









## CONTRIBUTED PAPER

# Evaluating scent detection dogs as a tool to detect pathogenic *Phytophthora* species

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## Abstract

The fungal genus *Phytophthora* includes an array of destructive plant pathogens that have had severe impacts on native, agricultural, and horticultural systems worldwide. Preventing the spread of *Phytophthora* species is critical for protecting vulnerable plants and ecosystems; yet detection remains a challenge due to their microscopic size, broad host range, and latent and cryptic expression in host plants. We tested the effectiveness of trained detection dogs to discriminate the odor of a target *Phytophthora* species, from among other non-target odors, by conducting a multi-choice experiment. We tasked two dogs with discriminating the scent of *P. agathidicida*—the causal agent of the lethal root rot disease “kauri dieback”—from two non-target *Phytophthora* scents (*P. cinnamomi* and *P. multivora*), and four non-target control treatments. We assessed the dogs' scent detection abilities by measuring the sensitivity and precision of their indications toward the target scent over 120 randomized trials. The dogs had a combined sensitivity of 68.6% (CI: 64.1–72.9) and precision of 52.2% (CI: 48.1–56.4), meaning they often identified *P. agathidicida* when it was present but also signaled on the other non-target *Phytophthora* species. Moreover, we characterized the nature of false positive indications made for non-target scents, which has important implications for how future multi-choice experiments should be conducted. Our study shows that detection dogs are likely to be an adequate first-pass detection tool for *Phytophthora* within a wider biosecurity framework. However, further research is warranted before dogs can be deployed for this purpose in the field.

## KEYWORDS

Aotearoa-New Zealand, biosecurity, detection dogs, kauri dieback, multi-choice experiment, *Phytophthora agathidicida*, plant pathogens, scent

# 1 | INTRODUCTION

Trained detection dogs are a mobile, real-time detection technology that can be used to identify scents in an increasingly wide range of contexts (Angle et al., 2016; Grimm-Seyfarth et al., 2021; Woollett Smith et al., 2014). Dogs have an acute sense of smell that can be used to identify chemical vapors at low concentrations, as well as an ability to scan large areas efficiently and non-destructively. This provides a superior alternative to many other visual and auditory detection approaches (Bennett et al., 2020). Within a conservation setting, detection dogs have been deployed for over 100 years to detect rare and cryptic species (Woollett Smith et al., 2014). Targeted searches have occurred successfully for a broad range of taxa, including mammals (e.g., Long et al., 2007), birds (e.g., Cheyne, 2011), reptiles (e.g., Browne et al., 2015), invertebrates (e.g., Ward et al., 2016), plants (e.g., Mclean & Sargisson, 2017), and pathogens (e.g., Cristescu et al., 2019). This success has driven an impetus for using detection dogs to survey other cryptic species that are considered detection priorities (Bennett et al., 2022). In a biosecurity context, detection dogs are used for surveillance and detection of low-density populations where detection is difficult, either to find the remaining survivors of an eradication, or during a high-priority pest incursion response (Kim et al., 2020).

Plant pathogens in the *Phytophthora* genus are high priority species for pest managers (Scott et al., 2019). *Phytophthora* species are often highly destructive, severely impacting native, agricultural, and horticultural systems worldwide (Scott et al., 2013). For example, *Phytophthora infestans* was the causal agent of the Irish potato famine of the 1840s and remains a substantial source of crop damages and losses, totaling more than USD\$6 billion in damages annually (Goss et al., 2014). Similarly, *Phytophthora cinnamomi* is regarded as one of the most devastating plant pathogens worldwide, infecting a wide range of woody plant species in productive and natural environments (Burgess et al., 2017), resulting in significant ecological impacts (Sena et al., 2018). Surveillance is used as the primary intervention method to ensure detection and to prevent *Phytophthora* from spreading into high-value areas. Yet detecting pathogens, such as *Phytophthora* species, is particularly challenging due to their microscopic size, broad host range, and latent and cryptic expression in host plants (Scott et al., 2013; Scott et al., 2019).

Recent research has demonstrated the efficacy of detection dogs to locate and identify plant pathogens (e.g., Gottwald et al., 2020; Mendel et al., 2018). Dogs may therefore also prove useful as a field-based survey tool for *Phytophthora* species. A first step in deploying

dogs for this purpose is to evaluate their performance as a scent detection tool (Johnen et al., 2013; Porritt et al., 2015) using standardized measures of precision (i.e., species specificity) and sensitivity (i.e., effectiveness) (Bennett et al., 2020; Rutter et al., 2021). Such measures are essential for interpreting survey data, understanding the comparative effectiveness of different survey and detection tools, and optimizing resources for cost-effective planning. However, the effectiveness of detection dogs for detecting *Phytophthora* species has yet to be evaluated (although see Swiecki et al., 2018 for a preliminary first attempt).

Here, we evaluate the effectiveness of trained detection dogs to discriminate the odor of a target *Phytophthora* species, *P. agathidicida*, from among a range of non-target odors, within a controlled laboratory setting. *Phytophthora agathidicida* is the causal agent of kauri dieback (Beever et al., 2009)—a lethal root rot disease that threatens the survival of kauri (*Agathis australis*), a conifer species endemic to Aotearoa-New Zealand (Ecroyd, 1982). This tree species is considered an ecological engineer due to its role in structuring plant community composition (Wyse et al., 2014) and is a taonga (or “treasure”) for indigenous Māori people (Black et al., 2018). Less than 1% of original old-growth kauri forest now remains as most kauri trees were burned or logged over the past 200 years (Steward & Beveridge, 2010) and, although these forests are now protected, kauri dieback threatens the survival of remnant populations (Bradshaw et al., 2020).

*Phytophthora agathidicida* is vectored by the movement of soil and soil-water (Bassett et al., 2017)—motile zoospores encyst on the fine root structures of host kauri, which then produce root-penetrating hyphae that colonize the trees' vascular tissue (Bellgard et al., 2016). Because infected trees are often symptomless in the early stages of infection, surveillance must rely on diagnostic tools to verify the presence of *P. agathidicida*, using either soil baiting assays or direct DNA detection methods (Beever et al., 2010). Although both these approaches are routine, they have limited functionality in the field—due to the need for specialized laboratory equipment, multiple weeks to assert pathogen presence (for baiting assays), and intensive labor requirements—which ultimately hinders surveillance.

Adaptive management procedures have been enacted as part of Aotearoa-New Zealand's national Kauri Dieback Programme to reduce soil movement among kauri stands (Bradshaw et al., 2020). Surveillance is undertaken to assist in identifying potential *P. agathidicida* vectors and pathways, and in identifying infected trees with latent symptoms (Froud et al., 2022). Dogs are now being trained to detect *P. agathidicida* with the intention of providing the Kauri Dieback Programme with an additional

surveillance tool. To evaluate the discriminatory abilities of dogs for this purpose, we conducted a “multi-choice” experiment (Johnen et al., 2013; Porritt et al., 2015) that involved presenting two trained detection dogs with a series of randomly selected odor samples, including one target *Phytophthora* (*P. agathidicida*), two non-target *Phytophthora* species that are commonly found in Aotearoa-New Zealand soils (*P. cinnamomi*, and *P. multivora*), and four non-target control treatments (oat grain, latex glove, dry dog kibble, and distilled water). We assessed the dogs' ability to discriminate *P. agathidicida* from among the non-target odors by measuring the sensitivity and precision of their indications toward the different samples. Finally, we characterized the nature of any false positive indications by comparing performance outcomes, and identifying patterns, to gain further insight into dogs' scent matching abilities.

## 2 | MATERIALS AND METHODS

### 2.1 | Dog selection and training

Two dogs—a female English springer spaniel (“Pip,” age 5) and a male jagdterrier (“Mawhai,” age 6)—were selected to be part of the trial (Figure S1) based upon their previous experience detecting vertebrate pest species in the Conservation Dogs Programme (Department of Conservation, 2019), their motivation for rewards, and their strong drive to work. Each dog was trained separately by an experienced handler (K.J. trained Pip; B.S. trained Mawhai) to associate *P. agathidicida* scent with a food reward (i.e., operant conditioning; Blackman, 1974) and to ignore the other treatment samples. Toy rewards were not used in this instance because they resulted in overstimulation for both dogs. The training process (including the facility used) was the same as the experimental trial where a group of target and non-target odors was randomly assembled for each dog to assess (see Section 2.3 for complete details), except during training the number of samples was varied to aid reinforcement. Training occurred intermittently over the course of 18 months (due to existing handler work and COVID-19 restrictions).

### 2.2 | Experimental trial

#### 2.2.1 | Odor selection and sample preparation

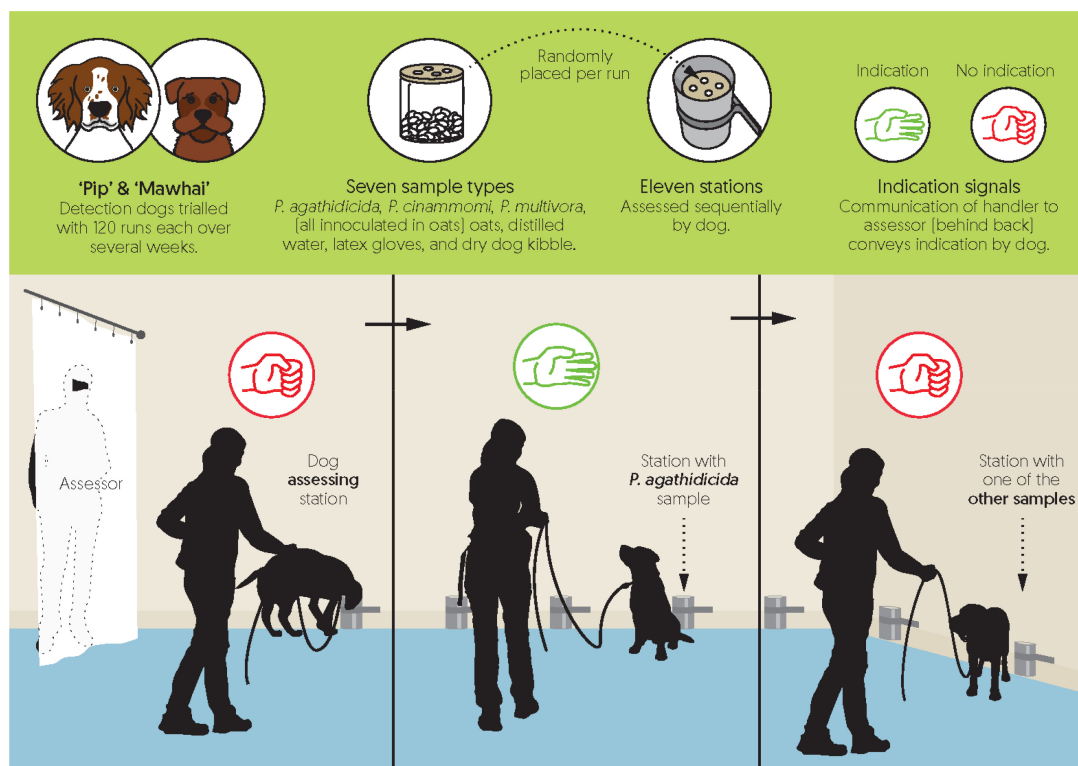
The seven scent treatment samples (hereafter “treatment samples” or “samples”) selected for the experimental trial comprised one target odor—*P. agathidicida*—and six

non-target odors: *P. cinnamomi*; *P. multivora*; sterilized oat grain; latex glove; dry dog kibble; and distilled water (Figure S2). Before commencing the study, the target and non-target *Phytophthora* species were inoculated in sterilized oat grain by Rangahau Ahumāra Kai—Plant and Food Research NZ Ltd. This process involved first growing clean *Phytophthora* cultures on V8-agar plates; colonized agar cubes (or uncolonized agar cubes for the controls) were then transferred to 100 mL sterilized squexagonal glass jars (Cospak Pty Ltd) containing 50 mL of sterilized oat grain soaked in water and incubated at 20°C for ~2 weeks. This facilitated thorough colonization of the oat grains. The *Phytophthora* and oat grain treatment samples were left sealed and stored in boxes at room temperature away from direct sunlight until they were used in the experimental trial.

The seven sample types were prepared each working day prior to commencing the experimental trial. Sample types not made by Rangahau Ahumāra Kai—Plant and Food Research NZ Ltd, including latex glove (one glove), dog kibble (50 mL equivalent), and distilled water (50 mL), were placed in 100 mL sterilized squexagonal glass jars, and all sample types were fitted with sterilized perforated metal lids. These non-target sample types were each transferred from separate, larger containers using separate equipment (e.g., gloves, measuring cups) to ensure there was no risk of cross-contamination. The number of samples prepared each working day was based on the number of trial runs being conducted (see Section 2.2.2, below, for complete details). Following use in the study, the days' prepared samples were promptly discarded; *Phytophthora* and oat grain samples were autoclaved and disposed by Rangahau Ahumāra Kai—Plant and Food Research NZ Ltd while the other sample types were disposed of as landfill waste. Reused jars and lids were sterilized in boiling water and with a pressure cooker, respectively.

#### 2.2.2 | Experimental design

We conducted the experiment over 15 working days from November 2021 to March 2022 at an Auckland Council research facility. Pip and Mawhai were separately tasked with assessing 11 different stations, each of which contained a randomly allocated treatment sample (Figure 1; Video S1). Each station comprised a hollow PVC tube (10 cm in diameter, 25 cm in length) permanently fastened to the wall (Figure S3). The length of the tube was such that, when the sample jar was placed inside, the handler could not discern its contents. Stations were 1.0 m apart to reduce odor interference. Seven stations were placed against one wall while the remaining four



**FIGURE 1** Overview of the experimental design used to assess each dog's ability to discern the scent of *P. agathidicida* inoculated in oats from among other non-target scents. The panels describe how the dog handler communicated indications made by the detection dog to the assessor.

were placed against the adjacent wall due to size constraints of the trialing room.

The dogs' task was to indicate on all stations containing oat samples inoculated with *P. agathidicida* while ignoring the other sample types. Each dog was individually led around the room on a lead by K.J. (who has extensive experience working with both dogs) and was required to assess each station sequentially by smelling the sample placed inside the station. There was no time-limit on how long the dog could spend at each station, but they were permitted to visit the station only once before moving on to the next station. The dogs took turns assessing the suite of samples and only one dog was present in the trialing room at a given time.

The experiment was conducted by one assessor (Z.T. C), who was obstructed from view by a curtain (Figure 1). The curtain had a viewing window so that the handler could always be seen by the assessor but also ensured that the assessor did not pass on non-verbal cues to the dogs. The assessor recorded whether an indication had occurred at a station, based solely on the declarations of the handler. The placement of each sample at each station was intentionally withheld from the handler. As the dog and handler progressed sequentially from station one to station 11, the dog would indicate whether it believed

the sample placed inside contained the target odor by sitting down. The handler, whose back was facing the assessor with their hand placed in a fist at their lower back, would then convey this indication to the assessor by opening their hand so that their palm was clearly visible. If the dog's indication was correct, the assessor would verbally respond with "correct"; the handler would then signal to the dog using a clicker device and reward them with food, before moving on to the remaining stations. If the dog had falsely indicated on the station, the assessor would remain silent, and the handler would abstain from rewarding the dog.

The experiment was organized into 120 trial runs (or "runs"), where each run contained a suite of 11 random samples selected through random number generation (totaling 220 target samples and 1100 non-target samples; Table S1). The number of target samples was selected using a power analysis, where 211 samples returned an estimated 90% probability that the lower confidence interval estimate would have  $\geq 60\%$  sensitivity (see Section 2.3.1, below, for complete details). The number of non-target samples was scaled to include five non-target samples for every one target sample (i.e., 20% prevalence) because *P. agathidicida* was thought to have a similar prevalence in Aotearoa-New Zealand's most heavily affected areas at



the time of this study (Hill et al., 2017; although see Froud et al., 2022 for updated estimates).

The number of runs selected on each working day was agreed upon before commencing the study but was contingent on both dogs' willingness to perform. Because both dogs were led through the experiment by the same handler, and because both dogs assessed the same suite of randomized samples, the order of the trial runs was also randomized for each dog so that the handler could not predict the contents of an individual station. After a dog completed an individual run, the assessor cleaned all PVC tubes using unscented, alcohol-based cleaning wipes, and then replaced the suite of samples with another, randomly selected suite of samples until all runs were completed for both dogs for the working day.

## 2.3 | Statistical analysis

### 2.3.1 | Performance measures

All statistical analyses were conducted using the R statistical environment (version 4.0.2; R Core Team, 2017). We used performance measures identified by Bennett et al. (2020)—including measures of precision and sensitivity—to evaluate the discriminatory ability of each dog. Precision is defined as the proportion of all indications made for a true target, and assesses how well the dog distinguishes the target scent from other odors. Sensitivity is defined as the proportion of targets found relative to the total number of targets available, and assesses how often the dog will identify the target odor when present in a multi-choice test. In other words:

$$\text{Precision} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}}$$

and

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}}$$

We computed 95% confidence interval (CI) estimates for these measures using the two-sided Clopper–Pearson interval (Thulin, 2014), via the epiR R package (version 2.0.41; Stevenson et al., 2022).

### 2.3.2 | Characterization of false positive indications

In addition to measuring the discriminatory ability of each dog, we also characterized their false positive

indications. Such a characterization provides insight into the dogs' scent-matching abilities (Wasser et al., 2009), which is key to evaluating their efficacy as a prospective detection tool. We first (i) determined whether there was a relationship between individual sample stations and the number of false positive indications made by each dog. In instances where both dogs falsely indicated at a station more than expected (defined below), we then (ii) investigated whether the previous stations influenced this outcome. (For example, had both dogs disproportionately falsely indicated at station no. 7, we would then determine whether this outcome was influenced by the samples at station nos. 1–6.) Finally, we (iii) investigated whether the dogs falsely indicated on the same runs more than would be expected due to chance, using the same stations identified in (ii).

We investigated (i) using simple linear regression; standardized residuals for each observation—and the distribution of errors across our datasets—indicate this was an appropriate choice for our study. We calculated a regression line for each dog and 95% CI using Wald Intervals. We considered a station to have more false positives than expected if the total number of false positive indications observed for a dog exceeded the linear model's 95% CI (sensu Fewster et al., 2000). We then used Fisher's exact test to investigate (ii) for these identified stations by comparing the observed and expected false positive indication frequencies. Our contingency tables specifically compared binary false positive presence-absences against the number of *P. agathidicida* samples (Tables S2 and S3). Finally, we investigated (iii) by comparing the total number of false positive indications made by both dogs for stations identified in (ii) against 100,000 simulated trials, using bootstrapped *p*-values. Each simulated trial consisted of 120 randomly generated runs for each dog for the identified station, using a binomial distribution, where the probability of a false positive for each run was the overall false positive proportion in each dog's observed data. The simulated data provided a distribution of the number of times that both dogs would falsely indicate on a single station. For each of the 100,000 trials, it was noted whether the number of times both dogs falsely indicated was equal to or greater than the number in the observed data. The proportion of times this happened in the simulated data represents a *p*-value for the null hypothesis (of no communication).

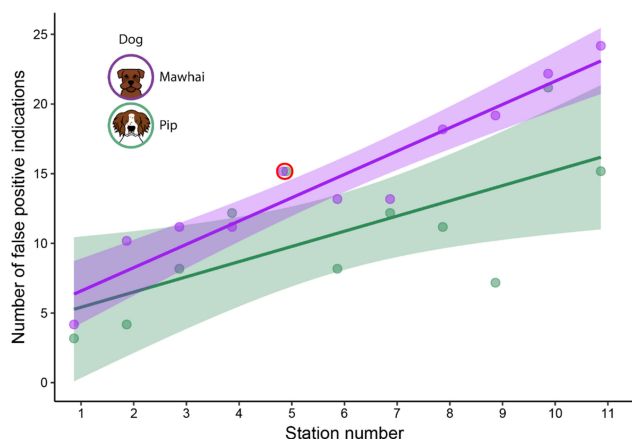
## 3 | RESULTS

### 3.1 | Performance measures

Our multi-choice experiment consisted of 120 trial runs with 220 randomly placed *P. agathidicida* target samples.

**TABLE 1** Summary of the indications made by each dog throughout this study with resultant performance measures.

Dog	Indications toward target				Performance measures (%)	
	True positive	False positive	True negative	False negative	Sensitivity	Precision
Pip	157	116	984	63	71.4 (64.9–77.2) <sup>a</sup>	57.5 (51.4–63.4)
Mawhai	145	160	940	75	65.9 (59.2–72.1)	47.5 (41.8–53.3)

<sup>a</sup>Indicates 95% confidence intervals.**FIGURE 2** Simple linear models describing the relationship between station number and false positive indications, for each dog taking part in the study. The overlapping points circled in red show the single instance where both dogs falsely indicated more than expected, based on the 95% CI.

Pip and Mawhai found and indicated on 157 and 145 target samples, respectively, resulting in a sensitivity of 71.4% and 65.9% for each dog (Table 1). Moreover, Pip falsely indicated on 116 non-target samples while Mawhai falsely indicated on 160, resulting in a precision of 57.5% and 47.5%, respectively, for each dog (Table 1). Nearly all false positive indications occurred on non-target *Phytophthora* species (comprising 88.8% and 76.9% of all occurrences for Pip and Mawhai, respectively) with the remainder occurring on the oat control. Although the estimated precision and sensitivity were higher for Pip than for Mawhai, these differences were not statistically significant (Wilcoxon rank-sum test: sensitivity  $W = 5506$ ,  $p = .60$ ,  $r = -.04$ ; precision  $W = 5886$ ,  $p = .21$ ,  $r = -.08$ ). Overall, the combined sensitivity and precision estimates were 68.6% (CI: 64.1–72.9) and 52.2% (CI: 48.1–56.4), respectively.

### 3.2 | Characterization of false positive indications

We found a significant linear trend between the sample station and the number of false positive indications made by each dog, indicating that as the sample station number

increased, false positive indications increased proportionally (Pip:  $F_{(1,9)} = 7.97$ ,  $p = .019$ ,  $\omega = 0.38$ ; Mawhai:  $F_{(1,9)} = 89.51$ ,  $p < .001$ ,  $\omega = 0.90$ ; Figure 2). Based on the 95% CI of the linear models characterizing this relationship, station no. 5 yielded more false positive indications than expected for each dog (Figure 2). Therefore, we investigated whether indications made at station nos. 1–4 influenced this outcome, and whether both dogs falsely indicated more than would be expected due to chance at station no. 5 for the same runs.

Pip and Mawhai falsely indicated at station no. 5 fifteen times each across the entire study (Figure 2). Most of these indications occurred when there were no *P. agathidicida* samples present at station nos. 1–4, comprising 46.6% and 53.3% of cases for Pip and Mawhai, respectively (Tables S2 and S3). Nonetheless, the observed and expected frequencies were similar for all categories within the contingency tables, and so we found no association between the absence of *P. agathidicida* at station nos. 1–4 and false positive indications at station no. 5, for either dog (Fisher's exact test:  $p > .05$ ). However, runs without any *P. agathidicida* samples present ( $n = 13$ ) resulted in a disproportionately high number of false positive indications (Pip:  $M = 1.77$ ,  $SE = 0.56$ ; Mawhai:  $M = 2.62$ ,  $SE = 0.87$ ) relative to runs with at least one *P. agathidicida* sample present (Pip:  $M = 0.87$ ,  $SE = 0.08$ ; Mawhai:  $M = 1.18$ ,  $SE = 0.11$ ; Figure S4). This difference was statistically significant for both dogs (Pip:  $t(14.47) = -3.34$ ,  $p < .05$ ,  $r = .66$ ; Mawhai:  $t(-3.47) = 3.47$ ,  $p < .05$ ,  $r = .68$ ). Put simply, samples of earlier stations likely did not affect indications occurring at later stations, although in extreme cases where a run had no *P. agathidicida* samples, both dogs falsely indicated a disproportionate number of times.

Of the 15 false positive indications made at station no. 5 by both dogs, eight of these occurred on the same runs (Table S4; where five false positive indications were made on *P. multivora* and three were made on *P. cinnamomi*). Our simulation of 100,000 experimental trials (each with 120 runs) returned 67 runs where both dogs falsely indicated eight or more times at station no. 5 (Table S5), equating to a 0.067% probability of occurrence under the null hypothesis. Our simulation therefore provides strong evidence that the observed false positive indications made at station no. 5 were not due to

chance (therefore we reject the null hypothesis of no communication between dogs,  $p = .067$ ).

## 4 | DISCUSSION

Our multi-choice experiment revealed that Pip and Mawhai are more sensitive to *P. agathidicida* than they are precise. In other words, the dogs often found and identified *P. agathidicida* when it was present but also signaled on other non-target *Phytophthora* species. This outcome is preferable to the alternative (i.e., low sensitivity with high precision; Saito & Rehmsmeier, 2015) in that effective biosecurity relies on successful detection to thwart the spread and establishment of invasive species (Bassett et al., 2016; Holcombe & Stohlgren, 2009). True targets that remain undetected (i.e., false negative indications; Table 1) following a survey therefore have the potential to compromise conservation outcomes, while non-targets falsely identified as being true targets remain relatively benign, within this context. (Although rewarding a dog for indicating on a false target can undermine its training and taking actions to address false targets can increase operational costs, e.g., by increasing the volume of lab tests to confirm *Phytophthora* presence.) Sensitivity of scent detection dogs varies drastically within the published literature—ranging from 20% to 100% (Bennett et al., 2020)—with value-added contributions being made to biosecurity and conservation across the entirety of this distribution. We, therefore, recommend that trained detection dogs are adequate discriminants of *P. agathidicida* within this laboratory setting and should proceed to further testing as a field-based survey tool. We stress more work is needed before dogs can be deployed for biosecurity purposes, though.

The utility of scent detection dogs is dependent on their ability to address inadequacies of an existing surveillance toolbox. As part of a wider biosecurity strategy, dogs have the potential to play an important role in determining the presence of *Phytophthora* species. For *P. agathidicida*, we suggest their strength will lie in identifying asymptomatic infected kauri trees and infected soils (Kiro, 2022), as well as high-risk goods (e.g., earthmoving equipment) being transported inter-regionally, which remains a major challenge. Moreover, kauri dieback monitoring is currently prioritized using a risk assessment based on individual trees (Froud et al., 2022); free-ranging detection dogs can efficiently scan large areas and therefore have the potential to monitor areas that are currently inaccessible (Woollett Smith et al., 2014). Overall, the performance measures we calculated indicate that dogs are likely to be an adequate first-pass detection tool; that is, a detection tool used in conjunction with other methods highly sensitive

to *P. agathidicida*—such as soil baiting assays or direct DNA detection methods (Beever et al., 2010; Bellgard et al., 2016)—to verify pathogen presence following initial indication.

We found three important trends regarding the false positive indications made by both dogs. First, there was a significantly positive association between the sample station number and the number of false positive indications. Although difficult to disentangle, we speculate this relationship could be due to the dogs' strong motivation for rewards, which spurred them to indicate more frequently toward the end of a given trial run as the perceived number of reward opportunities began to dwindle. Such positional bias is common in multi-choice experiments (Lazarowski et al., 2020) and can be counteracted using a circular carousel-style sample array, where there is no discernible start or end point (Johnen et al., 2017), or by rewarding the dog for correctly identifying a non-target sample. We recommend future studies incorporate at least one of these approaches in their experimental design.

Second, although we found that samples of earlier stations likely did not affect the indications occurring at later stations, we did find a disproportionate number of false positive signals occurred on runs without any target *P. agathidicida* samples (Figure S4). This may indicate that the dogs were expecting at least one target sample to be present within a given trial run, the absence of which may have resulted in frustration, ultimately translating to behavioral compromises (Fadel et al., 2016). Grimm-Seyfarth et al. (2021) found that frustration resulting from low target densities was a major contributor of reduced performance outcomes in studies where dogs were used as detection tools. We recommend special attention is paid during training to ensure each dog can satisfactorily resist indicating on non-target samples before proceeding to the testing phase of a multi-choice experiment.

The third important trend we found was an unexpectedly high number of false positive indications made by both dogs on the same trial runs at station no. 5 (Figure 1), which may provide evidence for chemical communication. Dogs can detect volatile organic compounds at very low concentrations (Angle et al., 2016) and so it is possible that one dog alerted the other through changes in its saliva or exhaled breath as a response to a perceived target (Jezierski et al., 2008). We suggest future studies can eliminate this problem by conducting multi-choice experiments in a well-ventilated area and by using new samples (or new containers) for each dog after every trial run. Alternatively, this trend may have been due to discrepancies in how the dogs were trained relative to how the study was conducted. Training often took place using a smaller subset of samples, which may have imprinted a preference for this station if it

was unknowingly used as a reliable reward source (Johnen et al., 2015). Such preferences can be corrected by setting up samples in a quasi-random fashion to target the dog's individual deficiencies during training (Jezierski et al., 2008).

Our study represents the first in-depth investigation into the efficacy of dogs as a scent detection tool for invasive *Phytophthora* species. Yet more work is needed to consider detection dogs a viable field-based survey tool for this plant pathogen. We recommend two additional testing stages are needed; adequate performance outcomes must be returned in both stages to warrant field deployment. First, dogs must demonstrate their ability to discern the scent of *Phytophthora* within soils, which emit an array of volatile organic compounds (Insam & Seewald, 2010), and can obfuscate or mask a target scent, thereby compromising performance outcomes. The test soil should contain biologically realistic sources of *P. agathidicida*, such as colonized root fragments. Second, detection dogs must demonstrate their ability to find and identify *P. agathidicida* in the field. Field conditions introduce yet another layer of complexity that both the dog and handler must contend with, including a wider variety of non-target odors and distractions relative to laboratory conditions. Such testing should occur in areas where *Phytophthora* presence/absence has been confirmed, and *P. agathidicida* prevalence closely resembles real-world conditions, so that training can be properly reinforced. We also recommend effort (i.e., time spent searching a unit area) and cost-effectiveness are calculated in this second stage as part of a robust performance measurement (Bennett et al., 2020).

## AUTHOR CONTRIBUTIONS

Ellery J. McNaughton, Murray P. Fea, Kerryn Johnson, Brian Shields, Alistair S. Glen, and Zachary T. Carter conceived the study; Zachary T. Carter and Kerryn Johnson collected the data; Zachary T. Carter and Jessica McLay analyzed the data; Zachary T. Carter led in writing the manuscript; all authors revised the text and gave final approval for submission.

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## DATA AVAILABILITY STATEMENT

Data and code available via the Figshare Digital Repository (<https://doi.org/10.17608/k6.auckland.21561705>).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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