TIAKINA KAURI
KAURI PROTECTION

Approved soil and root sampling method for *Phytophthora agathidicida* detection

Tiakina Kauri

2023

Version 1.0 (01/06/2023)

Biosecurity New Zealand
Ministry for Primary Industries
Manatū Ahu Matua

Tiakina Kauri - approved soil and root sampling method for *Phytophthora agathidicida* detection

Purpose and background

The method below describes the operating procedure for sampling roots and soil in the field for the purpose of *Phytophthora agathidicida* (PA) detection, which was standardised and approved by Tiakina Kauri. Soil and roots obtained using this method may be used to amplify PA using the standard soil baiting method, followed by downstream analysis using the loop-mediated isothermal amplification (LAMP) or morphological assessment.

The approved methods (Version 1.0) were agreed by attendees at a 17 March 2023 workshop to standardise the soil and root sampling method. Some components of the methods require additional evidence to determine if further standardisation is necessary, and changes will be made as part of continuous improvement as evidence becomes available. The next review of the approved method is due in 2024.

	Name
Drafted by:	Lindsay Bulman (Technical Advisory Group lead)
Reviewed by:	Karyn Froud (Biosecurity Research) Lauren Waller (Tiakina Kauri)
Workshop attendees:	Chris Beard (Northland Regional Council) Tony Beachamp (DOC) Yue Chin Chew (Auckland Regional Council) Gavin Clapperton (Kauriology) Lee Hill (BioSense Ltd.) Fredrick Hjelm (BioSense Ltd.) Taoho Patuawa (Te Roroa|Kauriora) Kim Parker (Waikato Regional Council) Peter Russell (Better Biosecurity Solutions) Suliana Teasdale (DOC)

	Name	Role	Signature/date
Approved by:			

Note: *Phytophthora agathidicida* is an unwanted organism and must not be spread or propagated without the permission of a chief technical officer. This precludes any laboratories that are not CTO approved from baiting soils collected using this method.

Protocol for Soil and Root Sampling

General Principles

1. This protocol is for sampling under general surveillance to determine *P. agathidicida* (PA) presence. The method may change in cases of research and/or validation, or extreme environmental conditions (e.g. a drought year or extended wet/flood period).
2. Contact should be made with the diagnostic testing laboratory prior to sampling to agree on sample size requirements.
3. Procedures that affect the laboratory result will, wherever possible, have clearly defined specifications that are to be followed nationally. Those procedures are generally under the design, equipment and sample collection steps.

1. Procedures that reduce risk to kauri will have suggested minimum standards and guidelines for best practice to be followed, but mana whenua and local authorities will determine what their acceptable level of risk is and define procedures accordingly. Those procedures are under the hygiene and sterilisation steps.
2. Data recording, labelling and data management are crucial to maintain data integrity and comparison of results across locations.
3. It is important surveyors are well trained and inexperienced surveyors supervised until they become skilled. It is expected that all surveyors understand the principles of forest hygiene and are willing and able to practice them.

Table 1 – Summary of SOPs. **Instructions in bold must be performed as directed.**

Step	Procedure	Mandatory operating procedure	Suggestions and guidelines	Outstanding questions and comments
Design	No. samples	**8 for general surveillance**		
	Distribution	**4 evenly spaced points 1m from stem, one in each quadrant, and 4 samples guided by qualitative descriptions of where PA is most likely to be. If there are no obvious likely locations, use cardinal points at canopy dripline.**	PA is more likely to be found in wet depressions in the soil and close to bleeds	Qualitative descriptions to be defined as agreed at a later meeting.
	Data recording		Record whether sub-samples were taken from targeted or cardinal points. Other metadata to be collected will be specified later.	Metadata that are mandatory to collect will be agreed at a later meeting,
	Sampling order	**Plan sampling before you start: Start away from lesions to minimise potential pathogen spread but ensure samples closest to the lesion are taken**.		
	General environment	**Avoid very wet or extremely dry conditions (I.e., so dry that soil crumbles when sampling). Wet conditions make it more likely to spread the pathogen, it is less likely to detect the pathogen in dry soils**		Need to confirm whether or not pathogen detection is less likely in dry conditions, optimum soil temperature, across different soil types
	Route planning	**Avoid kauri areas enroute to sampling sites to the extent that it is possible. Work/walk upslope of Kauri and avoid river crossings where possible.**		
Equipment	Digging tool	**The digging tool must be able to be easily cleaned and checked for cleanliness.**	Entirely stainless steel trowels are preferred because they are easiest to clean (but these are difficult to find). It must have minimal features that trap soil. Serrated trowels (available from	

			www.kings.co.nz and minipicks cut the roots cleanly and minimise root damage. Minipicks may be preferable on dry, rocky sites. Gladwrap wrapped around the handle of the tool avoids having to clean the handle at the accepted frequency (i.e. between sub-samples, trees, or sites/stands. The minipick head detaches from the handle easily, clean between both parts well. A small pair of tongs may be helpful for handling parts.	
	Collection bag and labelling	**Use Snaplock medium storage bags (220x250mm) for one lab, or Snaplock large storage bags (260x380mm) when the sample will be split between two labs The label must be clear, safe and permanent.**	Bag size is important because it limits the volume sampled. Place the label between the inner and outer bag if the sample is double bagged, or write on the bag prior to adding the sample and avoid the combination of sharpie and ethanol. Aluminium tags may also be used, placed inside the sample bag. Use capital letters, zeroes and sevens have strikes, underline 9s and 6s. Other optional procedures to help avoid sample confusion and improve clarity include: Taking a photo of bag and label (helpful during large surveys) and bringing a prewritten inventory of samples to be collected into the field.	Volume to be confirmed by labs
Sample collection	Depth	**Sample from under the litter surface to 10-15cm, without collecting irrelevant material on the top of the soil (e.g. leaf litter).**		
	Target fine root material	**Target fine root material, especially nodules and necrotic roots.**		
	Soil quantity	**Fill the bag 2/3 filled with loosely packed soil/fine roots.**	Use a medium bag to send for one lab/test, and a large bag for two labs/tests (bag size specified above)	
Hygiene and Sterilisation	General principles	**Route planning is key – err on the side of caution**.	The frequency of cleaning between trees or sub-samples will be determined	Detail and figures similar to the existing guideline on route planning in the final SOP will be added.

		Limit the number of people and movement in the kauri hygiene zone and no more than two people in the drip line. **Footwear, euipment and other personal items that could have soil on their surface must be cleaned and disinfected before and after entering sites.** **Remove all soil before applying sterilant.**	by those responsible for the forest and land. Guidelines for cleaning trowels and footwear are provided below. Hypochlorite is a good sterilant, but it breaks down quickly in the container so concentration will be unknown. Sterigene is safe, but does not destroy all life stages of the pathogen. 70% ethanol is effective, but has safety issues. Material safety data sheets and training must be provided if using meths.	Need a vehicle treatment protocol. A table with different methods, timing, etc will be added. A table with solution dilutions is needed.
	Equipment placement	**Equipment must be kept off the ground. Avoid kneeling on the ground.**		
	Trowel treatment	**As a minimum, trowels must be cleaned or replaced between stands.** **Remove soil using a clean cloth or paper towel and use 70% ethanol to clean.** **Allow to air dry before using again.**	Best practice is to replace or clean trowel between every subsample taken. Often that is not possible or practical, so replace or clean between trees. Place Gladwrap on trowel handle to help keep it clean and replace after every tree.	
	Footwear treatment	**Booties must be changed or shoes must be cleaned between Kauri hygiene zones (Noting that hygiene zones should be avoided when travelling to the extent that it is possible).**	Booties are recommended, but may not always be suitable due to H&S issues. Do not reuse booties unless they have been boiled for 4 minutes. Always have someone else clean your shoes. Freezing boots for 48 hours at -18C reduces spore viability by around 20%.	
	Gloves		Gloves are preferred but not mandatory.	
Storage and transportation	Temperature	**Chilly bin not below 10^{o}C or above 25^{o}C, not in fridge.**	**.**	