splitting	Remove individual samples onto a separate bench for soil splitting. Washdown between samples and denature between batches (avoids spill).	X	√	√	√

Transfer to baiting containers	Remove individual samples onto a separate bench for soil transfer. Use a NEW container or DNA denature washed container for each sample. Washdown between samples and denature between batches (avoids spill)	X	√	√	√
Air drying	Separate containers by batches, apply double sided tape to bench between batches to stop invertebrate movement (also avoids dust/knocking). Include at least 2 negative control soils in a random location within each batch of samples to detect cross contamination.	X	√	√	√
Moist incubation	Remove individual samples onto a separate bench for moist incubation spray (avoids splash)	X	√	√	√
Needle extraction	Use ethanol to sterilise forceps and flame until red hot between samples to denature DNA. Replace ethanol between batches.	X	√	√	√
Needle labelling	Double check label. Label is written from lid to base.	X	√	√	√
DNA extraction and testing					
Needle cutting	Use ethanol to sterilise forceps and flame until red hot between samples to denature DNA. Replace ethanol between batches. Use new section of tissue paper on cutting surface between samples. Denature clean between batches	√	√	√	√

Pipette DNA into plate well	Calibrate pipettes 3 monthly. Include a weak positive control to detect lower titre target and cross contamination. Typically, this can be x100 higher than the limit of detection.	√	√	√	√
Recording results	Double check sample ID.	√	√	√	√

If both the morphological test and the LAMP test are undertaken in parallel, there are several pairs of results that can arise with differing validation requirements depending on the geographical criteria set for validation (Table 5).

		Morphological test			
		Present		**Not detected**	
		Within PA geographic criteria	***Outside PA geographic criteria***	***Within PA geographic criteria***	***Outside PA geographic criteria***
DNA-based LAMP test	**Detected**	Confirmed detection	Positive screening test. New sample validation required.	Confirmed detection	Positive screening test. Validation required.
	Questionable	Confirmed detection	Positive screening test. New sample validation required.	Suspect screening test. Validation required.	Suspect screening test. Validation required.
	Not detected	Confirmed detection	Positive screening test. New sample validation required.	Not detected	Not detected

Table 5 Diagnostic scenarios for validation of screening test results when both LAMP and Morphological bioassays are used, stratified by known PA-site informed geographic criteria.

APPENDIX 3

COMMUNICATION PLAN

It is recommended that this communication plan template is completed in partnership with mana whenua and land management agencies when designing surveillance activities (i.e. prior to undertaking surveillance). It will be particularly useful in areas believed to be free or partially free of PA.

Depending on the reason for undertaking surveillance, all or only parts of this Plan may be required. Data sharing agreements that have been signed previously may already cover all or parts of the communication plan. These details should be discussed prior to initiating any surveillance activity.

AWARENESS OF PLANNED SURVEILLANCE ACTIVITIES

Who should be aware of surveillance activities:

- *List agencies that need to be aware of surveillance including within the lead agency, mana whenua partners, supporting agencies, communities.*
- *Do you need signs at trailheads to alert the public of surveillance activities?*
- *If there are private property owners at forest boundaries make them aware of planned surveillance activities.*

How and when will you raise awareness among key stakeholders:

- *Describe the key awareness messages amongst key stakeholders including communities and the most suitable means (for your contexts) to communicate these prior to surveillance.*

DATA MANAGEMENT

How should surveillance data and test results be managed:

- *Describe any data sharing agreements among partners that are involved in the surveillance including the lead agency, mana whenua partners, supporting agencies, community groups. If no agreements exist, describe how test result data and confirmed detection data will be managed.*
- *What platforms will be used to hold the data and how will access be managed?*

NOTIFICATION OF POSITIVE SCREENING TESTS AND CONFIRMED DETECTIONS: PARTNERS

How awareness of this process will be maintained to ensure effective and timely sharing of notifications:

- *There are three potential outcomes of a test: detected, undetected, questionable. Describe the notification process for each result.*
- *At what point should key stakeholders be notified of diagnostic test results (i.e. immediately following initial diagnostic test result, following validation, etc.)? Think about the consequences of a leaked result or withheld results.*
- *Describe the process for reporting positive results for* P. agathidicida *from the diagnostics service provider to the key stakeholders, including contact names and details.*

· *Identify the trees/sites/areas where positive detections will require validation prior to undertaking surveillance activities (i.e., where the pathogen has never been found before, culturally sensitive areas, areas where management actions aimed at containment will be especially onerous).*

· *Describe expectations for reporting results, including timeframes to notify key stakeholders for a positive screening test in areas believed to be free of* P. agathidicida*.*

· *How will you ensure confidentiality is maintained when reporting results?*

· *Describe the process (e.g. phone calls, email, urgent online hui) and timeframes for the lead agency to notify partners and key stakeholders of a positive (unvalidated) test result.*

· *Detail who, within and external to the lead agency, will be informed. Include how often and at what points in the investigation they should be updated.*

· *Describe communication plan for a confirmed detection after validating the screening test. Identify any external agencies or stakeholders who may be needed to assist directly.*

· *Isolates from positive detections in new areas (i.e., where PA has not been detected previously) should be isolated and sent to the International Collection of Microorganisms from Plants (ICMP) for long term preservation and storage*

NOTIFICATION OF POSITIVE SCREENING TESTS AND CONFIRMED DETECTIONS: KEY STAKEHOLDERS

· *Detail how and at what point (e.g. during validation a positive test result vs a confirmed detection) a potential detection of P. agathidicida will be communicated to the wider community.*

· *Consider key messages for a draft comms plan.*

· *Consider preparing key messages should information be made public prior to the planned communication (e.g. prior to a confirmed detection).*

ACTION PLAN FOR A CONFIRMED DETECTION

· *Describe the type of urgent actions that may be undertaken and their objectives (e.g. track closures, site investigation to understand introduction pathways (incorporated in the additional sample collection) including tracing of planted kauri, adjustment of forest management plans (weed/pest control operations, planned maintenance), treatment etc.). A table of scenarios in Appendix A might be helpful.*

· *Describe the process (e.g. phone calls, email, urgent online hui) and timeframes for the lead agency to agree with partners (and key stakeholders if required) to implement the action plan. Describe the approval process.*

· *Detail who has the authority to approve funding and resources to implement the actions.*

REFERENCES

Froud K, Chew YC, Kean J*, et al.*, 2022. 2021 Waitākere Ranges kauri population health monitoring survey. *Auckland Council technical report, TR2022/8.*, Pp. 265.