Progress report: kauri dieback detector dog training

Dr Imogen Bassett

Auckland Council Biosecurity

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Executive Summary

Current methods for detecting kauri dieback disease (*Phytophthora agathidicida*) have a number of limitations. Scent detection dogs have huge value in other fields of biosecurity, due to their ability to produce instantaneous diagnoses in the field for relatively low cost. This report summarises progress to date on a pilot project to scope and trial a scent detection dog for kauri dieback disease. A literature review indicated that scent detection dogs are capable of detecting a range of microbial taxa with levels of accuracy comparable to those of scent detection dogs used for other taxa, and broadly comparable to current methods used for detecting kauri dieback disease. However background literature was unable to answer some important questions, including whether dogs are able to distinguish between closely related microbial taxa (required in order to ensure kauri dieback disease can be distinguished from other *Phytophthora* spp.), and whether the dormant oospore stage would be detectable.

Scent detection training was piloted with a Labrador dog 'Paddy'. Training involved kauri dieback disease cultured on oat grains, along with three control treatments (uninoculated oat grains; grains inoculated with *P. cinnamomi*; grains inoculated with *P. multivora*). Paddy's sensitivity (kauri dieback samples positively identified) in a replicated handler-blinded test was 87% on the first attempt and 100% on second attempt. Specificity (negative samples correctly identified as negative) was 96%. These results are very encouraging and strongly indicate that Paddy is able to identify kauri dieback disease and to differentiate it from other common *Phytophthora* species.

Further training was initiated to expose Paddy to kauri dieback disease in more complex situations, initially using a mixture of greenhouse-grown inoculated kauri seedling roots and the potting mix soil in which they were grown. However, this phase of training was cancelled due to Paddy's inability to focus on the samples and commands. Paddy had evidently been suffering boredom for some time, apparently reflecting the confined conditions in which training was undertaken in order to comply with Unwanted Organism permit restrictions. This is considered to be the most likely cause of his failure to progress well in this phase of training. More flexible permit conditions are required in order to effectively progress this project to the next stage.

Project background

Pathogens present numerous challenges as invasive species, not least of which being their microscopic nature and consequent difficulties in identifying presence/absence. Current detection methods for kauri dieback disease have a number of constraints including, *inter alia*:

- Considerable time-lag between sample collection in the field and subsequent laboratory diagnosis.
- Inability to delimit infection in the field at a fine landscape scale.
- Cost (c.$135 per sample).

Scent detection dogs have demonstrated huge value in other fields of biosecurity, due to their ability to produce instantaneous diagnoses in the field for relatively low cost (c.$2,000 per year to maintain a dog), as well as their ability to engender publicity and positive public engagement. Possible uses for a kauri dieback detection dog include delimiting of disease in the field; hygiene accreditation checks for nurseries; public advocacy and engagement while checking disease status of footwear and other equipment at wharves or events. However, the feasibility of a scent detector dog with respect to these and other scenarios involving kauri dieback disease was unknown. This project was

therefore initiated to 1) undertake a background literature review to scope project feasibility, and, given a promising outcome from the literature review 2) undertake pilot training with a dog "Paddy".

Literature review

Dogs and biological scent detection

Dogs have highly developed olfactory systems and are therefore used to aid detection of a wide variety of living and non-living material. In wildlife ecology and biosecurity dogs have proved successful in species-specific detection across a range of taxa including vertebrates (e.g. Cablk and Heaton 2006; Gsell et al. 2010), invertebrates (e.g. Brooks et al. 2003; Pfiester et al. 2008; Lin et al. 2011; Zahid et al. 2012), weeds (e.g. Godwin 2010), and pathogens (Martin 2011; Mittleman 2016).

Scent detection dogs have previously been trained for tree pathogens, although there are relatively few details of these projects available in the accessible literature. Dogs are used to detect the Huanglongbing bacteria in orange orchards in America in order to reduce further spread of the disease (Mittleman 2016). Similarly dogs have at least shown promise in preliminary pilot work aimed at detecting two fungi, *Leptographium* and *Heterobasidion*, which infect pine tree roots and cause Southern Pine Decline (Martin 2011; American Kennel Club 2015).

Dog have been more extensively used to detect human pathogens or diseases, both in samples of bodily fluids or breath (e.g. Cornu et al. 2011) and within indoor environments (Wolfgang et al. 2001; Kauhanen et al. 2002). Kauhanen et al. (2002) tested the ability of dogs to detect a range of microbial taxa on pieces of timber hidden inside a domestic building; three wood rot fungi (*Serpula lacrymans, Coniophora puteana* and *Antrodia sinuosa*), five other fungal taxa *(Cladosporium herbarum, Trichoderma viride, Botrytis cinerea, Penicillium verrucosum* and *Aspergillus niger*), and five strains of the bacteria *Streptomyces* sp. Overall, dogs displayed a low false positive rate (90% specificity) when detecting microbial growth in an indoor environment, and this value is likely to be conservative because the study's authors considered that at least some false positives were attributable to poor hygiene during the study setup (Kauhanen et al. 2002). False negatives occurred somewhat more frequently than false positives. Sensitivity (number of positive samples correctly detected) was only 75% overall (Kauhanen et al. 2002). Other detection methods for kauri dieback also show a trend of more frequent false negatives than false positives (Stacey Hill pers. comm. 18th February 2014). Therefore, while false negatives are of concern, it is possible that they may not be any more of an issue for sniffer dogs than for existing technology, and could be mitigated by follow-up laboratory based confirmation where deemed appropriate. In some instances dogs were more successful in detecting fungi than bacteria (Kauhanen et al. 2002). These authors do not, however, detail whether the dogs were able to search for a specific individual pathogen rather than simply discriminating between infected and non-infected material.

Studies examining dogs' ability to detect cancer from urine samples or exhaled breath tend to report levels of sensitivity and specificity similar to those described above for identification of indoor wood microbes; negatives correctly identified around 80-90% of the time, and positives correctly identified in around 70% of cases (e.g. Cornu et al. 2011; Ehmann et al. 2011, 2012; Buszewski et al. 2013).

Johnen et al. (2013) reviewed all literature on scent detection by dogs across all target taxa/diseases, and found that for studies in which success rates had been rigorously tested,

sensitivity ranged from 88-100% and specificity from 91-99%. Consistent with this overall trend, Rhys (Auckland Council's Argentine ant dog) is able to correctly identify Argentine ants approximately 90% of the time (Brian Shields pers. comm. 19th February 2014). Therefore there are reasonable grounds to explore the potential of a kauri dieback sniffer dog, based on known capability of detector dogs with respect to other taxa. What does not appear to have been tested for any type of pathogen is the ability of dogs to discriminate among closely related pathogen taxa.

Microbial Volatile Organic Compounds (MVOCs)

Microbes emit volatile organic compounds (MVOCs) during growth, and the chemical profile of these emissions varies among microbial species (Fischer et al. 1999; Korpi et al. 1999; Polizzi et al. 2012). Gas chromatography and mass spectrometry (GC-MS) can therefore be used to produce species-level identification of microbes such as moulds and crop pathogens based on emission profiles (Vickram et al. 2005; Polizzi et al. 2012), although not all studies have successfully identified species-specific MVOC profiles for their study organisms (Lui et al. 2005; Back et al. 2010). GC-MS has previously been used to identify several MVOCs which could be consistently used as markers for detecting the presence of *Phytophthora cinnamomi* (Qiu et al. 2014). However, MVOC production is known to be biologically dynamic, and the MVOC profile of a given pathogen species or strain may vary depending on a range of environmental factors such as substrate, duration of incubation/fungal life history stage, nutrient availability, temperature, and moisture (Morath et al. 2012; Polizzi et al. 2012). Although Qiu et al. (2014) identified MVOCs consistently associated with *P. cinnamomi* on a variety of culturing substrates, these authors also identified other MVOCs which were only produced by the pathogen on certain culturing media. Similarly, Lui et al. (2005) identified several MVOCs which could be used to discriminate between healthy vs disease-inoculated potatoes, but found that individual compounds did not occur consistently across all of their experimental replicates.

Arthropods are known to be able to detect and respond to MVOCs (Morath et al. 2012), and similarly, MVOCs are likely to be key to the ability of dogs to detect microbes and cancer (Cornu et al. 2011). Dogs have some notable advantages compared with analysis of chemical profiles via GC-MS. Dogs are cheaper, more mobile, and also have been shown to be able to provide binary yes/no answers to questions such as 'does this patient have cancer?' even when GC-MS results contain large amounts of complex and seemingly inconsistent information, and the specific compounds of interest are unknown (Buszewski et al. 2013).

Kauri dieback dog: The unknowns

The literature review indicated that dogs may plausibly be able to identify a pathogen such as kauri dieback disease. However, several questions which have material bearing on project feasibility cannot be answered based on existing literature. Chief among these are:

- Can dogs distinguish among closely related microbial taxa? Ability to discriminate *Phytophthora agathidicida* from closely related *Phytophthora* species such as *P. cinnamomi* and *P. multivora* would be essential for a detector dog to be of practical use for field surveillance.

- Can dogs detect kauri dieback across varied substrates within the complex odour environment of a forest?

- Can dogs detect oospores? Ability to detect this inactive life stage would be essential for a detector dog to be of practical use for field surveillance.

Progress to Date

Training is being undertaken by Stacey Hill and Brian Shields with the Labrador dog "Paddy", supplied by Mark Veet. Paddy is trained using a 'click/reward' system of positive reinforcement. The first stage of training involved basic obedience training.

The second phase of training involved *P. agathidicida* cultured on oats, contained in small glass jars. Identical jars containing uninoculated oat grains, or containing oats inoculated with either *P. cinnamomi* or *P. multivora* were used as controls. During training, jars were opened and placed within terracotta pots covered with wire mesh to enable Paddy to smell the contents while preventing him from coming in physical contact with the pathogen. Strict hygiene protocols were in place throughout training and testing, including a trigene bath at the facility entry/exit point.

Paddy's training was temporarily delayed when he received a bite from another dog, which required some recovery time, leading to a total of c.5 months for the initial training period. Following the initial training, handler-blinded testing was conducted to assess Paddy's ability to discriminate between jars of oats inoculated with kauri dieback, con-generic pathogens, or no pathogens. The test assessor laid out a row of four jars; one jar each of *P. agathidicida*, *P. cinnamomi*, *P. multivora* and uninoculated oats. Each jar was contained in a terracotta pot as described above for training. The positioning of these jars was randomized, and the dog handler was not privy to the layout to prevent Paddy from picking up on subtle body-language cues from the handler. Paddy and the dog handler were then allowed access to the randomized jars. Following exploration of the jars, Paddy sat at a jar to indicate presence of kauri dieback. This process was repeated 15 replicate times, thus exposing Paddy to a total of 15 kauri dieback samples and 45 negative samples.

In this test, Paddy's sensitivity (kauri dieback samples positively identified) was 87% on the first attempt and 100% on second attempt. This discrepancy reflected the test assessor intervening prematurely in some instances of incorrect sample identification. The dog handler felt that given more time to work with the dog he would have been able to determine that the dog was 'trying it on' rather than firmly indicating a positive sample. This highlighted the need to develop for the next phase of testing an agreed procedure for the handler to indicate when they are confident of the dog's determination, and only at this point should the test assessor indicate whether the choice was correct or not. Specificity (negative samples ignored i.e. correctly identified as negative) was 96%. Of those few negative samples incorrectly identified, con-generic pathogens were not identified any more commonly than the uninoculated controls, suggesting that Paddy is able to differentiate *P. agathidicida* from other *Phytophthora* species. These results are very similar to the levels of sensitivity and specificity evidenced by detection dogs working with other taxa in previous studies.

The promising results in this initial pilot suggested that it was worth investing in further work to extend Paddy's training to situations which are more challenging and more 'real-world' than the inoculated oats, in order to elucidate the remaining questions.

Another round of training was initiated using greenhouse-grown inoculated kauri seedling roots and potting mix, and Conservation Dog accreditation testing was planned for mid-April 2016. However, Paddy was persistently disinterested in the soil and root mixture and had trouble focusing on the training. Consequently the decision was recently made to cancel the remainder of this phase of training and post-pone the Conservation Dog test.

The Unwanted Organism (UO) status and microscopic nature of kauri dieback disease present several logistical challenges for this project. The permit conditions associated with handling a UO have required that all training to date has occurred within a small barn facility in order that the UO is strictly contained. While this was adequate for initial training, remaining confined to the barn is now a serious constraint to project success for two reasons. The first and most critical is that Paddy is becoming bored with the barn environment and the repetitive nature of the training exercises which can be delivered in this venue. It has been evident for some time that boredom is making it harder for the dog handlers to maintain Paddy's focus on his tasks. Boredom is one of the more probable explanations for the failure of the most recent training exercise using soil and roots.

Next Steps

Samples of the soil/root mixture will be re-tested to confirm that they indeed still have detectable *P. agathidicida* within them. Assuming that this is the case, further work is required to explore the reasons for Paddy's disinterest, to determine among the following alternative explanations:

- Paddy may be a capable kauri dieback detector dog who is simply under-performing at present due to boredom. This option is consistent with his previously demonstrated capability in the first phase of training.
- Kauri dieback disease may be detectable by dogs, but Paddy may be the wrong dog for the job. While he has demonstrated proof-of-concept for detection, Paddy himself is unproven as a working dog in the field.
- Kauri dieback may not be a suitable target for dog detection. However, while the situations in which a dog may be useful in the field remain untested, based on proof-of-concept it seems probable that a focused detection dog would demonstrate usefulness in at least some field situations.

To further develop our understanding of Paddy's potential it is now necessary to devise training and testing exercises which test his abilities in a more varied environment. **Therefore to facilitate a successful move to the next phase of the project, it is important that a new UO permission can be negotiated which allows for more flexibility to work outside of the barn.**

If a more flexible UO permission is possible, then a further training regime will be developed to elucidate Paddy's detection ability in more complex conditions and narrow down the situations in which he can/can't usefully detect the pathogen (e.g. on footwear/machinery; in contaminated soil; in potted plants; in a kauri forest).

Science outputs

- In November 2015 an oral paper on progress to date was presented at the New Zealand Ecological Society conference (Bassett et al. 2015; see Appendix).
- A manuscript documenting this project will be written and submitted to P & I for review prior to submission for journal publication.

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Appendix: Paper presented to NZ Ecological Society conference

Title: Assessing the potential of detector dogs for use in forest pathogen management: Paddy the kauri dieback dog

Authors: Bassett IE[1], Hill S[1], Shields B[1], Vette M[2], Avery K[2], Horner I[3]

[1]Auckland Council, Auckland.

[2]Mark Vette's Animals on Q, Auckland

[3]The New Zealand Institute for Plant & Food Research Limited, Hawke's Bay.

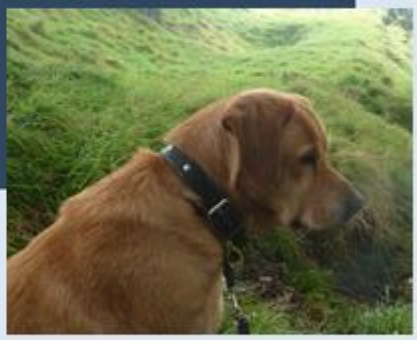
Abstract:

In wildlife ecology and biosecurity, dogs have proved successful in species-specific detection across a range of taxa including vertebrates, invertebrates and weeds. Detector dogs can also have huge value in positively engaging the public with biosecurity issues. While dogs do not appear to have been used to detect microbes in ecological contexts, they have been used successfully to detect plant pathogens in crops as well as human pathogens or diseases, both in samples of bodily fluids or breath and within built environments. However there are few examples of detector dog programmes which have tested for the ability to discriminate among closely related microbial taxa. If dogs can successfully target plant pathogens within complex forest environments, they could offer relatively cheap real-time diagnosis with considerable potential value for large scale management programmes of forest pathogens such as *Phytophthora* spp. In what is possibly a world first, Paddy the Labrador is being trained to detect kauri dieback disease (*Phytophthora agathidicida*). This talk will outline the process of Paddy's training to date, including results from handler-blinded specificity and sensitivity testing from stage one of his training. This includes testing his ability to distinguish *P. agathidicida* from the con-generic and widely occurring *P. cinnamomi* and *P. multivora*. Next steps in the training process will be described, scaling up to more ecologically complex scenarios. Potential advantages and constraints of forest pathogen detector dogs will be discussed within the context of the kauri dieback management programme case study.

Detector dogs for use in forest pathogen management: Paddy the kauri dieback dog

Imogen Bassett, Stacey Hill, Brian Shields, Mark Vette, Kirsty Avery, Ian Horner

@ImogenBassett



Plant & Food RESEARCH

Mark Vette

Auckland Council

Kauri dieback disease

Phytophthora agathidicida (Oomycetes)

Soil- and water-borne pathogen.

Incurable; management focus is on spread prevention.

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Current detection methods

- Soil samples taken from around infected trees.
- Time-lag involved in lab-based baiting.
- Laboratory test costs c.$135/sample (c.f. annual cost of dog c.$2K).
- Unable to delimit at fine scale.

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Dogs and micro-organisms

Dogs used to detect both fungi and bacteria e.g.

- Indoor wood-rotting fungi.
- Faecal contamination of stormwater.

ELSEVIER

ENVIRONMENT INTERNATIONAL

Validity of detection of microbial growth in buildings by trained dogs

Eeva Kauhanen[a], Mikko Harri[b], Aino Nevalainen[a], Timo Nevalainen[c,d,*]

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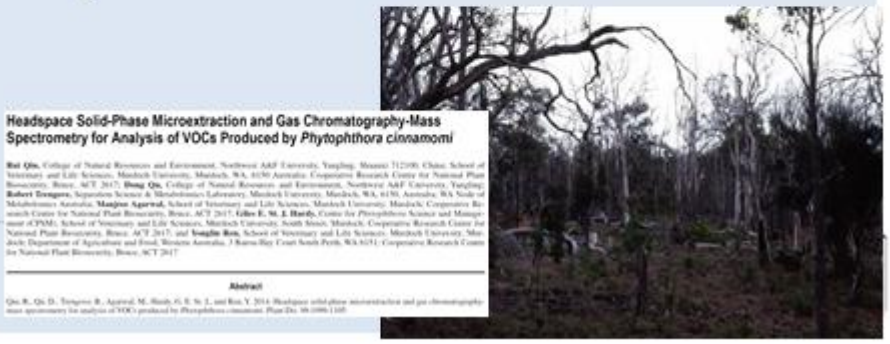
Volatile Organic Compounds

- *P. cinnamomi* infection (Jarrah dieback) associated with identifiable VOC profiles.
- But, dynamic, influenced by host, substrate, life-stage etc.



Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry for Analysis of VOCs Produced by *Phytophthora cinnamomi*

Rui Qiu, College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, China; School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, 6150 Australia; Cooperative Research Centre for National Plant Biosecurity, Bruce, ACT 2617; **Dong Qu**, College of Natural Resources and Environment, Northwest A&F University, Yangling; **Robert Dongen**, Separation Science & Metabolomics Laboratory, Murdoch University, Murdoch, WA, 6150, Australia; WA Node of Metabolomics Australia; **Manjree Agarwal**, School of Veterinary and Life Sciences, Murdoch University, Murdoch; Cooperative Research Centre for National Plant Biosecurity, Bruce, ACT 2617; **Giles E. St. J. Hardy**, Centre for *Phytophthora* Science and Management (CPSM), School of Veterinary and Life Sciences, Murdoch University, South Street, Murdoch; Cooperative Research Centre for National Plant Biosecurity, Bruce, ACT 2617; and **Yonglin Ren**, School of Veterinary and Life Sciences, Murdoch University, Murdoch; Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court South Perth, WA 6151; Cooperative Research Centre for National Plant Biosecurity, Bruce, ACT 2617

Abstract

Kauri dieback dog: the unknowns

- Can dogs distinguish among closely related microbial taxa?
- Feasible within the complex odour environment of a forest?
- Would oospores (life-stage predominating in soil) be detectable?

Meet Stacey and Paddy…

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Russell Blackstock Russell Blackstock is a senior reporter at the Herald on Sunday.

Introducing: NZ's first kauri dieback sniffer dog

5:00 AM Sunday Jul 28, 2013

Environment Pets & Animals

Mark Vette with Paddy. Photo / Doug Sherring

A former rescue dog has been trained as New Zealand's only specialist kauri dieback sniffer dog.

Three-year-old golden labrador Paddy was so emaciated when he was rescued from the home of an injured hoarder in Auckland, all his ribs could be counted. The dog was infested with fleas and was aggressive.

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Training

Pathogen cultured on cereal.

3x controls

- Uninfected cereal
- Cereal with *P. cinnamomi*
- Cereal with *P. multivora*

Click/reward system.

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Pilot test results

Stratified random, handler-blinded testing.

- **Sensitivity**
  (positive samples correctly identified)
  = 87% on first try, 100% on second attempt.
- **Specificity**
  (negative samples correctly ignored)
  = 96%

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Where to now?

Comprehensive test with current set-up.

Scaling up to more complex situations.

- Inoculated vs clean potting mix.
- Greenhouse plants/forest soil.
- A kauri forest...
- Practical applications?
- Other forest pathogens.

Aiming for Conservation Dog NZ certification.

Acknowledgements

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Photo credits: mpi.govt.nz; wsj.com (Glen Sanders); iinet.net.au (Ray Wills); Jeremy Warden; Brian Shields; Stacey Hill; Mark Veet; Alastair Jamieson.