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Full-length Review

FULL-LENGTH REVIEW – Pathogens & Pathogenicity

The Fatty Acid Methyl Ester (FAME) profile of *Phytophthora agathidicida* and its potential use as diagnostic tool

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One sentence summary: The fatty acid methyl ester (FAME) profile of *P. agathidicida* was characterized and assessed for its potential use in detecting *P. agathidicida* in soil.

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ABSTRACT

Phytophthora diseases cause devastation to crops and native ecosystems worldwide. In New Zealand, *Phytophthora agathidicida* is threatening the survival of kauri, an endemic, culturally and ecologically important tree species. The current method for detecting *P. agathidicida* is a soil bating assay that is time-consuming and requires high levels of expertise to assess, thus limiting the analytical sample throughput. Here, we characterized the fatty acid methyl ester (FAME) profile of *P. agathidicida*. We also compared it with the FAME profile of *P. cinnamomi* and assessed the efficacy of FAME analysis as a diagnostic tool for detecting the pathogen in soil samples. In FAME analysis, the total fatty acid content is isolated from a sample and converted to FAMEs for analysis, a process that takes less than a day. Unique fatty acid acyl chains can serve as biomarkers for specific organisms. We detected 12 fatty acids in *P. agathidicida*, two of which (20:4ω6 and 20:5ω3) show promise as potential *Phytophthora* specific biomarkers. Collectively, these findings advance our fundamental understanding of *P. agathidicida* biology and provide a promising technique to increase the rate of sample processing and the speed of pathogen detection for *P. agathidicida* in soil.

Keywords: *Phytophthora*; *Phytophthora agathidicida*; diagnostics; fatty acid methyl ester analysis

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INTRODUCTION

Phytophthora agathidicida is a recently identified plant pathogen that is threatening New Zealand's native kauri trees (*Agathis australis*; Weir *et al.* 2015; Bellgard *et al.* 2016). It is the causative agent of kauri dieback disease, which has spread throughout most regions within the natural range of kauri (Bradshaw *et al.* 2020). Kauri trees are massive and can live for thousands of years. Because of this, there can be a latency period from infection to the expression of symptoms (Bradshaw *et al.* 2020). Thus, detection of *P. agathidicida* in soil, before the onset of disease symptoms, is critical for managing the spread of kauri dieback. Currently, there are limited tools available to control the spread of disease, and improved surveillance and diagnostics is an urgent priority for research (Bradshaw *et al.* 2020).

Phytophthora can spread in a variety of ways, including by water, root to root contact and movement of contaminated plant tissue or soil (Erwin and Ribeiro 1996). The lifecycle of *Phytophthora* species is commonly characterized by the production of vegetative mycelial growth and various spore types (Erwin and Ribeiro 1996). Zoospores are short-lived, motile spores that initiate infection in roots (Bradshaw *et al.* 2020). Oospores are thick-walled, dormant spores that can survive in soil for years (Bradshaw *et al.* 2020). Each lifecycle stage plays a critical role in disease spread. In New Zealand, there are at least 30 known species of *Phytophthora* that cause disease in agricultural, horticultural and indigenous forest settings (Scott and Williams 2014). For example, *P. cinnamomi* often co-occurs with *P. agathidicida* at sites of infected kauri (Waipara *et al.* 2013).

Currently the primary method used to detect *P. agathidicida* in soil is a baiting assay (Beever *et al.* 2010). Soil-baiting is a method that is used for detection and isolation of many different *Phytophthora* species (Erwin and Ribeiro 1996; O'Brien, Williams and Hardy 2009). This method is effective but has limitations; it is slow (2–3 weeks) and requires a high level of expertise to correctly identify the colony and spore morphology that distinguishes *P. agathidicida* from other *Phytophthora* species (Beever *et al.* 2010; Bradshaw *et al.* 2020). These limitations reduce sample throughput, which limits the capacity to monitor the geographic spread of *P. agathidicida* in a timely manner. More rapid DNA-based molecular diagnostic tools are in development, but so far have limited effectiveness for detecting *P. agathidicida* in soil samples (Than *et al.* 2013; McDougal *et al.* 2014; Winkworth *et al.* 2020).

Fatty acid methyl ester (FAME) analysis is potentially an alternative or complementary tool for the detection of organisms in soil. FAME analysis has been used extensively to characterize microbial community structure in soils and has also been applied as a diagnostic tool for the detection of specific organisms in environmental samples (Cavigelli, Robertson and Klug 1995; Drenovsky *et al.* 2004; Rastogi and Rajesh 2011; Yousef *et al.* 2012). In FAME analysis, total lipids are extracted from an organism or environmental sample, acyl chains are released as fatty acids and converted to their corresponding methyl esters with subsequent gas chromatography–mass spectrometry (GC–MS) analysis (Welch 1991; Drenovsky *et al.* 2004). Organisms differ in the types and quantities of acyl chains that are components of their lipids (White *et al.* 2002; Ehrhardt *et al.* 2010). Consequently, the presence of or varying ratios of specific FAMEs can indicate the presence and quantitative abundance of particular taxa in a sample, in which case the FAME or ratio of FAMEs may be considered a biomarker. For example, the neutral lipid 16:1ω5 is widely used as an indicator of arbuscular mycorrhizal fungi in soils (Olsson 1999). Specific to *Phytophthora*, it was shown that soils with *P. sojae* zoospores added showed increased levels of 18:2ω6, 20:4ω6 and 22:1ω6, indicating that increased ratios of these fatty acids to background fatty acids may potentially serve as a biomarker for the presence of *P. sojae* in soil (Yousef *et al.* 2012). Additionally, A significant advantage of a FAME approach, relative to soil baiting and PCR-based methods, is the potential to quantify the biomass of the pathogen in the sample. Finally, the characterization of FAMEs is relatively rapid; the process from a sample to quantitative detection of a biomarker can take less than a day.

Here, we present the results of FAME analysis of *P. agathidicida* across several key lifecycle stages, including mycelia, oospores and zoospores. Additionally, a comparison of FAME profiles of *P. agathidicida* and *P. cinnamomi* reveals that many fatty acids are conserved between the two species; no fatty acids unique to *P. agathidicida* were identified. However, elevated ratios of the long-chain polyunsaturated fatty acids 20:5ω3 and 20:4ω6 were observed in soil samples with *P. agathidicida* oospores added. This suggests that FAME analysis may be a useful diagnostic tool for the detection of *Phytophthora* species in soil.

MATERIALS AND METHODS

Culture conditions and mycelia production

Phytophthora agathidicida NZFS 3770 and *P. cinnamomi* NZFS 3910 (obtained from Scion, Rotorua, NZ) were maintained regularly on 10% clarified V8 (cV8) agar plates in the dark at 22°C. Mycelia is a common tissue source of lipids for FAME profiling of *Phytophthora* species (Larkin and Groves 2003; Duan, Riley and Jeffers 2013). Soil microbes may adjust the relative concentrations of membrane lipids to acclimate to different growth temperatures (Griffiths *et al.* 2003; Yousef *et al.* 2012). Therefore, we initially characterized the basic fatty acid profile of *P. agathidicida* mycelia at two different temperatures. We selected 16°C as a value similar to mean annual temperature in the host's range and 22°C as *P. agathidicida* shows optimal growth *in vitro* culture at this value. Mycelial mats for lipid extraction were grown in liquid 10% cV8 broth for 48 h at 16°C and 22°C in the dark. Mycelia were separated from the agar plug from which growth was initiated and washed in deionized water to remove residual cV8 broth.

Oospore production

Phytophthora agathidicida oospores were produced as described in Fairhurst *et al.* (Fairhurst, Deslippe and Gerth 2021). Briefly, three 3 mm agar plugs were taken from the leading edge of mycelial growth on agar plates and inoculated in 15 mL of 4% w/v carrot broth containing 12 μg/mL β-sitosterol and grown in the dark at 22°C for 2 weeks. The resulting mycelial mats were harvested and oospores were isolated by homogenization using a tissue homogenizer for 2 min followed by sonication for 1 min on ice. The homogenized mixture was sequentially filtered through 100 and 40 μm filters to separate the oospores from mycelial fragments. The concentration of the filtered oospore suspension (oospores/mL) was estimated using a disposable hemocytometer by averaging three separate counts.

Zoospore production

Phytophthora agathidicida zoospores were produced as described in Lacey *et al.* (2021). Briefly, mycelial mats were initially grown in 2% w/v carrot broth supplemented with 15 μg/mL β-sitosterol for 30 h. The mycelial mats were then washed with 2% w/v soil

solution and incubated under light for 14 h. The soil solution was removed and the mycelial mats were washed with water. Zoospore release was induced by adding ice-cold water and incubating the mycelial mats at 4°C for 20 min. After sufficient zoospore release, the concentration of spores was estimated using disposable hemocytometers by averaging three separate spore counts.

Fatty acid extraction, conversion to FAMEs and GC-MS analysis

For FAME analyses, mycelia (~200 mg) from *P. agathidicida* and *P. cinnamomi* were grown and prepared as described above. For *P. agathidicida* oospore and zoospore analyses, each spore type was produced as described above. A total of 250 000 zoospores or 100 000 oospores in 250 μL was used for lipid extraction. For the detection of oospores in soil, we collected five soil samples from forest and garden locations in Wellington, NZ, a region outside the native range of kauri and presumably free of *P. agathidicida*. Soil samples were collected with a trowel, which was sterilized with 70% ethanol between samples. Soil samples were sealed in plastic bags and placed on ice for transport to the laboratory. Samples were then freeze-dried and stored at –20°C. Laboratory grown oospores were added in 250 μL aliquots to 0.5 g subsamples of soil sample A. The mixtures of soil and laboratory-grown oospores were subsequently subjected to lipid extraction and FAME production.

For all conditions, lipid extraction and conversion to FAMEs was carried out as described in Duan, Riley and Jeffers (2013) with slight modifications. Initially, fatty acids were released and saponified by the addition of 3.75 M NaOH dissolved in 50% $MeOH_{(aq)}$ (1 mL) to the sample and incubation at 80°C for 30 min with occasional mixing. Next, free fatty acids were methylated by addition of 62.5% 6 N HCl: 37.5% MeOH solution (2 mL) and incubation at 80°C for 10 min. Phase separation was then carried out by the addition of 1 mL of a 1:1 mixture of *n*-hexane and methyl-tert butyl ether with vigorous mixing by vortex for 30 s. After sufficient phase separation, the top organic phase was transferred to a new glass tube and washed with 3 mL of 0.3 M $NaOH_{(aq)}$. The top organic phase was again transferred to a new tube and concentrated under a stream of nitrogen gas. The remaining FAMEs were then resuspended in 150 μL *n*-hexane and transferred to 2 mL GC vials with inserts and processed immediately or stored at –20°C until analysis.

Samples were analysed on a Shimadzu GCMS-QP2010 Plus. The GC column used was a Restek RXI-5SilMS (30 m × 0.25 mm × 0.25 μm) and He was the carrier gas. An aliquot (2 μL) of the sample was injected (8:1 split ratio) at 260°C with a column flow rate of 1.09 mL/min and linear velocity of 39.6 cm/s. The GC conditions were as follows: 2 min hold at 150°C followed by a 10°C increase per min to 280°C with a final 10 min hold at 280°C. The MS transfer line temp was 260°C. MS analysis began after 4 min and ended at 25 min with 3 scans/s. Ions between 40 and 600 m/z were detected. The EI ion source operated at 70 eV ionization energy. Chromatograms were analysed using Shimadzu GCMSsolution Software. FAMEs were annotated using mass spectral matching to the National Institute for Standards and Technology (NIST) 2011 database. These annotations were confirmed based on a comparison of the retention time and fragmentation pattern with those of authentic FAME standards (Larodan). Fatty acids are named using standard nomenclature (fatty acid chain length: number of double bonds followed by an ω, finally the carbon number from the methyl terminus where the first double bond starts, e.g. linoleic acid ((9Z,12Z)-octadeca-9,12-dienoic acid) is 18:2ω6).

FAMEs were quantified in two ways. First, the relative amount of each *P. agathidicida* FAME was determined as a % of the total fatty acid content. To do this, the % of each FAME was determined by integrating the area under the peak representing each FAME on the chromatogram. The values were then converted to percentages with all identified peaks per sample combined to total 100%. Thus, each FAME is calculated as a fraction of 100% of the total FAMEs identified per sample. Second, we quantified molar amounts of 20:4ω6 and 20:5ω3 in soil samples using a 19:0 fatty acid standard as the internal standard. These two fatty acids were chosen for quantification due to the difference in ratio of 20:5ω3 to 20:4ω6 when comparing soil samples and cultured *P. agathidicida*. For use as an internal standard, 100 nmol of 19:0 fatty acid was added directly to soil samples prior to lipid extraction and conversion to FAMEs. For quantification, response factors for 20:4ω6, 20:5ω3 and 19:0 were first determined and relative response factors between 20:4ω6/19:0 and 20:5ω3/19:0 were subsequently determined (Dodds *et al.* 2005). For each condition, five biological replicates were performed, where each replicate represents a separate extraction, analysed on a separate day. Statistical analyses and graphical representations of the data were performed and generated using Prism GraphPad (Version 8.2.1).

Statistical analyses

In each experiment, treatments were applied to five biological replicates. To be considered a biological replicate, samples of *Phytophthora* life-cycle stages must have been isolated on different days. Replicate soil samples consisted of individual aliquots of a homogenized bulk soil sample. In all figures, errors bars indicate standard deviation of the five replicates per treatment. We used *t*-tests to determine the statistical significance of treatment effects. To account for multiple comparisons within experiments, we applied Bonferroni's correction to adjust *P*-values. All statistical analyses and graphical representations of the data were performed using Prism GraphPad (Version 8.2.1).

RESULTS

Comparison of FAME profiles of *P. agathidicida* and *P. cinnamomi*

A total of 12 fatty acids were regularly detected from *P. agathidicida*. The five most abundant fatty acids were 14:0, 16:0, 18:2ω6, 18:1ω9 and 20:5ω3, constituting greater than 75% of the total fatty acid content (Fig. 1). Comparing *P. agathidicida* mycelia grown at 16 and 22°C revealed slight changes in relative fatty acid amounts. Of the five most abundant fatty acids only 18:2ω6 was significantly increased at 22°C compared to 16°C (Table S1, Supporting Information). We also examined the FAME profile of *P. cinnamomi* mycelia at 16 and 22°C (Fig. 1). The FAME profiles of *P. agathidicida* and *P. cinnamomi* were largely similar. A total of 11 fatty acids were detected in *P. cinnamomi*, all of which overlapped with fatty acids present in *P. agathidicida* (Fig. 1). The five most abundant fatty acids were also consistent between both species at 16 and 22°C (Fig. 1). Of the five most abundant fatty acids 18:2ω6, 18:1ω9 and 20:5ω3 were significantly different between *P. agathidicida* and *P. cinnamomi* at both 16 and 22°C (Table S1, Supporting Information). Interestingly, the fatty acid 22:1ω9 was regularly present in *P. agathidicida* but not detectable from *P. cinnamomi*. However, previous studies have shown that

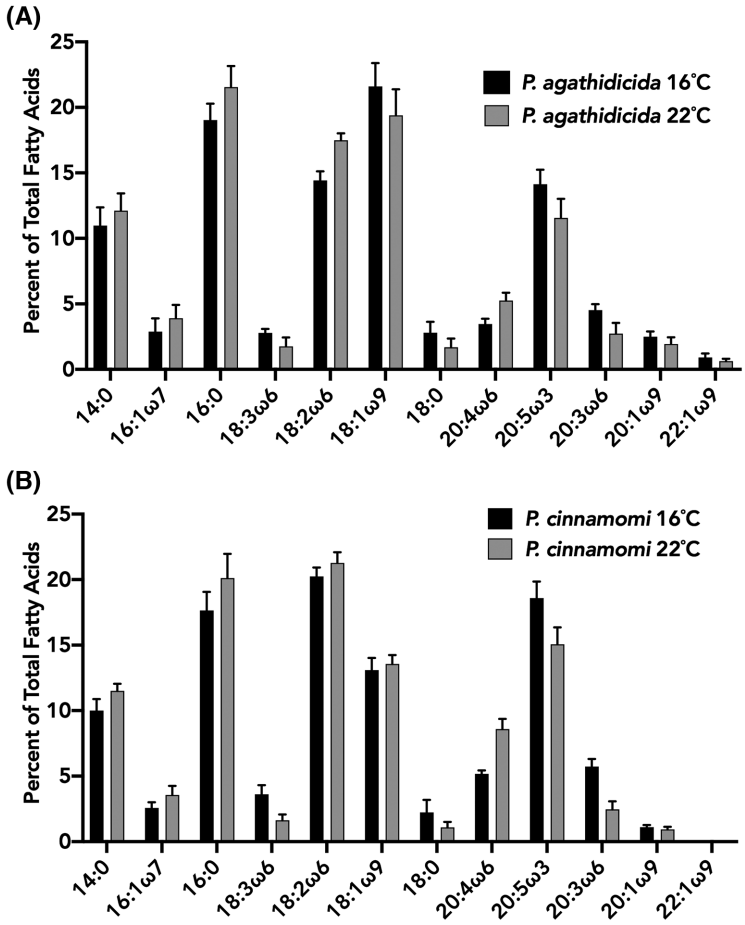

Figure 1. Fatty acid profile of *P. agathidicida* and *P. cinnamomi* at varying temperatures. FAMEs were produced and analysed from mycelia of *P. agathidicida* **(A)** and *P. cinnamomi* **(B)** grown at 16 and 22°C. The % of each fatty acid is the average relative % of five biological replicates. Error bars indicate standard deviation.

this fatty acid is present in *P. cinnamomi* at low relative percentages (Duan, Riley and Jeffers 2013). In general, our fatty acid profile for *P. cinnamomi* was similar to a previously published report that spanned multiple isolates of this species (Duan, Riley and Jeffers 2013). Duan *et al.* detected fifteen fatty acids from *P. cinnamomi*. The four additional fatty acids detected in that study comprised less than 2% of the total fatty acid content.

Comparison of FAME profiles of various *P. agathidicida* lifecycle stages

Environmental conditions can affect the relative abundances of oomycete life-cycle stages in soil (Erwin and Ribeiro 1996). We therefore compared the FAME profiles of *P. agathidicida* oospores, zoospores and mycelia (Fig. 2). Compared to mycelia, the FAME profiles of oospores and zoospores were less diverse in quality and quantity of acyl chains that were present. The five most abundant fatty acids (14:0, 16:0, 18:2ω6, 18:1ω9 and 20:5ω3) were the same for each lifecycle stage. Significant differences for each of these fatty acids between lifecycle stages can be found in Table S2 (Supporting Information). Each fatty acid that was detected from the mycelial samples was also detected in oospores; however, the long-chain unsaturated fatty acids were in lower quantities relative to shorter chain length fatty acids when compared with mycelia. In mycelia, three polyunsaturated, long-chain fatty acids (20:4ω6, 20:5ω3, 20:3ω6) and two mono-unsaturated, long-chain fatty acids (20:1ω9, 22:1ω9) were detected and all except 22:1ω9 constituted >1% of the total FAME profile. In oospores and zoospores, 22:1ω9 was not detected. In zoospores, 18:3ω6 and 20:1ω9 were also not detected. Overall, oospores and zoospores produced lower quantities of long-chain fatty acids. Different growth media are known to induce changes in the FAME profiles of *Phytophthora* species (Larkin and Groves 2003; Duan, Riley and Jeffers 2011), and may have contributed to the variation we observed among life-cycles stages. However, these effects are difficult to quantify since no single laboratory procedure exists to produce oospore and zoospores in *P. agathidicida*.

Assessment of the FAME profile of soils with and without *P. agathidicida* oospores added

To determine if the identified acyl chains could serve as biomarkers for identifying *P. agathidicida* in soil, we first examined the fatty acid profile of five field-collected soil samples. In each soil sample, we characterized the 12 fatty acids produced by *P. agathidicida*. Of the 12 *P. agathidicida* fatty acids, eight were readily detectable in varying quantities across samples (Figure S1, Supporting Information). The addition of oospores to the soil revealed that no unique *P. agathidicida* fatty acids were detectable (Figure S2, Supporting Information). However, the relative % of the long-chain polyunsaturated fatty acid 20:5ω3, which was present in low quantities in soil (Figure S1, Supporting Information) and is relatively abundant in *P. agathidicida*

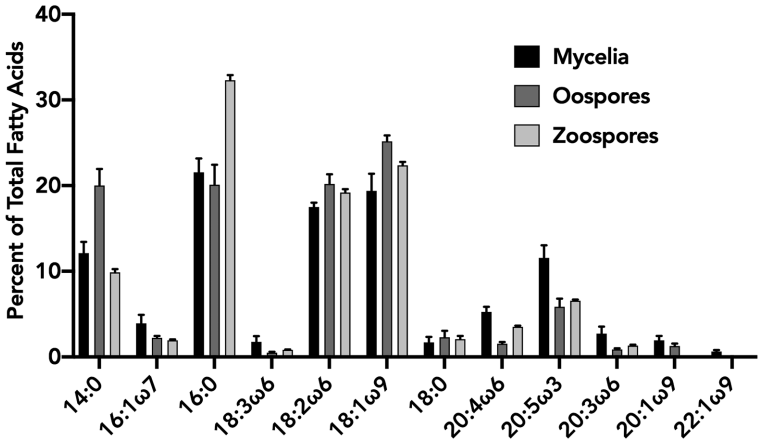

Figure 2. Fatty acid profile comparison of different *P. agathidicida* lifecycle stages. FAMEs were produced and analysed from *P. agathidicida* mycelia, oospores and zoospores. The % of each fatty acid is an average relative % of five biological replicates. Error bars indicate standard deviation.

Table 1. Ratios of 20:5ω3 to 20:4ω6 fatty acids in soil, soil with *P. agathidicida* oospores added, and *P. agathidicida* oospores alone.

	Soil alone	25 k oospores in soil	50 k oospores in soil	100 k oospores in soil	200 k oospores in soil	100 k oospores alone
20:5ω3/20:4ω6	0.92 ± 0.30	1.06 ± 0.24*	1.12 ± 0.34	1.41 ± 0.37**	1.4 ± 0.44*	4.05 ± 0.78**

The ratios of 20:5ω3 to 20:4ω6 were determined by dividing the concentration of 20:5ω3 by 20:4ω6 for each condition across five biological replicates with the average and standard deviation shown. A single * indicates a statistical difference when compared with soil alone at $P < 0.05$, while ** indicates a statistical difference at $P < 0.005$.

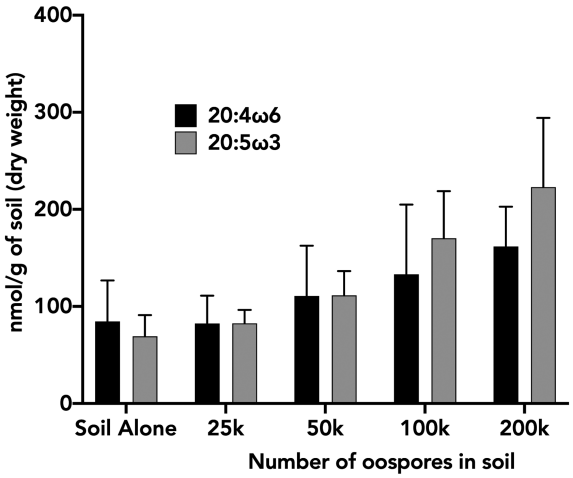

Figure 3. Quantification of 20:4ω6 and 20:5ω3 fatty acids in soil samples with *P. agathidicida* oospores added. Oospores were added at varying concentrations to 0.5 g of soil containing a 19:0 fatty acid internal standard. FAMEs were then produced and analysed from each sample, and the concentration of 20:4ω6 and 20:5ω3 was determined as nmol/g of soil. The values are an average of five biological replicates. Error bars indicate standard deviation.

(Fig. 1), increased linearly with increasing oospores added to soil (Figure S2, Supporting Information). In contrast, the % of 20:4ω6 remained relatively stable with increasing numbers of oospore added (Figure S2, Supporting Information). With this observation, we determined the molar quantity of 20:5ω3 and 20:4ω6 and examined the ratios of these fatty acids in each sample. The ratio of 20:5ω3 to 20:4ω6 in soil samples alone is <1 (Table 1). In *P. agathidicida* oospores, this ratio is >4 (Table 1). Thus, the presence of *P. agathidicida* in soil should lead to an increase in the ratio of 20:5ω3 to 20:4ω6 relative to *P. agathidicida*-free soil. As oospores were added to soil, the molar quantity of 20:5ω3 and 20:4ω6 both increased (Fig. 3 and Figure S2, Supporting Information). However, this change was greater for 20:5ω3 than for 20:4ω6 leading to a greater ratio of 20:5ω3 to 20:4ω6 as oospores increased (Table 1). In *P. agathidicida*-free soil, the 20:5ω3 to 20:4ω6 ratio was 0.92. This ratio shifted to 1.06 at the lowest concentration of added oospores (25 000), and increased to 1.4 at the highest concentration of added oospores (200 000). At 25 000, 100 000 and 200 000 added oospores, the ratio of 20:5ω3 to 20:4ω6 was significantly greater than that of soil alone (Table 3).

DISCUSSION

Rapid and reliable diagnostics are essential when trying to manage diseases in both humans and plants. In the case of kauri dieback, tracking the spread of the *P. agathidicida* is particularly difficult due to the latent onset of disease symptoms (Bradshaw *et al.* 2020). In this study, we present the first FAME profile of *P. agathidicida*. We also assessed the potential of FAME analysis as a tool for detecting *P. agathidicida* in soil. A significant advantage of a FAME approach relative to soil baiting and PCR-based methods, is the potential to quantify the pathogen in the sample. Overall, the FAME profile of *P. agathidicida* is largely consistent with the FAME profiles of other *Phytophthora* species. The five most abundant *P. agathidicida* fatty acids (14:0, 16:0, 18:2ω6, 18:1ω9 and 20:5ω3) are also the five most abundant fatty acids in six other species of *Phytophthora* (Larkin and Groves 2003; Duan, Riley and Jeffers 2013). The similarity of *P. agathidicida* and other studied *Phytophthora* species is particularly interesting as *P. agathidicida* falls into the recently categorized (but as of yet understudied) *Phytophthora* clade five (Weir *et al.* 2015); our data suggest that lipid profiles may be conserved across different clades of *Phytophthora*.

While we identified no unique FAME biomarker for *P. agathidicida*, our data reveal that the addition of increasing amounts of *P. agathidicida* oospores to soil leads to an increase in the ratio of 20:5ω3 to 20:4ω6. Similar results have been observed with *P. sojae* where long-chain polyunsaturated fatty acids were detected above background soil levels when zoospores were added to soil (Yousef *et al.* 2012). Combined, this suggests that the ratio of 20:5ω3 to 20:4ω6 could be a useful biomarker indicating the presence or absence of *Phytophthora* species in forest soils. However, other oomycete genera may also influence the ratios of 20:5ω3 to 20:4ω6 in soil. For example, of the *Plasmopara* species examined by Spring and Haas, there was a high degree of variability in FAME profiles with only *Plasmopara halstedii* producing high levels of 20:5ω3 (Spring and Haas 2002). *Pythium* species also produce variable FAME profiles. *Pythium aphanidermatum* produces low levels of 20:5ω3, whereas *Pythium irregulare* and *Pythium ultimum* produce high levels of 20:5ω3 and 20:4ω6 at ratios close to 1:1 (Cheng *et al.* 1999; Spring and Haas 2002; Larkin and Groves 2003). Overall, of the *Phytophthora* species with FAME profiles reported in the literature, all produce high ratios of 20:5ω3 to 20:4ω6, while non-*Phytophthora* oomycetes present more variable FAME profiles. Thus, high 20:5ω3 to 20:4ω6 ratios are likely to indicate the presence of *Phytophthora*, but other oomycetes may also be detected.

The work presented here highlights the potential of the ratio of 20:5ω3 to 20:4ω6 to function as a tool for the detection of *Phytophthora* in soils. Nevertheless, further studies and analyses are needed to optimize FAME analysis as a diagnostic tool that can be effective in assessing field samples. Our results suggest that basic qualitative FAME analysis is not sufficient for differentiating *P. agathidicida* from other *Phytophthora* species and potentially other oomycetes. However, previous studies indicate that FAME analysis can be used to distinguish not only *Phytophthora* species but isolates within a species as well (Larkin and Groves 2003; Duan, Riley and Jeffers 2013). For example, cluster analysis based on FAME profiles was used to differentiate cultured *P. cactorum, P. citrophthora, P. cinnamomi, P. cryptogea* and *P. nicotianae* (Duan, Riley and Jeffers 2013). In another study, using similar techniques, individual isolates of cultured *P. infestans* were differentiated (Larkin and Groves 2003). This suggests that a more detailed characterization of *P. agathidicida* FAMEs may provide sufficient information for distinguishing *P. agathidicida* from other *Phytophthora* species. Additionally, utilizing extraction techniques that target specific groups of lipids such as phospholipids or neutral lipids may help to further enrich target fatty acids such as 20:5ω3 and reduce background signals from complex environmental samples like soil (Drenovsky *et al.* 2004). These techniques and analyses could lead to a determination of the threshold of detection for *Phytophthora* in infected soil samples.

Despite the current limitations of FAME analysis, detecting *Phytophthora* on a genus level and possibly other oomycete species is useful and can potentially be used in conjunction with soil bating assays or other molecular diagnostics for species confirmation. For example, large scale soil sampling and screening of samples via FAME analysis could be used to identify samples with 20:5ω3: 20:4ω6 >1. These samples could subsequently be subjected to the soil-baiting assay to confirm the presence of *P. agathidicida*. This is similar to the 'funnel and filter' model used for screening soils contaminated with *P. ramorum* (Smart *et al.* 2021). In that example, soils were pre-screened for the presence of *Phytophthora* using an immunosorbent assay. Soils positive for *Phytophthora* were then examined using qPCR to determine if *P. ramorum* was present.

In contrast to FAME analysis, several DNA based diagnostic tools have been explored for use in detecting *P. agathidicida* and other *Phytophthora* species. These include both conventional qPCR and loop-mediated isothermal amplification (LAMP) methods (Than *et al.* 2013; McDougal *et al.* 2014; Hansen *et al.* 2016; Winkworth *et al.* 2020). Recently, Winkworth *et al.* (2020) combined aspects of the existing *P. agathidicida* soil-baiting assay with LAMP to reduce the overall sample processing time. LAMP is a DNA amplification-based method that uses primers to amplify regions of the genome-specific to an organism of interest (Wong *et al.* 2018). Efficient amplification can lead to a detectable signal, such as color-change, that can often be assessed quickly and on-site spectrophotometrically. LAMP assays efficiently detected *P. agathidicida* immediately following baiting (Winkworth *et al.* 2020). This variation on standard soil-baiting effectively eliminated the final week of the assay, shortening the process to 2 weeks. It is unclear whether or not LAMP would be useful at directly testing soil samples for the presence of *P. agathidicida*.

Currently, our knowledge of the geographical spread of *P. agathidicida* is limited primarily to the presence of visible disease symptoms combined with soil-baiting assays (Bradshaw *et al.* 2020). More thorough knowledge of the full range of *P. agathidicida* could potentially help answer questions regarding disease resistance in kauri trees and environmental factors that lead to infection. The knowledge gap is mainly due to the lack of a quick, cost-effective and simple diagnostic tool. While effective, soil-baiting is not amenable to high-throughput, widespread testing. Both FAME analysis and DNA based molecular diagnostics are promising but have limitations. Further development of these techniques in conjunction with soil-baiting would help to expand our knowledge of the distribution of *P. agathidicida* in New Zealand enabling better management of kauri dieback disease.

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SUPPLEMENTARY DATA

Supplementary data are available at FEMSLE online.

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Conflicts of Interest. None declared.

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