Forestry An International Journal of Forest Research

Institute of Chartered Foresters

Forestry 2017; **00**, 1–9, doi:10.1093/forestry/cpx019

Ingestion of infected roots by feral pigs provides a minor vector pathway for kauri dieback disease *Phytophthora agathidicida*

I.E. Bassett[1]*, I.J. Horner[2], E.G. Hough[2], F.M. Wolber[3], B. Egeter[4,5], M.C. Stanley[6] and C.R. Krull[7]

[1]*Auckland Council, Private Bag 92300, Auckland 1142, New Zealand*
[2]*The New Zealand Institute for Plant & Food Research Limited, Private Bag 1401, Havelock North 4157, New Zealand*
[3]*Massey University, Tennent Dr, Palmerston North 4474, New Zealand*
[4]*Department of Zoology, University of Otago, Dunedin, New Zealand*
[5]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, 4485-661 Vairão, Portugal*
[6]*Centre for Biodiversity and Biosecurity, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[7]*Institute for Applied Ecology New Zealand, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand*

*Corresponding author. E-mail: Imogen.Bassett@aucklandcouncil.govt.nz

Received 4 December 2016

Identifying vectors of soil-borne forest pathogens is crucial in limiting disease spread. *Phytophthora agathidicida* causes kauri dieback disease, killing kauri (*Agathis australis* (D.Don) Lindl.), Aotearoa-New Zealand's largest endemic tree. Currently incurable, management focuses on spread prevention. Feral pigs (*Sus scrofa*) are implicated in spread, through ingestion of infected material; congeneric *Phytophthora cinnamomi* is known to survive pig gut passage. We investigated *P. agathidicida* survival of pig gut passage in a captive feeding experiment, and assessed *P. agathidicida* incidence in feral pig stomachs from DNA. We detected viable *P. agathidicida* from a kauri root retrieved from captive-fed pig faeces, providing proof of concept that pigs can internally vector *P. agathidicida*. However, we detected only one positive, despite processing a total of 11.2 m of passaged roots and 800 millet (*Pennisetum glaucum* (L.) R.Br.) seeds, from 12 pigs. We did not detect *P. agathidicida* in any of 184 wild-caught feral pig stomachs, but did detect five other *Phytophthora* species including *P. multivora* and *P. cinnamomi*. Ingestion of contaminated material by feral pigs is probably a minor pathway for *P. agathidicida*, and a higher risk pathway for *P. cinnamomi*. We highlight the need to test *Phytophthora* species individually, as pathways of spread may be considerably different between related species.

Introduction

Invasive pathogens, like invasive plants and animals, have benefited from increases in human movement and habitat modification (Kelly *et al.*, 2003; Brasier, 2008; Meentemeyer *et al.*, 2008). However, pathogens present their own particular suite of challenges, not least of which is the difficulty in detecting and identifying microscopic invaders, and consequently in determining invasion pathways and vectors. Vectors influence the frequency and destination of transfer events as well as the number of individual propagules per transfer event (Lockwood *et al.*, 2007). Furthermore, the speed with which a vector delivers the invasive species to a new site often affects propagule survival, with reduced transit time associated with increased likelihood of establishment (Ruiz and Carlton, 2003). Given that propagule pressure (i.e. the number of individuals introduced and the number of introduction events) is a key determinant of invasion success (Colautti *et al.*, 2006), identification of vectors is crucial in managing the spread of unwanted pathogens.

Phytophthora spp. (Oomycetes) have long been recognized as major pathogens of agricultural crops, including having caused the failure of potato (*Solanum tuberosum* L.) crops resulting in the Irish famine of the 1840s (Judelson and Blanco, 2005). *Phytophthora* spp. are also responsible for catastrophic effects in a diverse range of forest ecosystems, including Sudden Oak Death (causative agent *P. ramorum*) and Jarrah Dieback (causative agent *Phytophthora cinnamomi*) (Rizzo and Garbelotto, 2003; Hansen, 2008).

In Aotearoa-New Zealand, *Phytophthora agathidicida* is the causal agent of kauri dieback disease (Weir *et al.*, 2015). *P. agathidicida* is considered likely to be non-native to Aotearoa-New Zealand (Beever *et al.*, 2009). It infects kauri trees (*Agathis australis*: Araucariaceae), causing root and collar rot, resin-exuding lesions, severe chlorosis, canopy thinning and widespread tree mortality (Beever *et al.*, 2009; Waipara *et al.*, 2013). Since its first record on the offshore island of Aotea-Great Barrier in 1974 (Gadgil, 1974; Beever *et al.*, 2009), *P. agathidicida* has more recently been detected in many of the remaining

mainland kauri forests (Waipara *et al.*, 2013). Mature kauri forests have already been considerably reduced in extent by historical logging and fire (Ecroyd, 1982); thus kauri dieback disease represents a serious threat to the continued functional persistence of kauri trees and associated ecosystems. Kauri produce deep banks of leaf litter which over hundreds of years result in very acid soils, thereby serving as ecosystem engineers, exerting a profound influence on the surrounding vegetation and invertebrate communities (Tomlinson, 2007; Wyse, 2012; Wyse *et al.*, 2014). In addition, kauri are Aotearoa-New Zealand's largest endemic tree, and are of great cultural significance to both indigenous and non-indigenous New Zealanders. Therefore, improved management of kauri dieback disease is an important component of conservation management in Aotearoa-New Zealand.

Forest *Phytophthora* species are generally not able to be removed from contaminated sites, and kauri dieback disease is currently incurably fatal. Therefore, preventing further disease spread is currently the most effective management approach available. As with many other *Phytophthora* species, *P. agathidicida* is vectored by movement of soil and soil-water, often facilitated by human activity (Kliejunas and Ko, 1976; Beever *et al.*, 2009). Reducing human-mediated dispersal through initiatives such as footwear cleaning stations is a key component of current kauri dieback management (Pau'uvale *et al.*, 2011; kauridieback.co.nz). In addition to human activity, other animals have also been shown to be capable of vectoring soil-borne forest pathogens. Pathogen transfer may occur externally on the animal (Kliejunas and Ko, 1976; Krull *et al.*, 2013a), or internally through ingestion and gut passage (Keast and Walsh, 1979; Li *et al.*, 2013).

Pigs (*Sus scrofa*) are one of the main species of large vertebrates present in kauri forests. Krull (2012) estimated feral pig density in Tāmaki Makaurau-Auckland's Waitākere Ranges at 8.23 ± 0.7 pigs/km^2. Pigs have been implicated in *P. agathidicida* spread through both internal and external mechanisms (Krull, 2012). Their omnivorous diet and the soil disturbance caused by their feeding activity bring them into close contact with infected roots and soil, more so than other large vertebrates such as deer (*Cervus* spp.) and goats (*Capra hircus*). In a podocarp/broadleaf forest in Aotearoa-New Zealand's central North Island, 82.4 per cent of pigs' annual diet was obtained from the ground (as opposed to above-ground browsing), 30.6 per cent specifically from rooting activity for plant roots and invertebrates (the latter principally earthworms) (Thomson and Challies, 1988). Pigs root under infected kauri trees, thereby ingesting infected plant and soil material (Hill and Davis, 2011). Krull *et al.* (2013a) were unable to detect *P. agathidicida* on feral pigs' trotters or snouts, probably partly attributable to the relative insensitivity of detection tests available for *P. agathidicida* at the time. However, they did detect *P. cinnamomi* on trotters, and using Bayesian modelling and expert opinion about the probability of transfer concluded that there was a 35–90 per cent probability that pigs do vector *P. agathidicida* externally. Kliejunas and Ko (1976) also found pigs capable of vectoring *P. cinnamomi* on their trotters, while Li *et al.* (2013) demonstrated that *P. cinnamomi* was capable of infecting seedlings after 7 days' passage through a pig's gut.

We aimed to elucidate the potential role of pigs in vectoring *P. agathidicida* internally through ingestion of infected plant material. Specifically, we aimed to (1) determine whether, following ingestion of infected material, viable *P. agathidicida* could be detected from pig faecal samples either by laboratory isolation and/or by inoculation of kauri seedlings; (2) assess the relationship between gut passage duration and detection rates of viable *P. agathidicida* and (3) explore the prevalence of *P. agathidicida* in gut samples from feral pigs killed within a *P. agathidicida*-infected forest.

Methods

Determining viability following pig gut passage: pig maintenance and experimental methodology

To determine whether viable *P. agathidicida* could be detected from pig faecal samples, we undertook a captive feeding experiment, including two treatments and a negative control. The two treatments were *P. agathidicida*-infected oat (*Avena sativa* L.) and millet grains and *P. agathidicida*-infected kauri roots, each mixed in a matrix of standard farmed-pig food. Infected kauri roots used in the study were sourced from deliberately infected greenhouse grown kauri seedlings. Root fragments varied in size from 1 to 50 mm long, and ca.1–3 mm wide. The use of two infected feed types was intended to elucidate the effect of gut passage duration on *P. agathidicida* viability, as the cereal grains (not a natural part of feral pig diet) were expected to passage more rapidly than the kauri roots, which are consumed by feral pigs in the wild. The negative control feed consisted of uninfected grains and kauri roots mixed with standard feed.

Refining detection techniques

Studies were conducted to confirm that known *P. agathidicida* could be detected from a matrix of pig faeces and to refine the detection methods required to achieve this. Samples of *P. agathidicida*-colonized kauri feeder roots and oat grains were inserted into the faeces of domestic pigs and left to incubate at 20°C. After 24 h, 15–20 root fragments (~5–10 mm long) and oat grains were extracted from the faeces, surface-sterilized in 50 per cent ethanol for 30 sec, and then plated onto *Phytophthora*-selective agar (corn-meal agar amended with pimaricin, ampicillin, rifampicin, PCNB and hymexazol; PARPH). Comparable oat grains and roots that had not been incubated in pig faeces were plated at the same time.

The pig faeces containing the remaining *P. agathidicida*-colonized kauri roots and oat grains were divided into two portions. From the first portion, five samples, each ~80 mL, were put into 500-mL containers, flooded with distilled water, and baited with freshly germinated lupins (*Lupinus* L.) and fresh cedar needles (*Cedrus deodara* Loudon) (fresh baiting). From the second portion, five 80-mL samples were air-dried on paper towels for 5 days, placed in 500-mL containers then moistened with distilled water for 4 days, and then flooded and baited with lupins and cedar needles as above (extended baiting). After 48 h of exposure, lupin and cedar baits were plated onto PARPH.

Pig feeding trial

Thirteen standard white male farm pigs (*S. scrofa domesticus*), each weighing 15–18 kg at the start of the experiment were individually housed in 1.5 m × 1.5 m crates in a New Zealand Ministry for Primary Industries-approved containment facility. The pigs were acclimatized for 1 week prior to the experiment. During this time pigs were maintained on 1 kg daily of standard feed comprised of fish meal, soybean meal, soybean oil, wheat and vitamins. Water was supplied *ad libitum* throughout the experiment. The evening before the test feed, pigs were fed only 200 g of food to enhance interest in the test feed. Ostomy frames and

bags to collect faeces were fixed to the pigs' shaved bottoms using glue and duct tape.

For the test feed, six pigs each received 700 g of standard feed plus 100 g of *P. agathidicida*-infected oats and millet. A further six pigs each received 700 g of standard feed plus 30 g of *P. agathidicida*-infected kauri roots. One pig received a negative control feed comprising 700 g of standard feed plus 100 g of a mix of uninfected oats, millet and kauri roots. Pigs received no additional food until either the test diet was consumed or 24 h had passed, at which time normal feeding resumed. Ostomy bags were changed and faeces collected twice daily. Faeces from each pig were bulked into seven categories, representing from 0–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h. Faeces were flushed extensively with water using a 1.6-mm mesh sieve, and samples including fragments of root, oat/millet grains and coarser fractions of the faeces were retrieved from the sieve. Sub-aliquots of raw faeces were also retained to provide for the possibility of loss of spores through the sieving process. Solid waste material from this study was incinerated. Liquid waste and contaminated surfaces were thoroughly disinfected with Trigene™.

Baiting of faeces was not carried out in the pig feeding trials, based on the detection failure when baiting raw faeces laced with *P. agathidicida*-infested roots and oat grains while refining detection techniques. Instead, only direct plating or baiting of roots or millet that had been thoroughly washed to remove faeces was carried out. Up to 20 millet seeds or 30 kauri root fragments from each faecal sample were extracted from the rinsed faecal samples using forceps, rinsed again, then surface-sterilized in 50 per cent ethanol and directly plated onto PARPH. Root fragments were plated whole, unless their shape precluded good contact with the agar, in which case they were cut in half before plating. The remaining portion of rinsed seeds or roots were placed in 23-mm diameter cell wells. Up to 10 wells, each with five seeds, and up to 30 wells with root fragments were then flooded with sterile distilled water. Cedar needle baits were then floated on the surface of each cell and incubated at 20°C. After 4 days baits were removed, surface-sterilized in 50 per cent ethanol and plated onto selective media. Wells were then re-baited with fresh cedar needles, incubated for a further 5 days, then plated as above. Agar plates were monitored for 7 days, and any *Phytophthora*-like colonies were sub-cultured onto V8-agar for identification. All root fragments that were either plated or baited were measured. Pearson's correlation co-efficient was calculated to test the relationship between root fragment length and gut passage duration. The control samples that were retained in the laboratory for validation of inoculum viability were plated and baited using the same techniques listed above.

Seedling inoculation trial

A 5- to 10-g sample of sieved faeces from each pig at each sample time was retained for tree inoculation. These were pooled into 0–48, 48–96, 96–144 and 144–168 h samples for each of the six pigs in each inoculum type (oats/millet or kauri roots). Each pooled sample was divided into two, with half mixed with potting mix, placed in a 0.85-L planter bag, and a *Phytophthora*-free 6-month-old kauri seedling planted on top, after adding a 5–10 mm layer of potting mix to avoid direct contact between the roots and the faeces. The other portion was inserted into a hole made around the root system of a 2- to 3-year-old nursery-grown kauri seedling in a 1.5-L planter bag. All seedlings were then flooded for 24 h, and then grown under normal watering conditions in a glasshouse maintained at 18–25°C. Periodically, seedlings were flooded for 24 h to facilitate zoospore production and infection. Seedlings were monitored weekly for 26 weeks, looking for any sign of infection symptoms.

All the 6-month-old seedlings were grown for 26 weeks following inoculation, then harvested. Roots were washed and scored for signs of root disease. Root samples (10 per seedling) were surface-sterilized and plated onto *Phytophthora*-selective agar, and other samples (with attached soil) were flooded and baited for up to 7 days with lupin radicles and cedar needles. After 2 days baits were surface-sterilized and plated onto PARPH, which was subsequently checked for *Phytophthora*-like colonies. Samples were re-baited with cedar needles for a further 5 days as above. To sample the faeces-inoculated 3-year-old seedlings after 26 weeks, a portion of roots was cut from each planter bag, and plated or baited as described for the 6-month-old seedlings. The 3-year-old seedlings were retained in the glasshouse for a further 3 months and re-sampled as above.

Assessing prevalence of P. agathidicida *in stomach contents of feral pigs*

Auckland Council contract pig hunters collected a total of 274 pig stomach samples as part of a cull of feral pigs across a 1600 ha area in Waitākere Ranges, West of Tāmaki Makaurau-Auckland, North Island, Aotearoa-New Zealand through all seasons from October 2008 until September 2011. The hunters collected a sample of stomach contents by slicing through the abdomen of the pig, opening the stomach with a knife and stirring the contents. An 80-mL sample container was then three-quarters filled with stomach contents and topped up with 75 per cent ethanol for preservation. Of the stomach samples, only 184 could be analysed due to containers leaking during storage and subsequently drying out.

The presence of plant material was visually confirmed for all the samples used in this study prior to analysis. Stomach samples were homogenized using a rotor-stator homogenizer (IKA Ultra-Turrax T25; IKA Labortechnik, Staufen, Germany) to remove potential subsampling bias and to provide a mechanical breakdown of tissue, aiding the DNA extraction process, following the methods in Egeter *et al.* (2015). Two DNA extraction methods were trialled: one using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and one using the Mo Bio PowerPlant Pro DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA). In each case, up to 20 mg (Qiagen Kit) and 50 mg (Mo Bio Kit) of material was taken from homogenized pig stomach contents for subsequent DNA extraction, using ethanol-washed and flamed dissection instruments. To provide a general test of DNA extraction efficacy, universal primers targeting plant DNA were employed (Cox2-1/Cox2-2; Duminil *et al.* 2002). To detect the presence of *Phytophthora* a highly sensitive nested polymerase chain reaction (PCR) approach was used, using two primer combinations (18Ph2F/5.8 S-1 R and ITS6/5.8 S-1 R) and targeting only the genus *Phytophthora* (Scibetta *et al.*, 2012). Details of PCR reagents and thermal profiles used are provided in the Appendix. Aerosol-resistant pipette tips were used throughout and products were separated by gel electrophoresis using SYBR Safe (Life Technologies, Carlsbad, CA, USA). All PCRs were carried out in duplicate i.e. there were 184 samples, two extractions methods, two PCR protocols (targeting either plant DNA or *Phytophthora* DNA) and two duplicates for each, totalling over 1 472 PCR reactions. At least two wells in each 96-well PCR plate were blank negatives. Cultures of *P. cinnamomi* and *P. multivora* and DNA extracted from a *P. agathidicida* culture were sourced from The New Zealand Institute for Plant & Food Research Limited (PFR). To provide known positives for PCRs and to ensure that the extraction methods were suitable for *Phytophthora* spp., up to 10 mg of *Phytophthora* hyphae from each of the cultures were taken and subjected to DNA extraction using both kits. One universal eukaryote primer pair (Uni-minibarF1/Uni-minibarR1; Meusnier *et al.*, 2008), one universal vertebrate primer pair (12Sai/12Sbi; Simon *et al.*, 1994) and one other *Phytophthora*-specific primer pair (Yph1F/Yph2R; Schena *et al.*, 2006) were trialled, but did not yield clear amplifications and were excluded from the remainder of the study.

DNA from successful amplifications using the *Phytophthora*-specific assay was sequenced using an ABI 3730xl DNA Analyser (Applied Biosystems, Foster City, CA, USA). Sequencing was performed in both

directions and resultant sequences were inspected, trimmed and cleaned using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and Bioedit (Hall, 1999). All DNA sequences were clean, with <0.15 per cent ambiguous base positions, indicating that where amplification was successful, it was most likely that only one *Phytophthora* sp. was present. The presence of multiple *Phytophthora* spp. in individual samples would have necessitated additional methods in order to ensure all species could be successfully identified e.g. cloning, denaturing gradient gel electrophoresis and next-generation sequencing. The majority of the resultant trimmed sequences were 193–240 bp in length, while a few ranged from 90 to 176 bp ($n = 6$). Mega6 (Tamura *et al.*, 2013) was used to create sequence alignments (using the Muscle algorithm; Edgar, 2004). BLAST searches were undertaken for each sequence using the Megablast algorithm (Zhang *et al.*, 2000). A local library was constructed containing (1) the closest match in GenBank for each sequence, (2) the next closest matching species in GenBank and (3) a representative sequence of every New Zealand *Phytophthora* species in GenBank (identified by searching the nucleotide database for 'Phytophthora New Zealand 5.8 S'). The local library consisted of 74 sequences; 23 from pig stomach samples; 3 from *Phytophthora* cultures and 48 closely matching sequences from GenBank (from 27 *Phytophthora* species). Sequences were assigned to species based on a pairwise distance matrix, constructed using Mega6 (Tamura *et al.*, 2013), using uncorrected p-distances, following the recommendations of Collins and Cruickshank (2013), Srivathsan and Meier (2012) and Collins *et al.* (2012). Species names were assigned to sequences only where p-distances were less than 0.01. This was considered appropriate as the average inter-specific p-distance for all New Zealand *Phytophthora* species for the target region was 0.22 and the minimum inter-specific p-distance was 0.03 (apart from a p-distance of 0 between *P. citricola* and *P. multivora*; see Table 2 for reasons why these sequences were designated as *P. multivora*).

Results

Refining detection techniques

When faecal samples containing viable *P. agathidicida* in oat grains or kauri roots were run through the standard *Phytophthora* lupin and cedar baiting systems (as would be done for soil samples), *P. agathidicida* was not isolated, despite normally being readily detected via baiting from infected plant material or soil. This was the case with both baiting of the fresh faecal sample and when the faecal samples were air-dried, moist-incubated and baited, as per the standard *P. agathidicida* isolation procedure. These results indicate that the pig faeces environment inhibits a process in the baiting procedure, either oospore germination, sporangial production, or zoospore release and colonization of baits. This would not be a problem in a natural environment, as the faeces would break down, releasing dormant oospores or colonized tissue fragments into the soil for subsequent germination.

However, in control oats and roots not incubated in pig faeces, and when the *P. agathidicida*-colonized oats and roots were incubated in pig faeces for 24 h and then extracted from the faeces and directly plated onto agar, all the root fragments and a majority of the oat grains yielded viable *P. agathidicida*. Furthermore, baiting was successful when root fragments were thoroughly rinsed free of faecal material. This indicated that *P. agathidicida* can survive within plant tissue in the pig faeces environment and that direct plating or baiting of washed roots was a reliable method of detection of *P. agathidicida*, in line with high rates of detection which can normally be expected from soil or plant material. We, therefore, concluded that for the feeding trial we needed to extract then plate or bait thoroughly rinsed passaged plant material, rather than attempting to plate or bait directly from the faecal matrix.

Pig feeding trial

Millet seeds were clearly discernible and plentiful in the 24 h sample and up until 96 h. A few were discernible in the 120-h and 144-h samples, and none in the 168-h sample. The numbers plated at the various times reflect the ease of locating the millet seeds within the samples (Table 1). Oat grains were mostly digested after passage through the pig gut, and only a small number were plated or baited. A few small (mostly <6 mm long) kauri root fragments were visible in the 24-h samples. The 48- to 96-h samples contained large numbers of root fragments of a range of sizes. Numbers of roots declined from 48 h onwards, but there were still kauri roots clearly visible in the 168-h samples. Most of the root fragments in faecal samples collected 120 h or more after feeding were relatively large (average >15 mm long), with a positive correlation between root fragment length and gut passage duration ($P < 0.01$).

From a total of 308 root fragments baited, only one baited kauri root fragment yielded *P. agathidicida*. This sample was a 4-mm long, 1 mm wide fragment of kauri root that was collected from pig faeces passed within 24 h of feeding. None of the 580 kauri root fragments plated onto selective agar yielded *P. agathidicida* or any other *Phytophthora*, nor did any of the millet seeds either direct plated ($n = 265$) or baited ($n = 535$). The control samples that were retained in the laboratory for validation of inoculum viability readily yielded *P. agathidicida*, with re-culturing from 90 per cent of millet seeds and 40 per cent of kauri roots.

Seedling inoculation trial

None of the kauri seedlings died or showed any symptoms indicative of *P. agathidicida* infection in the 26 weeks following inoculation with pig faeces. There were no observable differences in the root health of control seedlings or seedlings inoculated with potentially contaminated pig faeces. None of the plated or baited roots of the 6-month-old seedlings yielded *P. agathidicida* or any other *Phytophthora*. None of the plated or baited roots of the 2- to 3-year-old seedlings yielded *P. agathidicida*. However, six isolates of *P. cinnamomi* and seven isolates of *P. multivora* were extracted from roots of 3-year-old trees, both from trees inoculated with pig faeces and from uninoculated trees. It is assumed that the source of these *Phytophthora* species was low-level contamination in the nursery which supplied the trees, as these species have been detected in seedlings from the same supplier in the past. The fact that no *Phytophthora* was detected in the 6-month-old seedlings grown in quarantine conditions and tested at the same time supports this assumption.

Assessing prevalence of P. agathidicida *in stomach contents of feral pigs*

Using the *Phytophthora*-specific nested PCR, DNA extracted from *Phytophthora* cultures was successfully and consistently amplified throughout all PCR runs, regardless of the type of extraction kit used, and resultant sequences for each culture matched the

Table 1 Millet seeds and kauri root fragments recovered from pig faeces after varying gut passage intervals, and assigned to either plating or baiting to detect *Phytophthora agathidicida* presence

	Gut passage duration							
	24 h	48 h	72 h	96 h	120 h	144 h	168 h	Total
Millet								
Number of seeds plated	70	120	49	22	2	2	0	265
Number of wells baited (5 millet seeds/well)	35	60	10	2	0	0	0	107
Kauri roots (plated)								
Number of root fragments	40	178	130	89	63	57	23	580
Total length (mm)	222	2036	1480	1166	996	996	398	7294
Maximum length (mm)	15	26	45	24	40	33	35	
Minimum root length (mm)	2	2	2	3	6	6	8	
Mean length (mm)	5.6	11.4	11.4	13.1	15.8	17.5	17.3	
Kauri roots (baited)								
Number of root fragments	36	149	84	39	0	0	0	308
Total length (mm)	181	2035	946	716	0	0	0	3878
Maximum length (mm)	21	38	25	38	–	–	–	
Minimum root length (mm)	1	3	2	8	–	–	–	
Mean length (mm)	5.0	13.6	11.3	18.4	–	–	–	

Up to 20 millet seeds or 30 kauri root fragments were tested from each pig at each gut passage time interval.

expected sequences in the local library. Based on the amplification of plant DNA (as plant material was visually confirmed to be present in all pig stomach samples prior to analysis), the overall DNA extraction success rate was 39.7 per cent (n = 73). The two extraction kits had similar success rates (Mo Bio 25 per cent; Qiagen 19 per cent); however, in only 14 cases did both kits result in amplifiable DNA from the same pig stomach sample.

A total of 23 samples tested positive for the presence of *Phytophthora* spp. (11 from Mo Bio extractions and 12 from Qiagen extractions). Six of these were from samples that did not result in amplification of plant DNA (although as noted above, plant material was confirmed as present in all stomachs). Overall, 79 of the 184 (42.9 per cent) samples resulted in the successful amplification of either plant or *Phytophthora* spp. DNA. *Phytophthora* spp. were, therefore, detected in 29.1 per cent of samples that tested positive for any DNA, and in 12.5 per cent of the entire sample set.

P. agathidicida was not detected in any of the feral pig stomach samples (Table 2). The *Phytophthora* detected belonged to five other species, three of which could be assigned species names (Table 2). The most frequently detected species were *P. multivora* (n = 11), *P. cinnamomi* (n = 5; Accession no. JX122743) and *P. kernoviae* (n = 5). Two other *Phytophthora* taxa were detected on one occasion each, but good matches (i.e. ≥99 per cent identical) were not found in either GenBank or the local library (Table 2).

Discussion

Our results confirm that *P. agathidicida* is capable of remaining viable following ingestion and gut passage by a pig. This provides proof of concept for internal vectoring of *P. agathidicida* by feral pigs, as has been previously documented for the congeneric *P. cinnamomi* (Li *et al.*, 2013). However, results suggest that internal vectoring by pigs may be a considerably lower risk pathway for spread of *P. agathidicida* than it is for *P. cinnamomi*.

Li *et al.* (2013) recovered viable *P. cinnamomi* from passaged material of all three of the inoculated host plant species used in their study, and from each of their three replicate pigs. Furthermore, they were able to inoculate seedlings successfully with *Phytophthora*-infected plant material which had undergone pig gut passage of up to 7 days' duration. In contrast, despite a much higher rate of animal replication (n = 12 pigs fed infected material), we detected only a single instance of viable *P. agathidicida* following gut passage, and none of the kauri seedlings became infected following inoculation. In retrospect it would have been useful to have included *P. cinnamomi* in our feeding trial as a positive control, to enable comparison of detection rates under the same experimental conditions. It is possible that our results may have under-detected viable *P. agathidicida* through the rinsing process, as we used a coarser sieve than Li *et al.* (2013). However, most *P. agathidicida* spores could be expected to be retained within the fragments of plant material. Notwithstanding this, the contrast between our results and those of Li *et al.* (2013) is striking and, we believe, unlikely to reflect solely methodological differences. Inter-specific differences in temperature tolerance are one possible contributor to the difference in results. Optimal growth of *P. agathidicida* on agar occurs at 22°C, with growth ceasing by 32°C (Weir *et al.*, 2015). In contrast, optimal growth of *P. cinnamomi* on excised roots was achieved at 25–30°C, with a maximum temperature for growth of 34°C (Shearer *et al.*, 1987). Furthermore, *P. cinnamomi* cultures have successfully recommenced growth following exposure to temperatures as high as 37–44°C (Shepherd and Pratt, 1974; I. Horner, unpublished data), whereas two mature cultures of *P. agathidicida*, each containing numerous oospores, could not be regenerated after 24 h of incubation at

Table 2 Sequence matches and species assignations for all DNA sequences obtained in the present study

Phytophthora reference culture/Pig stomach sample no. (P)	Assigned species	Closest match in local library	p-distance
P. agathidicida H261 PFR	*P. agathidicida*[1]	KP295318 (NZ) KP295315 (NZ)	0
P. multivora H455 PFR	*Phytophthora multivora*[2]	JX074737 (NZ) KP295299 LM651003 KU146526	0
Phytophthora cinnamomi H401 PFR	*Phytophthora cinnamomi*	JX122743 (NZ) KP183223 KT265815	0
P5, P6, P11, P30, P33, P61, P68, P74, P114, P137, P159	*P. multivora*[2]	JX074737 (NZ) KP295299 LM651003 KU146526 *P. multivora* H455 PFR	0–0.008
P4, P26, P118, P131, P155	*P. cinnamomi*	JX122743 (NZ) KP183223 KT265815 *P. cinnamomi* H401 PFR	0–0.006
P35, P37, P76, P117, P175	*Phytophthora kernoviae*	JN595854 (NZ) KJ755134	0–0.007
P138	*Phytophthora* sp.	HM004230 (*Phytophthora megasperma*)	0.02
P153	*Phytophthora* sp.	GU111588 (*Phytophthora cactorum*)	0.11

A species name was only assigned when the p-distance was less than 0.01. PFR denotes a sequence originating from cultures held by the New Zealand Institute for Plant & Food Research Limited, Havelock North. NZ denotes a sequence obtained from a sample originating from New Zealand.
[1]It should be noted that the *P. agathidicida* target sequence is in many cases identical to those of *Phytophthora castaneae*, *Phytophthora cocois* and *Phytophthora katsurae*, but these species have not been detected in New Zealand to date and were excluded from final analyses.
[2]Sequences matching *Phytophthora multivora* also matched a number of *Phytophthora citricola* sequences in GenBank (JX122742 [NZ], EU194425, AM235209, FJ801981, GU111598), however, *P. citricola* has been split into a number of species, the most abundant of which is *P. multivora*. Given that these sequences were identical to the *P. multivora* reference culture (p-distance = 0), this is the species name assigned.

37°C (I. Horner unpublished data). These data suggest that *P. agathidicida* is less robust than *P. cinnamomi* to temperatures in the range likely to be encountered during passage through the digestive system of pigs or other endothermic species. When refining detection techniques, we were able to culture *P. agathidicida* on agar following incubation in faeces, but we were unable to detect it via baiting. These results indicate that even without the high temperatures associated with gut passage, the presence of pig faeces also inhibits some part of the life cycle of *P. agathidicida* (but does not kill it). Further research would be useful to elucidate the mechanisms behind this inhibition.

Li *et al.* (2013) found viability rates of *P. cinnamomi* decreased with increasing gut passage duration. Because we detected only a single instance of *P. agathidicida* surviving gut passage, we were unable to explore the influence of gut passage duration on *P. agathidicida* survival rates. However, we hypothesize that, given the apparently lower tolerance of *P. agathidicida* to gut passage, increased transit time is similarly likely to be associated with decreased survival rates in this pathogen. That our positive sample was passaged within 24 h of feeding is consistent with this hypothesis. Feral pigs' movements vary widely depending on available resources, and movement studies of pigs in the Waitākere ranges have not been conducted. However, Australian studies observed mean 24 h home ranges of 1.4 ± 1.1 km^2 for males and 0.8 ± 0.4 km^2 for females (Saunders and Kay, 1991) and movements within a 3-h period that ranged from 1 to 6457 m, averaging 459 m (Caley, 1997). Assuming that feral pig movements are similar in the Waitākere environment, a 24-h passage period has the potential of moving *P. agathidicida* between catchments and into uninfected kauri stands. Gut passage times in feral pigs are likely to be variable depending on diet composition and probably differ from those observed in our captive feeding trial, potentially influencing the distances over which dispersal may occur.

Our analysis of DNA from feral pigs' stomachs suggests that *P. cinnamomi* and *P. multivora* are currently more likely to be ingested by feral pigs than *P. agathidicida*, within the 1600 ha of the Waitākere Ranges from which our stomach samples were sourced. This could reflect the distributions of the respective taxa; *P. cinnamomi* and *P. multivora* have very wide host ranges and are widespread within the Waitākere Ranges and other Aotearoa-New Zealand forests (Robertson, 1970; Scott and Williams, 2014; Auckland Council, unpublished survey data). In contrast, current knowledge suggests that *P. agathidicida* has a narrow host range and had a more limited distribution at the time that culling was undertaken (2008–2011), occurring mostly in isolated pockets or stands (Waipara *et al.*, 2013; Jamieson *et al.*, 2014). We conclude that ingestion by feral pigs is has

historically been likely to be a minor vector pathway for spread of *P. agathidicida*, and a higher risk pathway for spread of *P. cinnamomi* (and possibly also *P. multivora*, depending on its tolerance of gut passage). However, the distribution of *P. agathidicida* within the Waitākere ranges has extended considerably since 2001, and is expected to continue to expand. Therefore, the risk of ingestion of infected material by feral pigs is likely to increase.

Estimating the true occurrence of *Phytophthora* in feral pig stomach contents from these data is difficult, primarily because of a potential failure to detect *Phytophthora* DNA despite its presence in a sample. Such false negatives may be the product of degraded DNA, failure to extract DNA, co-extraction of PCR inhibitors and/or failure of PCR reaction. Monitoring the incidence of false negatives in diet samples is extremely difficult (Deagle *et al*., 2005). In the present study, we can be confident, given the higher sensitivity of the *Phytophthora*-specific nested PCR assay (detecting down to 1 fg total DNA (Scibetta *et al*., 2012)), that where samples yielded a positive amplification of plant DNA, but no amplification of *Phytophthora* DNA, this reliably indicated the absence of *Phytophthora* spp. However, for the samples that did not result in any DNA amplification, we cannot draw the conclusion that an absence of DNA detection equated to an absence of *Phytophthora* spp. Therefore, our minimum and maximum estimated occurrences of *Phytophthora* in feral pig stomach samples are 12.5 per cent and 29.1 per cent, respectively. It should be noted that although DNA extraction success was relatively low, this was not a substantial constraint of our study as we assessed the prevalence of false negatives. However, obtaining higher DNA extraction success rates would narrow the range of estimates obtained. We tested two commercial extraction kits, optimized for DNA extraction from either animal or plant tissue. Given the complex nature of pig stomach contents, future studies might consider testing the efficacy of soil or faecal DNA extraction kits to increase DNA extraction success.

Implications for forest management

Given the apparent sensitivity of *P. agathidicida* to the high temperatures associated with gut passage, we hypothesize that movement of infected soil on pigs' bodies is likely to play a more important role in vectoring *P. agathidicida* than does internal vectoring following ingestion. Further work should be undertaken to re-examine the potential for external vectoring, as detection methods have improved since previous sampling was undertaken (Krull *et al*., 2013a). Li *et al.* (2013) noted that the challenges in undertaking such research in a field context in order to validate the proof of concept demonstrated by Kliejunas and Ko (1976).

In the interim, our results indicate that a precautionary approach to forest management should treat feral pigs as being at least a minor vector of *P. agathidicida*, and a more important vector when other forest *Phytophthora* are also considered. However, although our results contribute to the body of evidence for feral pig impacts in forest ecosystems the low incidence of detection in our study also reinforces the importance of continued prioritization of managing human-mediated soil movement, as a well-established high risk pathway for spread of both *P. agathidicida* and *P. cinnamomi* (Pickering *et al*., 2010; Pau'uvale *et al*., 2011).

Physical damage to delicate kauri root systems through feral pig rooting behaviour may be of further importance to the establishment of root diseases in kauri, and feral pigs continue to be a significant vector of *Phytophthora* spp. and other plant pathogens via the external transport of soil adhering to their bodies (Krull *et al*., 2013a). Furthermore, feral pig management is of continuing importance in kauri and other forest ecosystems, given feral pig impacts on several aspects of forest health, including impacts on seedlings, macroinvertebrates, frogs, birds, leaf litter cover and nutrient cycling, as well as dispersal of weed seeds through ingestion and gut passage (Taylor *et al*., 2011; Krull *et al*., 2013b, 2016; Murphy *et al*., 2014; Beavon and Kelly, 2015; Krull and Egeter, 2016).

The benefit of biosecurity and forest health research into forestry management in Aotearoa-New Zealand has been demonstrated (see Turner *et al*., 2004) with benefits–cost ratios ranging from 103:1 to 172:1 showing significant benefits to the forest growing industry. Studies such as ours can highlight cost effective solutions for forest health management by determining likely vector pathways for pathogens. Our results, in contrast with those of Li *et al.* (2013) demonstrate the need for species-specific understanding of the ecology of *Phytophthora* and associated invasion pathways, in order to inform forest management internationally.

Acknowledgements

Thanks to MPI, especially Anna Rathé, for permission to undertake research on an Unwanted Organism. Thanks to feral pig hunters Bevan Jones, John Rule, Peter Jackson, Matt Oldfield and their teams. Thanks to Stacey Hill, Lee Hill, Jack Craw, Alison Davis, Nari Williams, Eric Neumann, Steve Hix, Cailín Roe and Patrick Morel for their assistance with various aspects of the project, and to Nick Waipara and Tony Beauchamp for comments which improved the draft manuscript. The authors also owe a huge debt of gratitude to Ana Carolina Lacerda, Emma Annear, Diana Cabrera Amaro, and Trent Olsen for pig care and preliminary processing of faeces. Thanks to Jolanda Roux, Sarah Wyse and an anonymous reviewer for comments which improved the quality of the manuscript.

Funding

Auckland Council, the Kauri Dieback Management Programme and conducted under University of Auckland animal ethics permit number 001 264.

Conflict of interest statement

None declared.

References

Beavon, M.A. and Kelly, D. 2015 Dispersal of banana passionfruit (*Passiflora tripartita* var. *mollissima*) by exotic mammals in New Zealand facilitates plant invasiveness. *New Zeal. J. Ecol.* **39**, 43–49.

Beever, R.E., Waipara, N.W., Ramsfield, T.D., Dick, M.A. and Horner, I.J. 2009 Kauri (Agathis australis) under threat from Phytophthora? In: *Phytophthoras in Forests and Natural Ecosystems. Proceedings of the Fourth Meeting of the International Union of Forest Research*

Organisations (IUFRO) Working Party. August 26–31 2007, Monterey, California, pp. 74–85.

Brasier, C.M. 2008 The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathol.* **57**, 792–808.

Caley, P. 1997 Movements, activity patterns and habitat use of feral pigs (*Sus scrofa*) in a tropical habitat. *Wildlife Res.* **24**, 77–87.

Colautti, R.I., Grigorovich, I.A. and MacIsaac, H.J. 2006 Propagule pressure: a null model for biological invasions. *Biol. Invasions.* **8**, 1023–1037.

Collins, R.A. and Cruickshank, R.H. 2013 The seven deadly sins of DNA barcoding. *Mol Ecol Res.* **13**, 969–975.

Collins, R.A., Boykin, L.M., Cruickshank, R.H. and Armstrong, K.F. 2012 Barcoding's next top model: an evaluation of nucleotide substitution models for specimen identification. *Methods Ecol. Evol.* **3**, 457–465.

Deagle, B.E., Tollit, D.J., Jarman, S.N., Hindell, M.A., Trites, A.W. and Gales, N.J. 2005 Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. *Mol. Ecol.* **14**, 1831–1842.

Duminil, J., Pemonge, M.H. and Peiti, R.J. 2002 A set of 35 consensus primer pairs amplifying genes and introns of plant mitochondrial DNA. *Mol. Ecol. Notes.* **2**, 428–430.

Ecroyd, C.E. 1982 Biological flora of New Zealand 8. *Agathis australis* (D. Don) Lindl. (Araucariaceae), Kauri. *New Zeal. J. Bot.* **20**, 17–36.

Edgar, R.C. 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic. Acids. Res.* **32**, 1792–1797.

Egeter, B., Bishop, P.J. and Robertson, B.C. 2015 Detecting frogs as prey in the diets of introduced mammals: a comparison between morphological and DNA-based diet analyses. *Mol. Ecol. Resour.* **15**, 306–316.

Gadgil, P.D. 1974 *Phytophthora heveae*, a pathogen of kauri. *New Zeal. J. For. Sci* **4**, 59–63.

Hall, T.A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acid S.* **41**, 95–98.

Hansen, E.M. 2008 Alien forest pathogens: *Phytophthora* species are changing world forests. *Boreal Environ. Res.* **13**, 33–41.

Hill, L. and Davis, A. 2011 *Pigs Associated with PTA Infected Kauri Trees*. Unpublished Report for Auckland Council.

Jamieson, A., Bassett, I.E., Hill, L.M.W., Hill, S., Davis, A., Waipara, N.W., *et al* 2014 Aerial surveillance methods to detect kauri dieback in New Zealand. *New Zeal. Plant Protect.* **67**, 60–65.

Judelson, H.S. and Blanco, F.A. 2005 The spores of *Phytophthora*: weapons of the plant destroyer. *Nat. Rev. Microbiol.* **3**, 47–58.

Keast, D. and Walsh, L.G. 1979 Passage and survival of Chlamydospores of *Phytophthora cinnamomi* Rands, the causal agent of forest dieback disease, through the gastrointestinal tracts of termites and wild birds. *Appl. Environ. Microb.* **37**, 661–664.

Kelly, C.L., Pickering, C.M. and Buckley, R.C. 2003 Impacts of tourism on threatened plant taxa and communities in Australia. *Ecol. Manage. Restor.* **4**, 37–44.

Kliejunas, J.T. and Ko, W.H. 1976 The occurrence of *Pythium vexans* in Hawaii and its relation to ohia decline. *Plant Dis. Res.* **59**, 392–395.

Krull, C.R. and Egeter, B. 2016 Feral pig (*Sus scrofa*) predation of a green and golden bell frog (*Litoria aurea*). *New Zeal. J. Ecol.* **40**, 191–195.

Krull, C.R. 2012 *Feral pigs in a temperate rainforest ecosystem: ecological impacts and management*. PhD. thesis, School of Biological Sciences, University of Auckland.

Krull, C.R., Waipara, N., Choquenot, D., Burns, B.R., Gormley, A.M. and Stanley, M.C. 2013a Absence of evidence is not evidence of absence: feral pigs as vectors of soil-borne pathogens. *Austral Ecol.* **38**, 534–542.

Krull, C.R., Choquenot, D., Burns, B. and Stanley, M.C. 2013b Feral pigs in a temperate rainforest ecosystem: disturbance and ecological impacts. *Biol. Invasions.* **15**, 2193–2204.

Krull, C.R., Stanley, M.C., Burns, B.R., Choquenot, D. and Etherington, T.R. 2016 Reducing wildlife damage with cost-effective management programmes. *PLoS. ONE*. **11**(1), e0146765.

Li, A.Y., Williams, N., Fenwick, S.G., Hardy, G.E.S.J. and Adams, P.J. 2013 Potential for dissemination of *Phytophthora cinnamomi* by feral pigs via ingestion of infected plant material. *Biol. Invasions.* **16**, 765–774.

Lockwood, J.L., Hoopes, M.F. and Marchetti, M.P. 2007 *Invasion Ecology*. Blackwell Publishing.

Meentemeyer, R.K., Rank, N.E., Anacker, B.L., Rizzo, D.M. and Cushman, J.H. 2008 Influence of land-cover change on the spread of an invasive forest pathogen. *Ecol. Appl.* **18**, 159–171.

Meusnier, I., Singer, G.A., Landry, J.-F., Hickey, D.A., Hebert, P.D. and Hajibabaei, M. 2008 A universal DNA mini-barcode for biodiversity analysis. *BMC. Genomics.* **9**, 214.

Murphy, M.J., Inman-Narahari, F., Ostertag, R. and Litton, C.M. 2014 Invasive feral pigs impact native tree ferns and woody seedlings in Hawaiian forest. *Biol. Invasions.* **16**, 63–71.

Pau'uvale, A., Dewan, C., Mora, H., Waipara, N., Bellgard, S. 2011 Kauri Killer on the Loose?—Study of Human Vectors and PTA Hygiene Treatments. In: LENS Junior Scientist Award Report. http://www.lenscience.auckland.ac.nz/webdav/site/lenscience/shared/webcontent/pdf/Alvina_Pau'uvale-Final%20Poster.pdf (accessed on 10 December, 2013)

Pickering, C.M., Hill, W., Newsome, D. and Leung, Y.-F. 2010 Comparing hiking, mountain biking and horse riding impacts on vegetation and soils in Australia and the United States of America. *J. Environ. Manage.* **91**, 551–562.

Rizzo, D.M. and Garbelotto, M. 2003 Sudden oak death: endangering California and Oregon forest ecosystems. *Front. Ecol. Environ.* **1**, 197–204.

Robertson, G.I. 1970 Susceptibility of exotic and indigenous trees and shrubs to *Phytophthora cinnamomi* Rands. *New Zeal. J. Ag. Res.* **13**, 297–307.

Ruiz, G.M. and Carlton, J.T. 2003 *Invasive Species: Vectors and Management Strategies*. Island Press.

Saunders, G. and Kay, B. 1991 Movements of Feral Pigs (*Sus Scrofa*) at Sunny Corner, New South Wales. *Wildlife Res.* **18**, 49–61.

Schena, L., Hughes, K.J. and Cooke, D.E. 2006 Detection and quantification of *Phytophthora ramorum*, *P. kernoviae*, *P. citricola* and *P. quercina* in symptomatic leaves by multiplex real-time PCR. *Mol. Plant Pathol.* **7**, 365–379.

Scibetta, S., Schena, L., Chimento, A., Cacciola, S.O. and Cooke, D.E. 2012 A molecular method to assess *Phytophthora* diversity in environmental samples. *J. Microbiol .Meth.* **88**, 356–368.

Scott, P. and Williams, N. 2014 *Phytophthora* diseases in New Zealand forests. *New Zeal. J. For.* **59**, 14–21.

Shearer, B.L., Shea, S.R. and Deegan, P.M. 1987 Temperature-growth relationships of *Phytophthora cinnamomi* in the secondary phloem of roots of *Banksia grandis* and *Eucalyptus marginata*. *Phytopathol.* **77**, 661–665.

Shepherd, C.J. and Pratt, B.H. 1974 Temperature-growth relations and genetic diversity of A2 mating-type isolates of *Phytophthora cinnamomi* in Australia. *Aust. J. Bot.* **22**, 231–249.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and Flook, P. 1994 Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87**, 651–701.

Srivathsan, A. and Meier, R. 2012 On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics.* **28**, 190–194.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013 MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729.

Taylor, D.L., Leung, L.K.-P. and Gordon, I.J. 2011 The impact of feral pigs (*Sus scrofa*) on an Australian lowland tropical rainforest. *Wildlife Res.* **38**, 437–445.

Thomson, C. and Challies, C.N. 1988 Diet of feral pigs in the podocarp-tawa forests of the Urewera Ranges. *New Zeal. J. Ecol.* **11**, 73–78.

Tomlinson A. 2007 *Invertebrate decomposer communities in northern New Zealand forests*. MSc. thesis, School of Biological Sciences, University of Auckland.

Turner, J.A., Bulman, L.S., Richardson, B. and Moore, J.R. 2004 Cost-benefit analysis of biosecurity and forest health research. *New Zeal. J. For. Sci.* **34**, 324.

Waipara, N.W., Hill, S., Hill, L.M.W., Hough, E.G. and Horner, I.J. 2013 Surveillance methods to determine tree health, distribution of kauri dieback disease and associated pathogens. *New Zeal. Plant Protect.* **66**, 235–241.

Weir, B.S., Paderes, E.P., Anand, N., Uchida, J.Y., Pennycook, S.R., Bellgard, S.E., *et al* 2015 A taxonomic revision of *Phytophthora* Clade 5 including two new species, *Phytophthora agathidicida* and *P. cocois*. *Phytotaxa.* **205**, 21–38.

Wyse, S.V. 2012 Growth responses of five forest plant species to the soils formed beneath New Zealand kauri (*Agathis australis*). *New Zeal. J. Bot.* **50**, 411–421.

Wyse, S.V., Burns, B.R. and Wright, S.D. 2014 Distinctive vegetation communities are associated with the long-lived conifer *Agathis australis* (New Zealand Kauri, Araucariaceae) in New Zealand rainforests. *Austral Ecol.* **39**, 388–400.

Zarzoso-Lacoste, D., Corse, E. and Vidal, E. 2013 Improving PCR detection of prey in molecular diet studies: importance of group-specific primer set selection and extraction protocol performances. *Mol. Ecol. Resour.* **13**, 117–127.

Zhang, Z., Schwartz, S., Wagner, L. and Miller, W. 2000 A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* **7**, 203–214.

Appendix

Table A1 Primers used in this study, along with their intended targets, target lengths, PCR reagent concentrations, PCR thermal profile conditions and associated reference

Primer Pair (5′–3′)	Target group, Target gene, Target length and reference	PCR reagent concentrations	PCR thermal profile
Cox2/1: TTTTCTTCCTCATTCTGATTT Cox2/2: CCACTCTATTGTCCACTTCTA	Plants CoxII 350–400 bp Zarzoso-Lacoste *et al.* (2013)	1 μL extracted DNA in a 25 μL reaction containing 1 × NH_4 (Bioline) buffer, 1 mM $MgCl_2$ (Bioline), 0.2 mM of each dNTP, 0.5 μM of each primer and 1.5 U BIOTAQ (Bioline)	An initial step of 95°C for 5 min, and then 40 cycles of 94°C for 60 sec, 48°C for 60 sec and 72°C for 60 sec, plus a final extension at 72°C for 10 min
18Ph2F: GGATAGACTGTTGCAATTTTCAGT 5.8 S-1 R: GCARRGACTTTCGTCCCYRC	Phytophthora ITS-5.8 S 490 bp Scibetta *et al.* (2012)	1 μL extracted DNA in a 25 μL reaction containing 1 × NH_4 buffer (Bioline), 1.5 mM $MgCl_2$ (Bioline), 0.2 mM of each dNTP, 0.5 μM of each primer and 1 U BIOTAQ (Bioline)	An initial step of 95°C for 2 min, and then 40 cycles of 95°C for 20 sec, 61°C for 25 sec and 72°C for 30 sec, plus a final extension at 72°C for 5 min
ITS6: GAAGGTGAAGTCGTAACAAGG 5.8 S-1 R: GCARRGACTTTCGTCCCYRC	Phytophthora ITS-5.8 S 260 bp Scibetta *et al.* (2012) Nested within above PCR	1 μL extracted DNA in a 25 μL reaction containing 1 × NH_4 buffer (Bioline), 1.5 mM $MgCl_2$ (Bioline), 0.2 mM of each dNTP, 0.5 μM of each primer and 1 U BIOTAQ (Bioline)	An initial step of 95°C for 2 min, and then 35 cycles of 95°C for 20 sec, 61°C for 25 sec and 72°C for 30 sec, plus a final extension at 72°C for 5 min