Mātauranga Māori and anti-microbials: Searching for new tools to control the spread of Kauri Dieback.

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Te Karakia o Te Atua

E tō mātou Matua i te rangi

Kia tapu tou Ingoa

Kia tae mai tou rangatira-tanga.
Kia meatia tau e pai ai ki runga i
te whenua, kia rite ano ki to te
rangi. Homai ki a mātou aianei
he taro mā mātou mo tēnei ra.
Murua o mātou hara

Me mātou hoki e muru nei i o te hunga e hara
ana ki a mātou. Aua hoki mātou e kawea kia
whaka-waia;

Engari whaka-orangia mātou, i te kino:
Nou hoki te rangatira-tanga, te kaha, me
te kororia, Ake, ake, ake.

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Ko te mihi tuatahi ki te atua a Ihu Karaiti, nānā i hanga, nānā i tango, nānā nei ngā mea katoa. Ko te mihi tuarua ki ngā hunga mate kua wheturangitia, haere, haere whakangaro atu rā. Hoki mai ki te kanohi ora, ko tātou e hikoi nei i ngā tapuwae a ō tātou mātua tipuna tēnā tātou katoa. E mihi ana ki te hunga kaipanui e arotake ana i tēnei mahi māhita. Tēnā koutou.

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E rere ngā wai kōpikopiko o Waiapū

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Abstract

Phytophthora are plant pathogens, well known for devastating thousands of ecologically, culturally and economically significant plant crops worldwide. In greek *Phytophthora* translates directly to ‘plant destroyer’. Though it is ‘fungus-like’, *Phytophthora* are eukaryotic oomycetes, more closely related to brown algae and diatoms. *Phytophthora* have three key lifecycle stages: oospores, zoospores, and mycelia.

Kauri are ancient conifer species dating back to the Cretaceous period (145 mya) and are now rapidly declining due to Kauri dieback caused by *Phytophthora agathidicida*. *P.agathadicida* causes root rot in Kauri trees and was first misidentified as *P. hevave* on Great Barrier Island in the early 1970s. Its origin is unknown however research argues it may have evolved from *P. infestans,* the pathogen that caused the Irish potato famine in 1845. For Te Āo Māori, Kauri are highly regarded tīpuna (ancestors) and Kauri Dieback is alarming to many Northern Iwi. Kauri wood and resin are highly sought and economically valuable resources. The Waipoua forest is the largest Kauri forest and the most impacted by Kauri Dieback. Over 25% of Kauri in the Waitākere ranges are either infected with *P. agathadicida* or are symptomatic, a percentage that is steadily increasing. A rāhui (temporary ban) was placed on the Waitākere ranges by local iwi Te Kawerau a Maki in 2018 as a preventative measure to reduce movement of *P. agathadicida* in soil. Apart from track closures, scrubbing and spraying equipment - before and after entering the forest - is the only tool of management. Sterigene disinfectant is the only treatment to reduce the spread of Kauri Dieback. Sterigene kills zoospores, mycelia and sporangium but is ineffective against *P. agathidicida* oospoores. Sexually produced oospores are responsible for the long-term survival of *Phytophthora* as they have a thick cell wall.

The first part of this thesis examines a range of commercially available disinfectants and their efficacy against *P. agathidicida* oospores. These results confirm that Sterigene and/or Trigene are not effective against *P. agathidicida* oospores. My results also show that 2% bleach, 1% Virkon, and 70% ethanol all reduce oospore viability. Napisan also reduced oospore viability, but also interacted with the viability stains, therefore further investigations are needed. Napisan is an oxygen bleach, commercially affordable and easily accessible in supermarkets. Unlike sterigene and bleach, Napisan is safe to use on clothes, wool and soft textiles. If effective against oospores and the other lifecycle stages, Napisan could be a promising solution to help reduce the spread of Kauri Dieback.

The second part of this thesis tested the efficacy of Kānuka extracts and essential oils against *P.agathidicida* oospores and mycelia. Results show Kānuka extract made from crude leaf and root material, was effective against oospores. Kānuka essential oil inhibited mycelial growth and reduced mat size significantly compared to water controls. Research from Lawrence et al (2019) found Kānuka extracts significantly reduced zoospore motility and germination and also inhibited mycelial growth. Kānuka is a very promising rongoā to eliminate *P. agathidicida* and future research should focus on using it as a topical spray on Kauri inoculated with the pathogen, in greenhouse trials. Ahakoa he iti te Kānuka, ka whakaora tonu te Kauri (Although Kānuka is small, it can heal Kauri). Lastly, this research merely touches on the importance of Mātauranga māori in the conservation and management of tāonga (natural treasures) in Aotearoa. This thesis aims to bridge the gap between mātauranga Māori and western science, where both worlds work together to save Kauri from extinction.

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Chapter 1
Introduction

1.1 *Phytophthora*

Phytophthora directly translates from Greek to English as ‘plant destroyer’. *Phytophthora* is a genus of eukaryotic micro-organisms most commonly referred to as water moulds. Based on morphology, *Phytophthora* was originally misclassified as fungi. However, genetic analyses have placed *Phytophthora* in the *Straminipilia* kingdom, class oomycetes. Therefore, *Phytophthora* are taxonomically more closely related to brown algae and diatoms (Judelson and Blanco, 2005). Some *Phytophthora* species have a broad host range *e.g., Phytophthora cinnamomi* can infect 5000 different plant species, meriting the name ‘biological bulldozer’ (Armstrong, 2018). *Phytophthora infestans* was the causal agent of the Great Potato Famine which was a catastrophic event in 1845 that caused the death of millions of people in Ireland (Henshall and Beresford, 2004; Bradshaw et al, 2020). Even in the present day, *P. infestans* is responsible for an estimated $5 billion USD in crop damage annually (Henshall, 2004; Judelson and Blanco, 2005). *Phytophthora ramorum* causes dieback of ecologically significant Oak trees in Oregon forests and extensive tree mortality has already led to altered forest landscapes on coasts in California (Hall, 2009).

P. capsici and *P. citrophthora* cause the greatest economic devastation on pepper and citrus plants within the Mediterranean. *P. citrophthora* is responsible for 15% of fruit losses per year and causes 8-30% infection on susceptible lemon cultivars (Biçici, 1990). *P.capsici* causes 100% drying and killing of pepper plants under poor drainage and irrigation conditions, thus affecting pepper-paste and pepper-spice productions. From 1952 to 1970 *P.cambiuora* killed 20 000 chestnut trees, known as ink disease (Bicici, 1990). Due to the pathogen’s aggressive nature to cause serious disease in numerous plant hosts, New Zealand plants are equally susceptible to the organism. *Phytophthora agathidicida* causes Kauri Dieback disease in *Agathis australis* (Kauri), the main subject of this thesis. Greenhouse soil trials found that *P. cinamommi* completely killed Kauri seedlings within 10 weeks of infection and inhibited the growth of mature Kauri (Horner and Hough, 2014). Plant biologists are aware of the huge threat *Phytophthora* poses to global plant biodiversity.

1.2 The life cycle of *Phytophthora*

Phytophthora are water and/or soil-borne pathogens that produce spores in both sexual and asexual stages of the life cycle (Weir et al, 2015; Bellgard et al, 2016). Zoospores, mycelia and sporangia are responsible for asexual reproduction (Figure 1). Zoospores are the motile stage and have flagella that actively propel them toward host plants via chemotaxis (Armstrong, 2018). Zoospores create cysts on host roots and form mycelial structures that produce either sporangium or oospores on the end of branches. Sporangia encase zoospores, restarting asexual reproduction (Armstrong, 2018). Oospores are the sexual life stage of *Phytophthora* and are responsible for its long-term survival due to a thick cell wall (Dick and Kimberley, 2012).

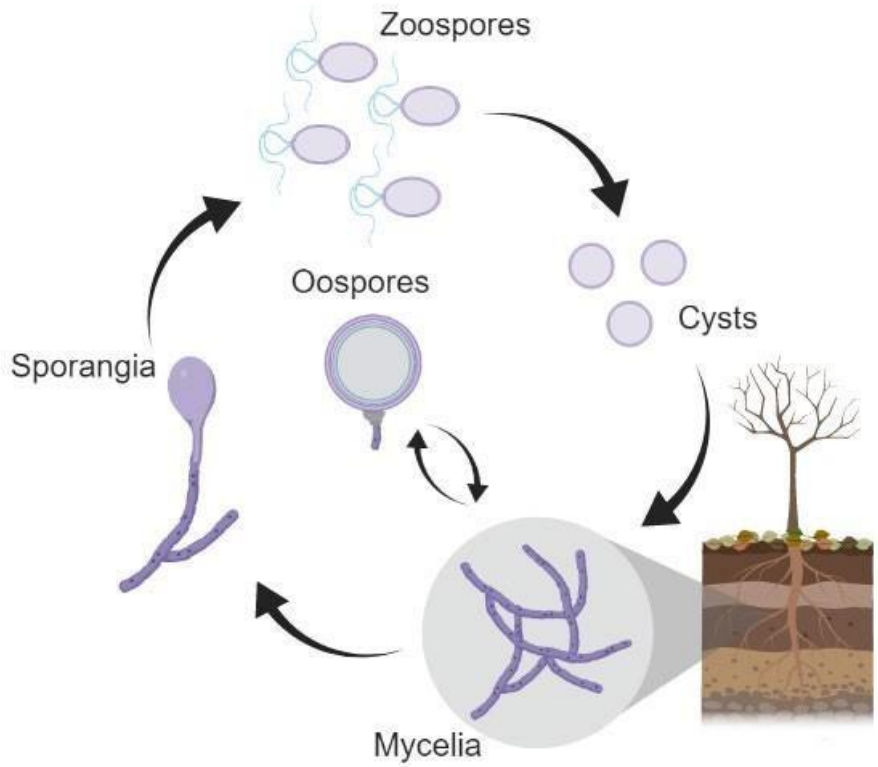


Figure 1. The life cycle of *Phytophthora*. Motile zoospores locate and move toward host roots via chemotaxis to infect and form cysts. Within cysts are hyphae and mycelia that penetrate host vascular tissue and derives nutrients from host roots. Oospores and sporangia form at the end of mycelial branches. Zoospores are contained within sporangium sacs and thus restarts the life cycle (Figure produced by Mike Fairhurst and used with permission).

Zoospores

Morphology and anatomy

Zoospores are laterally biflagellate short-lived and motile (Hickman, 1970; Weir et al, 2015; Bellgard et al, 2016). The standard characteristics of zoospore flagella are posteriorly directed whiplash, anteriorly directed tinsel (Hickman, 1970). Both flagella are active in propulsion (Couch, 1941). *Phytophthora* zoospores are ovoid, bluntly pointed and appear kidney-shaped.

Behaviour of zoospores

Zoospores of *P. agathidicida* swim in an irregular pattern with a rapid change in direction. Depending on the environmental condition, on average zoospores can remain motile for several days (Hickman, 1970). However, our lab tests found *P. agathidicida* zoospores remained motile in sterile water for 17 h (Lawrence et al, 2017). External factors such as nutrients, osmotic pressure, pH and surface membrane ability, likely affect zoospore motility.

P. agathidicida zoospores are chemotactically attracted to chemical exudates given off by finer feeder roots of Kauri trees, however, the exact isoflavones are yet to be identified (Bradshaw et al, 2020). Zoospores of other *Phytophthora* (especially those with a small host range) are extremely specific in attraction towards host plants. For example, *P. sojae* is known to infect only soybean hosts and can detect chemicals given off by host plants even at nanomolar concentrations (Tyler, 2002).

Upon reaching a host root, zoospores germinate to produce feeding tubes that grow chemotropically in the same direction penetrating host tissue (Figure 1.2 D) (Hickman, 1970). Zoospores then create cysts on the surface of roots, initiating the production of vegetative structures; mycelium and hyphae (Bradshaw et al, 2020).

Mycelia and hyphae

Mycelia and hyphal structures are created by zoospores and are also part of asexual reproduction (Figure 1.2 C). Cysts produce hyphae that penetrate and colonize vascular tissue of host roots (Bradshaw et al, 2020). Most *Phytophthora* species are poor saprophytes and their survival on the surface of roots is uncommon (Savita and Nagpal, 2012). The lag time between infection and lesion visibility is unknown (Horner & Hough, 2014; Herewini et al., 2018). *Phytophthora* can be cultured in the lab from hyphae only, however, in the environment, pathogen survival relies heavily upon the production of new spores (Judelson and Blanco, 2005). Different from fungi, *Phytophthora* requires the continual reproduction of spores as the host declines (Judelson and Blanco, 2005). Mycelia produce sporangia, cause

necrosis and allow the pathogen to derive nutrients from the host. Mycelia also produce oospores at the end of the structures via sexual reproduction (Bradshaw et al, 2020).

Sporangia

Sporangia are produced asexually by mycelia and release zoospores. According to lab observations, sporangia sacs contain typically around 6-10 zoospores (Figure 1.2 A). However, for some *Phytophthora spp,* sporangia contain 20-30 zoospores and are produced under a series of specific environmental conditions (Erwin and Ribeiro, 1996). Latijnhouwers and Govers (2003) found the expression of *P.infestans* G-protein β-subunit gene (*PiGPB1*) was involved in the production of sporangia and vegetative growth. The study showed mycelia with severely low levels of *Pigpb1* mRNA was associated with empty or malformed sporangium and reduced sporulation. In addition, temperature, exposure, time of incubation, light intensity and media used for growth are variables that can influence sporulation (Armstrong, 2018). Armstrong (2018) developed methods to improve the number of *P. agathidicida* zoospores by increasing sporangium production. Light intensity (40 watts) was a major determiner for increased sporangia in *P.agathidicida*. Sterols are known to increase asexual and sexual production for many *Phytophthora* species *in-vitro* by influencing membrane biogenesis of spores (Erwin and Ribeiro, 1996). It is proposed that sterol synthesis pathways in *Phytophthora* are insufficient and therefore *Phytophthora* may rely upon external sources of sterol (Armstrong, 2018). Sterols could potentially be taken from the host plant, an evolutionary adaption of *Phytophthora* (Nes, et al 1982).

Oospores

Oospores play a key role in the long-term survival and spread of *P.agathidicida* due to a thick cell wall (Figure 1.2 B) (Sneh and McIntosh 1974; Stack and Millar, 1985). On average, *Phytophthora* oospores have a wall volume index (oospore wall volume to total oospore volume) of 0.41. In comparison, *P. agathidicida* oospores have a slightly thicker wall volume index of 0.64 (Bradshaw et al, 2020). Oospores are largely produced within plant host tissue and readily dislodge from an infected host and disperse via water and soil movement (Bellgard et al, 2018). Other vectors of spread include humans, animals and vehicles. Oospores can also survive passage through stomachs of feral pigs (Bassett et al, 2017, Bellgard et al, 2019; Bradshaw et al, 2020). Oospores germinate to produce modified

mycelial stalks that generate sporangium sacs. Germination is influenced by nutrition, age, temperature, light, treatments and chemical treatments with enzymes (Widmer, 2010).

Oospore development

The general process of oospore development has been observed under light microscope and electron microscope and documented in seminal papers (Dastur, 1913; Pethybridge, 1913; Murphy, 1918; Blackwell, 1943; Vuji~i6, 1971; Hemmes and Garcia, 1975). The process can be generalized in three steps from Hemmes and Garcia (1975) and has been observed for many *Phytophthora spp*. The following development steps are recognized 1) penetration of the antheridia into the oogonium 2) oogonial expansion and wall thickening 3) outer and inner oospore wall development (Hemmes and Garcia, 1975).

The oogonial initial emerges as a growing orb by rupturing the antheridia. The middle and outer wall layers also rupture and remain near the base of the expanding oospore, giving its characteristic 'collar look' (Figure 1.2 B). The oogonium then becomes 'blocked off' from the mycelium by a 1-3 μm thick plug and the antheridium is separated from the parental hyphae by a septum (Hemmes and Garcia, 1975). The oospore cell wall then thickens from 200 nm to 800 nm. During oogonium expansion, the antheridial cell wall thickens considerably. After this phase (117hrs) a large oospore has formed inside of the oogonium, surrounded by a thin layer of cytoplasm (0.4 - 0.8 μm) separated by a unit membrane (Hemmes and Garcia, 1975).

A thick oospore cell wall begins to differentiate into several separate layers each approximately 0.7 -1.0 μm thick, the thickness is affected by the space in between each layer (Hemmes and Garcia, 1975). Lippman et al (1974) found that oospore cell walls are made up mostly of β-glucans. The electron-dense inner oospore cell wall is transparent and does not take up stain, thus the contrast between the oogonium and inner wall indicates a difference in chemical composition. In a mature oospore, the cytoplasm consists of a large central vacuole (ooplast) surrounded by many lipid-like bodies (Hemmes and Garcia, 1975).

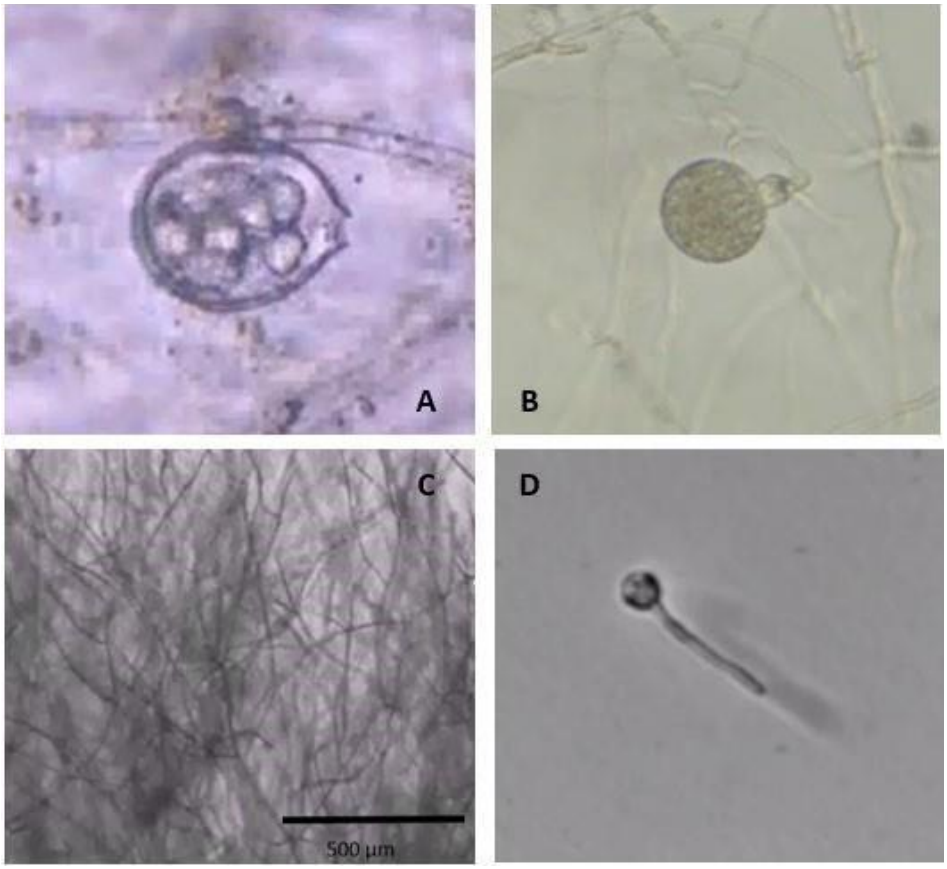


Figure 1.2 Stages of *P. agathidicida* under x40 magnification light microscope. A) Sporangium sac containing zoospores. B) Oospore with oogonium attached to mycelium. Ruptured cell wall of the antheridium forming the 'collar' base C) Mycelial mat under x4 magnification. D) Zoospore undergoing germination.

1.3 The impact of *Phytophthora* species in New Zealand

In New Zealand, many species of *Phytophthora* are biosecurity threats due to their impact on key plant species. *P. pluvialis* causes 'Red Needle Cast' in radiata pine (*Pinus radiata*), by initiating premature defoliation of mature needles. Radiata pine (*Pinus radiata*) is a dominant forest species making up 90% of New Zealand's 1.7-million-hectare forest plantation (Watt et al, 2019). Approximately 60% of Radiata pine is milled and exported, earning $5.6 billion in forestry exports and non-log activity contributed $2 billion to NZ GDP last year (Fox, 2019). Therefore, *P. pluvialis* has had a significant impact on New Zealand's plantation, agricultural and forestry economy (Scott, 2014).

P.cinnamomi causes root rot in hundreds of crops worldwide (Zentmyer, 1967) and in New Zealand, *P.cinnamomi* is responsible for a 20% decrease in yearly avocado yield (Armstrong, 2018). *P.infestans* causes potato late blight on crop yields in areas of high rainfall such as Pukekohe, Auckland (Hartill and Young, 1985).

All *Phytophthora* species mentioned above share similar life stages and characteristics, therefore general research on all species could potentially provide knowledge and insight into the control and management of another species. Efforts to manage Kauri dieback in the past has advanced from research on *P.ramorum* in USA forests (Bradshaw et al, 2020).

P. agathidicida causes rapid death of native Kauri trees (*Agathis australis)* and is the main focus of this thesis. The aggressive and rapid working nature of the pathogen earnt its declaration as an 'unwanted organism' under the biosecurity act (1993) in 2008. *Phytophthora agathidicida* belongs to a clade 5 sub-group of *Phytophthora,* a group that so far contains only four species isolated from Asia (Bradshaw et al, 2020). Currently, there is no existing cure and the main management tools involve phosphite treatment, containment within infected areas and hygiene procedures. As numbers of Kauri decrease, more biosecurity attention and cultural concern is given to the area by tangata whenua (indigenous people of the land), community groups and councils. Research is therefore highly important for the future safeguarding of Kauri.

1.4 Origins of *Agathis australis*

Araucariacecae have an ancient fossil record dating back to the Mesozoic period (Dargavel, 2015). They are distributed widely over both hemispheres and within this genre are *Araucaria*, *Agathis* and *Wollemia*. *Agathis* usually emerge from the canopy and are distributed around eastern Australia and northern New Zealand to Fiji and Malesia (Wilf et al. 2014). Depending on the species classification criteria, within the family are 13 to 21 species (Laubenfels, 1988). Morphological data present *Wollemia* and *Agathis* as sister taxa, whereas a cladogram based on gene sequences shows *Agathis* relating to *Araucaria* (Wilf et al. 2014). Based on data from preserved foliage, *Agathis* links back to the early Cretaceous period. Stockler et al (2002) argue that according to fossil records *A. australis* evolved from a New Zealand ancestor instead of a migrant Australian taxon (Wilf et al. 2014). There are 17 extant gymnosperms conifer species within the *Agathis* genus and *A. australis* is recognized as one of the first divisions of *Agathis* (Wilf et al. 2014; Bradshaw et al, 2020).

1.5 Mātauranga Māori

Indigenous knowledge is becoming increasingly recognized as playing a significant role in conservation and resource management worldwide. Kaitiakitanga (stewardship) is an understanding that we do not own the land, we belong to the land and while we are here, we are the sole guardians (Waka Huia, 2019). Māori have built banks of traditional ecological knowledge and values over many years of interaction with the environment (McKay, 2015). This knowledge has helped sustain livelihoods, whilst also protecting and conserving resources for ngā tamariki mō āpōpō (children of the future). Māori values such as; Whakapapa, Mana whenua, Whakapono, Āwhina, Kotahitanga, Tino rangatiratanga, manaakitanga and aroha most commonly appear in policy statements and regional council plans (Kanwar et al, 2016).

Due to increased urban development, large-scale agricultural farming, language loss and the displacement of Māori people, the role of kaitiaki has been disrupted. Co-management is, therefore, more applicable to modern-day environmental issues, where a combination of scientific methodology and Māori knowledge form a construct of collaborative resource management policies (Kanwar et al, 2017). This is implemented by the resource management act 1991 which encourages collaborative management and the incorporation of Maori values. Collaborative management and policy-making is occurring between indigenous people worldwide because it is proven to be a better approach than the current single approach and maximizes the ability to manage resources (Resource management act, 1991; McKay 2015). It is not a straightforward process and trust and respect between Iwi and partners need to be re-established.

Kauri dieback provides an opportunity for collaborative management between tangata whenua and Pākehā. Collaborative decisions between Auckland council and Te Kawerau a Maki to place a rāhui over the Waitākere rangers is a prime example of this. In addition, collaborative research with rongoā Māori practitioners and western science methods has produced promising results as outlined in this thesis.

1.6 Tīpuna Kauri - The significance of Kauri in Aotearoa

Māori believe Kauri are descendants of Tāne Mahuta, guardian of the forest. Certain Northern iwi speaks of Tāne transforming into a Kauri before separating his parents, Papatuānuku (mother earth) and Ranginui (sky father), thus indicating the strength of a Kauri (A. Kingi 2020, Pers comms., 6 May). Pūrākau (stories) from Tūhoe (Mātātua) speak of Tāne having 14 wives (13 depending on Iwi variation), his 4th wife was Hinemahou and she gave birth to Kauri and Tanekaha (H. Ratana 2020, Pers comms, May 7). Another pūrākau from Ngāti Hine and Ngāti Wai says Kauri and Tohorā (whales) were brothers.

Tangata rangatira or people of high status are often compared to Kauri. Whakatauaki (proverbs) have been written about Kauri and are continuously used in whaikorero (speeches) on marae ātea. A common Ngā Puhi whakatauki ‘kua hinga tētahi Kauri o roto I te Wāo-nui a Tāne’ - the Kauri of the realm of Tane has fallen. This whakatauki is often said at tangi or the death of a chief/ person of high significance to the whanau. Traditionally, Māori sought permission from Tāne Mahuta through karakia before felling a Kauri. Waka (sailing ships) were carved from Kauri wood, extremely buoyant and dense and one whole Kauri could create a waka big enough to carry approximately 200 men (Irwin et al, 2017).

Kauri are endemic to New Zealand (Black et al, 2018) and as mentioned above, have enormous cultural significance. The natural growth range of Kauri is in the Northern regions of New Zealand (i.e., Northland, Auckland, the Coromandel and the Bay of Plenty) and are significant among to Northern iwi, sports teams and communities. For example, the Northern region rugby team have had Kauri as their team emblem since professional rugby started in Northland (S. Dawson 2020, pers comms, 6 May).

Tāne Mahuta was the name given to the largest living Kauri on record located in the Waipoua Forest sanctuary. Its age is estimated at 2,500 years, its height is 51 m and girth 14 m. It has a timber volume of approximately 245 cubic meters, 100 times greater than the average plantation pine tree. Kawana Waititi, master carver of 10 years at Te Puia Māori arts and crafts institute, describes Kauri as one of the greatest carving woods among Tōtara and Matai. He says “Kauri is an amazing wood and is often used in whakairo (carving) because it is easy to carve and has a beautiful finish”. He estimated Kauri costs ~ $4000- $6000 per m^3 (K. Waititi 2020, pers comms, 25 Sept).

Ecologically speaking, Kauri are a foundation species in forest stands as they stabilize root structures, alter soil pH and provide refuge for native birds and invertebrates (Bradshaw et al, 2020). Kauri are also one of the largest growing and oldest living trees in New Zealand with an average trunk diameter of 4.4 m and an average life span of 600 years (Bradshaw et al, 2020). The oldest reported Kauri are well over 1500 years old (Ahmed & Ogden, 1987, Steward & Beveridge 2010,). Of all native conifers, Kauri store the largest pools of carbon (Silvester and Orchard, 1999; Bradshaw et al, 2020).

Pre- European settlement Kauri covered greater than 1 million hectares of forest, however over two hundred years of burning and logging this number has drastically decreased to less than 1% (Bradshaw et al, 2020). It is estimated that ~60,000 kauri trees have regenerated since this exploitation event and are now under protection since being recognized as a threatened species (Steward and Beveridge, 2010).

1.7 Economic benefits

Kauri are important to New Zealand's economy and in the past were considered more valuable than gold (Chikumbo and Steward, 2007). Kauri gum and timber earn most of its revenue, however, swamp Kauri fossils have recently emerged as a valuable worker timber with the potential to contribute to New Zealand's export market (Lorrey et al, 2017). The sustainability of this resource is questionable due to its limited availability (Lorrey et al, 2017).

Kauri produce very large amounts of resin in the heartwood and small resin canals located in the bark, leaves, surface leaves and other areas. Kauri resin is used for the manufacture of varnishes and linoleum, quite popular in the Indonesian region (Thomas, 1969). Kauri gum is harvested from the ground as fossil gum or on the tree as green gum. In 1939, exports of Kauri gum were 2,316 tonnes which were only a quarter of what was exported in the peak year 1899 (Harrison-Smith, 1940). From 1850 to 1950, 450,000 tons of Kauri gum was excavated and exported for 25 million pounds to manufacturers in Britain, Australia and the USA.

Models of growth and production of Kauri plantations in New Zealand estimated gross values of $220,678 to $236,250 per hectare (Steward et al, 2014). These prices are likely to maintain higher market prices than 'bulk wood' and have the potential to increase in the future as the resource becomes scarcer (Steward et al, 2014).

The Kauri Museum is a tourist attraction located in Northland and currently leads the heritage sector. Many people worldwide have visited the museum since 1962 and its identity is built on the story of Kauri, the industries and the people involved. The Museum has done well to attract and maintain visitor numbers throughout the years. The annual report for 2018-2019 shows the Museum had 40677 visitors in 2019 and 43276 in 2018. Accumulated funds were $6,584,158 in 2019 and $6,195,422 in 2018 (The Kauri Museum annual report 2018-2019).

1.8 Kauri Dieback disease

P. agathidicida is a lethal root rot pathogen. It targets and encysts on Kauri roots and penetrates plant cell walls with hyphae, interrupting sugar and water flow pathways. Eventually, the pathogen permeates through the entire root system, resulting in a leafless, colourless 'Kauri skeleton' (Figure 1.3), a process most commonly known as Kauri Dieback.

Figure 1.3. Dead kauri trees, infected with kauri dieback, at Trounson Kauri Park. RNZ radio.

Symptoms of an infected tree include excessive gum excretion at the collar and lower trunk region, known as gummosis or hyper-resinosis (Bradshaw, 2020). Other symptoms include shedding of bark or leaves and yellowing leaves. These symptoms are a result of the chronic phase of the pathogen where the tree initiates pathogen-mediated dysfunction of the outer vascular tissue (Bradshaw et al, 2020). The lag time between root infection and visible lesion development also assumed as the symptomless stage, is common amongst *Phytophthora spp*

and may be due to the pathogen suppressing host defense mechanisms (Denman et al, 2009; Bradshaw et al, 2020).

Above-ground disease symptoms have a latency period of approximately several years and infections on finer feeding roots are likely to have occurred far before then (Bradshaw et al, 2020). Tree mortality varies between 1 and 10 years from symptom development and this is highly influenced by environmental conditions, tree predisposition and amount of the pathogen (Bradshaw et al, 2020).

The origin of *P. agathidicida* is currently unknown; however, it was first misidentified as *Phytophthora heveae* in the early 1970s in Aotea Great Barrier Island (Gadgil, 1974; Bradshaw et al, 2020). It was later correctly identified on the mainland in 2006 within the natural range of Kauri stands in the upper North Island (Waipara et al, 2013). It was identified as a new *Phytophthora* taxon *agathis*, after a mismatch in DNA sequences was found (Bradshaw et al, 2020) thus it was officially given the name *Phytophthora agathidicida* in 2015 (Weir et al, 2015). Surveillance shows the widespread nature of the epidemic throughout the bay of plenty and Northland region (Figure 1.4) (Bradshaw et al, 2020). Waipoua is one of the most heavily infected forests in NZ and has the largest amount of Kauri stands (Beever et al, 2009; Waipara et al, 2013) The Waitākere ranges (located in west Auckland) had 19%- 58% infection of Kauri patches greater than 5 ha between 2011 and 2016 (Hill et al, 2017). Though there are no known cures, the epidemic had triggered a multicultural response across New Zealand (Bradshaw et al, 2020).

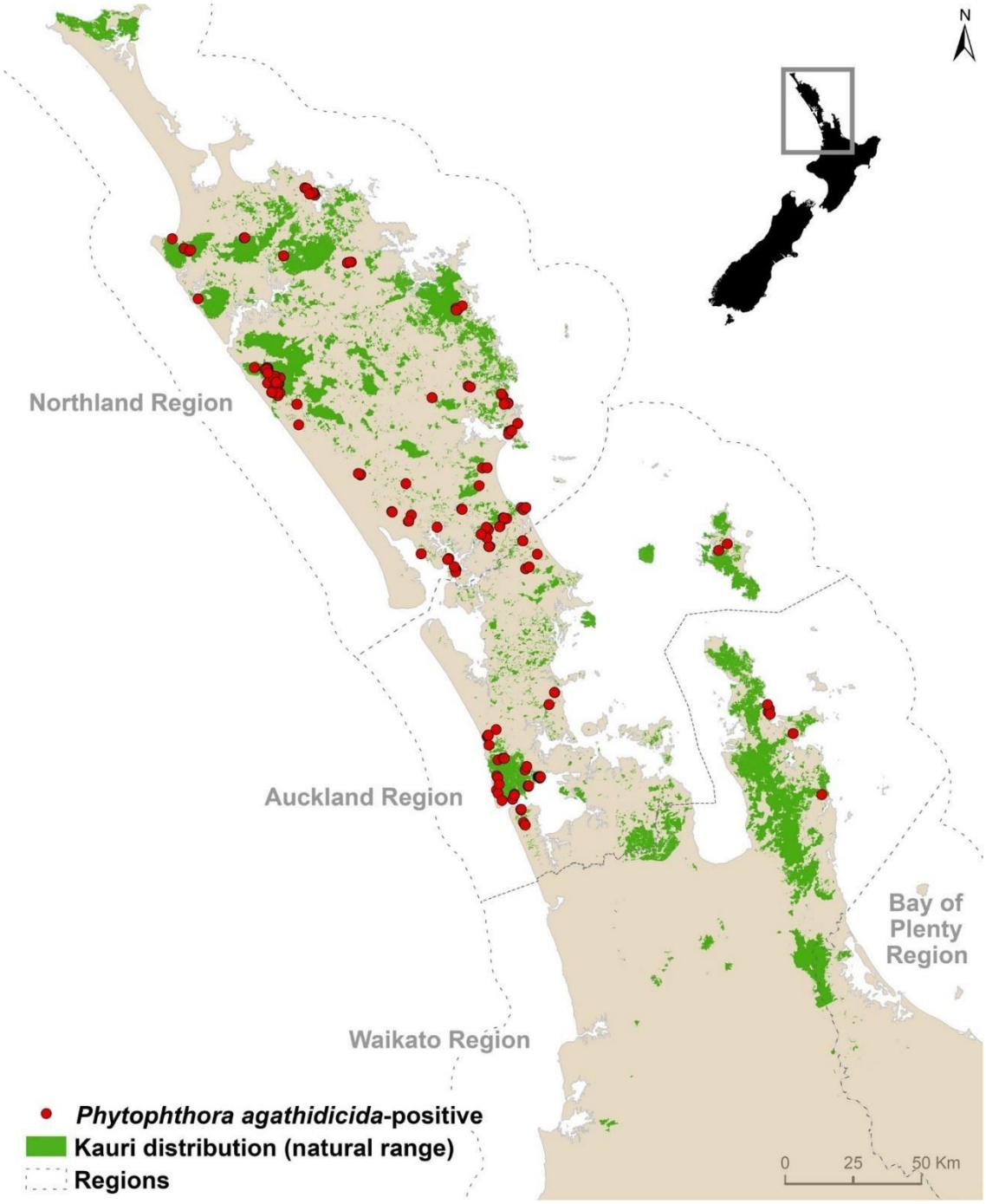


Figure 1.4. Kauri dieback spread across the North Island, red dots show the confirmed locations of *P. agathidicida* pathogen in native Kauri forests. Distribution map (Crown copyright) was created by Biosecurity New Zealand on August 14th 2019 using available Kauri dieback survey data. Accuracy, completeness, reliability or fitness of the map are not certain and MPI gives no warrant for liability. The small map was sourced from Wikimedia (Creative Commons CC0 1.0 Universal Public Domain) (Bradshaw et al, 2020).

1.9 Kauri Dieback management and spread

There are three main strategies for managing Kauri dieback at the Regional Council level: 1) Research 2) Behavioral change 3) Infrastructure. Each strategy covers a broad scope of challenges that fall beneath the umbrella of Kauri dieback management.

Research

Research is important for the expansion of knowledge and to identify best practices when managing Kauri dieback. This field explores Kauri Dieback treatments, data collection and surveillance. Phosphite injections are currently the only treatment for Kauri. Yet phosphite does not kill *P.agathidicida*, instead, it helps boost the immune system of Kauri enabling prolonged co-existence with the pathogen (Bellgard et al, 2019).

The Kauri Dieback Program was initiated in 2009 as a management tool, which includes standardized survey methods to determine the distribution of *P. agathidicida* in forests. Survey sites were selected based on high biodiversity, prioritizing culturally and ecologically significant Kauri. Risk assessments were also conducted to determine potential pathways of the pathogen for example fragmented forests, nurseries, Kauri plantations and sites of high soil disturbance (Bradshaw et al, 2020).

Detecting Kauri Dieback includes active surveillance such as soil sampling, aerial or ground based surveillance across regions and passive surveillance from public reports of symptomatic trees on private or public properties. Sampling considers the surrounding vegetation, hydrology of the site and proximity to known infections. Strict hygiene procedures are enforced and must be carried out while soil sampling due to high transmission potential and the shallow nature of fine feeder roots of Kauri (Beever et al, 2009). Due to the unknown lag-time of infection and lesion visibility, aerial surveillance alone does not suffice and soil sampling must be undertaken (Bradshaw et al, 2020).

Behavioural change

The behavioural change strategy focuses on education and creating awareness within communities and among target recreational user groups (*e.g.*, hunters, trampers, cyclists, conservation groups and scout groups). Kauri dieback is a recent occurrence and became a nuisance to communities that live within contaminated areas e.g., the community of Titirangi in the Waitākere Ranges. The Waitākere ranges are heavily infected with Kauri dieback (Figure 1.4). A combined decision between Te Kawerau a Maki (local iwi) and Auckland

council was made to place a Rāhui (temporary closure) on the Waitākere ranges in efforts to contain spread and allow for forest regeneration. The Waitākere ranges are a popular tourist and visitor attraction, especially over summer months. The placement of a Rāhui caused frustration within Titirangi and amongst tourist groups that could no longer visit the forest. Even after tracks were closed and fenced off, park rangers constantly repaired holes in the fences, made by those who did not agree with the closure. This highlighted the need for education and awareness, triggering a series of science talks and behavioural change projects.

A part of 'behaviour change' is researching strategies that have been successful in other contexts. For example, research from the USA found that the use of signage saying 'You are being watched' or 'Smile for the camera' in car parks and at bike stands were extremely important for compliance levels and reduced rates of theft. CTV footage revealed that standard 'barrel and grate' wash stations in the Waitākere ranges, had lower compliance than wash stations with 'airport level biosecurity' spray stations. Furthermore, park rangers observed forest visitors ignoring wash stations at the start of a track, especially if there was minimal or no signage. An observational study found that even when there was 100% compliance at wash stations, visitors often displayed incorrect compliance or partial compliance and were confused about instructions (Aley and MacDonald, 2018). Scrub, Spray, Stay is the 'easy to remember' slogan displayed on signage, along with general knowledge of Kauri dieback. Scrubbing shoes first was considered most important to encourage track users to clean shoes of soil rather than rely on sterigene disinfectant alone. Behaviours influencing incorrect compliance were 'sign blindness' and 'impatience'. Wash stations at popular tracks were often covered in mud, a factor that may have influenced compliance (Aley and MacDonald, 2018).

Kauri dieback ambassadors were trained and placed at the start of tracks to help educate visitors and encourage them to use wash stations correctly. Ambassadors were stationed at tracks that were popular and within close proximity to Kauri trees. There was 100% visitor compliance at tracks with ambassadors compared to tracks where there were none.

Scrub your shoes, spray your shoes and stay on the open tracks were the main messages echoed in biosecurity talks throughout primary and intermediate schools in Auckland. Children were encouraged to talk about their appreciation for the forest and some of the roles people play as kaitiaki to protect it from further degradation. Information of Kauri dieback was cycled throughout council organizations e.g., the botanical gardens, zoo's, libraries and at largescale outdoor events e.g., X trail, Kauri Karnival and Sculpture on the gulf. Michelle Hislop is a photographer based in Auckland who created a montage of Kauri dieback related

photographs. The project covered various ways Kauri dieback has affected people and their work. The project revolves around stories of people from different sectors such as forestry workers, community groups, recreational groups, iwi, scientists and artists. Photographs were suspended from trees in Albert Park, Auckland for summer months in 2019. This project aimed to educate viewers on Kauri dieback by eliciting emotion through true stories told by those who are most affected. Behaviour change is recognized as a slow-moving process but essential for the long-term management of Kauri dieback.

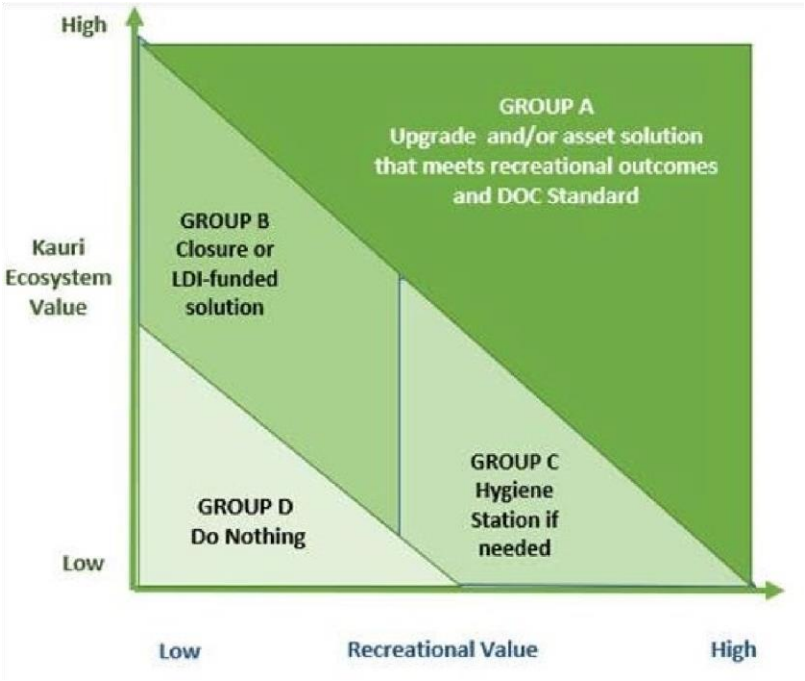
Infrastructure

Infrastructure includes the development and management of tracks and wash stations within Kauri lands. Tracks are upgraded by eliminating muddy areas (adding gravel) and installing boardwalks or geoweb on tracks to protect Kauri roots. In some cases, re-routing tracks to avoid Kauri roots is a better option, however, it is dependent on the location, popularity and proximity of Kauri to the track. Track closures occur when there is a high risk of spreading Kauri Dieback and are often permanent. Tracks that were prioritized for development or closure were determined using the track prioritization chart (Figure 1.5).

Cleaning stations are extremely important and visitor compliance was highly influenced by the wash station type (Aley and Mac Donald, 2018). An observational study was conducted by DoC to document people's behaviors when entering and leaving wash stations.

Two types of wash station were identified: a simple brush and grate, or a highly developed Mark II prototype which includes extensive signage (Figures 1.6 and 1.7).

The Mark II prototype was designed based on research of earlier designs and their compliance rates. It includes operational barriers and easy to use equipment that earlier designs lacked and studies found there was full compliance at Mark II prototypes (Aley and MacDonald, 2018). No observational studies documented compliance rates at old barrel and grate wash stations and there is no baseline data to compare Mark II prototype behavioral data. However, observations from Park rangers noticed a significant increase in compliance from earlier wash stations. By 2018, 20 sites had Mark II prototypes installed.



CATEGORY A	Parks with high or medium kauri ecosystem value AND high or medium recreational value that are prioritised for NETR funding for track/track network upgrade or asset solution. Temporary closure may be required until works can be implemented.
CATEGORY B	Parks with high or medium kauri ecosystem value BUT low recreational value where indefinite track closures would be appropriate. Local Board may choose to fund upgrades.
CATEGORY C	Parks with low kauri ecosystem value BUT high recreational value. Some protection may be needed, which could be provided in the form of Hygiene stations. Some track upgrades may be needed.
CATEGORY D	Parks with low kauri ecosystem value AND low recreational value. No action is recommended.

Figure 1.5 Track prioritization chart (Auckland council)

Figure 1.6 Barrel and grate wash station at Trounson park (Aley and MacDonald, 2018).

Figure 1.7. Mark II prototype wash station located on Hairamata track, Waikato. (Aley and MacDonald, 2018).

1.10 Knowledge gaps

P. agathidicida is a recently emerging pathogen and many knowledge gaps remain. For example, the plant host range of *P. agathidicida* is unknown. Its ability to infect and colonize other plants within Kauri forests, as a symptomless pathogen or a primary pathogen is also uncertain (Bradshaw et al, 2020). In other countries, multiple plant hosts play key ecological roles in the pathogen's epidemiology by acting as refugia or a sporulating host, terminal hosts or inoculum bridges (Crone et al, 2013). Lab tests show that oospore production in pine forest soils was higher than in Kauri forest soils, suggesting pine soils may act as potential reservoirs of *P. agathidicida* (Lewis, et al, 2019). In lab results also reveal that successful mycelial colonization of indigenous plants Rewarewa (*Knightia excelsa)* and Mingimingi *(Leucopogon fasciculatus)* (Ryder 2016). Other exotic species are also susceptible to mycelial inhabitation (Lewis 2018). Research into forest infections are ongoing however early predictions suggest *P. agathidicida* may infect Kauri related species Tānekaha (*Phylloclaydus trichominoides*) (Ryder, 2016). Agathis robusta – an Australian kauri – is not susceptible to *P. agathidicida* as glasshouse experiments suggest, however other agathis species have not been tested and susceptibility is therefore unknown (Ryder, 2016).

Effective procedures around wash station etiquette to limit *P. agathidicida* dispersal is an area that needs to be explored. 'How to' effectively discard Sterigene waste after visitor use is a concern for park rangers.

1.11 Thesis aims

Kauri are significant tīpuna in te āo Māori and are a major icon to Northland Iwi, recreational groups and communities. Kauri dieback is, therefore, a threat to Māoridom, New Zealand's cultural heritage, the forestry economy and forest biodiversity. In addition to current Kauri dieback management plans, more research is needed to contain and limit the spread of *P. agathidicida.*

The focus of my thesis research was to explore potential treatments that may be effective against *P. agathidicida.* In Chapter 3, I assess the efficacy of a range of commercially available disinfectants (Virkon, Napisan, Bleach, Ethanol, Sterigene and Trigene) against *P. agathidicida* oospores.

In Chapter 4, I explore potential Mātauranga-guided solutions. The main treatment for *P. agathidicida* infected Kauri are phosphite injections. The use of chemicals on tīpuna Kauri is frowned upon by many Māori. Iwi prefer traditional natural remedies to heal the environment. One of the branches of rongoā Māori is the production of plant remedies.
Chapter 4 explores Kānuka extracts and essential oils; and their efficacy against *P. agathidicida* oospores.

Chapter 5 touches on a 'Māori view' of the taiāo (environment) and its inhabitants. Conservation management that combines indigenous knowledge and western science has proven effective in many cases around the world. Overall, this thesis attempts to bridge the gap between Mātauranga Māori and western science to protect tāonga Kauri from extinction.

Chapter 2
Materials and Methods

2.1 Materials

Chemicals: Type I Ultrapure water was used for the preparation of all media and chemicals, unless noted otherwise. Water and/or media were sterilized by autoclaving at 121°C and 15 psi for 20 min. Chemical stock solutions were prepared in water and sterile filtered using 0.22 µm filters, unless otherwise noted. Chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) unless stated otherwise.

Isolate: *P. agathidicida* isolate 3770 was provided by Scion, Rotorua, New Zealand. This isolate was originally sourced from Great Barrier Island in 2006 from the host plant *Agathis autralis* (Studholme et al, 2016).

Plant extracts: Two sets of Kānuka were harvested, processed and tested separately. Kānuka (*Kunzea ericoides*) samples from plants of different ages were collected from Waima, New

Zealand (September 2019) by matua Ian Mitchell (Te Uri Taniwha, Ngāpuhi). Methanolic extracts of the samples were prepared by Dr. Jonathan Singh (Ferrier Institute, VUW) and fractionated according to hydrophobicity using acetonitrile (Table 1). The Kānuka fractions were tested in oospore viability assays.

Rongoā kānuka oil (KK oil) was also tested for inhibition of mycelial growth. The KK oil was produced by Hone Ratana from Kānuka harvested in Northland, Hokianga New Zealand on private property. The method used for preparation of this rongoā is the intellectual property of matua Hone Ratana. Those who wish to reproduce KK oil must contact him. Briefly, Kānuka leaves (150 kg) were heated and reduced in a 150 L homemade distillation apparatus, then re-distilled in a 25 L distillation apparatus for 2-6 h. The resulting KK oil was stored at 4 °C.

Table 1: Kānuka extracts from chemical fractionation

Sample Name	Mass (mg)	Type	Parent fraction	Comments
01A	5	Extract	n/a	Extract of young kanuka (4 yo)
01B	5	Extract	n/a	Extract of mature kanuka (40 yo)
03A	5	Fraction	01A	20% acetone/water fraction from young kanuka
03B	5	Fraction	01A	40% acetone/water fraction from young kanuka
03C	5	Fraction	01A	60% acetone/water fraction from young kanuka
03D	5	Fraction	01A	80% acetone/water fraction from young kanuka
03E	5	Fraction	01A	100% acetone fraction from young kanuka
04A	5	Fraction	01B	20% acetone/water fraction from mature kanuka
04B	5	Fraction	01B	40% acetone/water fraction from mature kanuka
04C	5	Fraction	01B	60% acetone/water fraction from mature kanuka
04D	5	Fraction	01B	80% acetone/water fraction from mature kanuka
04E	5	Fraction	01B	100% acetone fraction from mature kanuka
2212	5	Extract	n/a	Extract of young kanuka root (4 yo)
2210	5	Extract	n/a	Extract of mature kanuka root (40 yo)

2.2 Preparation of media

2.2.1 Clarified V8 broth and agar

To prepare clarified V8 (cV8) broth, 200 mL of Campbell's Original V8 juice was diluted with 300 mL of water and 2 g of calcium carbonate ($CaCO_2$) was added to adjust the pH to ~7. The mixture was centrifuged at 5000 × g for 10 min and the supernatant was filtered through a Whatman filter (Grade 1) to clarify the solution (cV8). Water was then added to a final volume of 1 L. The resulting clarified 20% (v/v) broth was then sterilized by autoclaving (Jeffers, 2006).

When preparing cV8 agar plates, agar was added at 15 g/L to the cV8 broth prior to autoclaving. After pouring, the plates were left to solidify overnight then stored at 4 °C.

2.2.2 PARP agar

This media is used for the selective isolation of *Phytophthora* from plant tissues (Jeffers and Martin, 1986; Ferguson and Jeffers, 1999). To prepare PARP agar, 17 g of pre-formulated cornmeal agar was mixed in 1 L of water. The media was autoclaved and then cooled 15-20 min before adding the antibiotics (Table 2). The agar plates were then poured and left overnight to solidify before storing in the dark at 4 C.

Table 2: Components of PARP agar.

Component	Final Concentration
Cornmeal agar	17 g/L
Ampicillin (500 mg/mL)	250 µg/mL
Rifampicin (20 mg/mL)[1]	10 µg/mL
Pimaricin (2.5% w/v)[2]	10 µg/mL
Pentachloronitrobenzene (40 mg/mL)[1]	100 µg/mL

[1]prepared in 100% DMSO.

[2]purchased as a 2.5% (w/v) aqueous solution (Sigma Aldrich).

2.2.3. Potato Dextrose Agar

Potato dextrose agar (PDA) was prepared per manufacturer's instructions (BD Difco) at 39 g/L and autoclaved.

2.2.4 Carrot Broth

To make 10% (w/v) solution of carrot broth, 50 g of frozen carrots were blended with 200 mL water using a standard kitchen blender. The resulting mixture was passed through four layers of muslin cloth to remove the bulk of the pulp, and then through Whatman filter paper (Grade 1) to further clarify the solution. The clarified broth was diluted with water to 500 mL (for a final concentration of 10% (w/v) and sterilized by autoclaving (Armstrong, 2018).

2.3 Routine culturing of *P. agathidicida*

P. agathidicida was routinely cultured as mycelia at 22 °C in darkness on cV8 agar. To maintain *P. agathidicida* cultures, a 5 mm plug of mycelial growth was placed in the center of a fresh cV8 agar plate and incubated at 22 °C in the dark for 5-7 d. This process was repeated a maximum of nine times. After nine passages on cV8 agar plates, the *P. agathidicida* culture was passaged through plant tissue (*i.e.*, pear) to preserve pathogenicity (Erwin and Ribeiro, 1996). For pear passaging, a 5 mm circular incision was made approximately $^1/_3$ from the bottom of a fresh pear. A plug of *P. agathidicida* mycelial growth was then placed in the pear incision. The incision was covered in plastic-wrap and the pear was incubated at 22 °C in the dark for 7 d. *P. agathidicida* was then re-isolated from the plant tissue by existing a ~5 mm plug from the leading edge of infection and placing the excised plant tissue on PARP agar (Jeffers and Martin, 1986). The PARP plates were incubated at 22 °C in the dark for 5-7 d. After incubation, a 5 mm plug was taken from the leading edge of the mycelial growth and transferred to a fresh cV8 plate, thus restarting the culturing cycle.

2.4 Oospore production

For oospore production, three plugs were taken from the leading edge of a mycelial mat, using aseptic technique and placed into a petri dish containing 15 mL of 10% (w/v) clarified carrot broth. For a typical purification, three dishes (nine plugs) were prepared in order to produce sufficient oospores. The dishes were incubated at 22 °C in the dark for 4-6 weeks.

After 4-6 weeks, the resulting mats of mycelia and oospores were transferred to 50 mL tubes and resuspended in 40 mL of sterile water. The suspension was homogenized for 2 min (Tissue homogeniser DS-160, DLAB) then sonicated at 20% amplitude for 1 min (500-watt Ultrasonic Cell Disruptor with a 5 mm probe, Sonics & Materials, Inc.). The resulting solution was then filtered through a 100 µm EASYstrainer filter (Greiner Bio-One), then a 40 µm EASYstrainer filter (Greiner Bio-One) to separate the oospores from mycelia. The filtered oospore solution was then centrifuged for 15 min at 22 °C and 1200 × g, to form an oospore pellet. Roughly 35 mL of supernatant was removed, leaving 5 mL remaining. The purified oospores were observed using brightfield microscopy on an Olympus CKX53 inverted light microscope. Final oospore concentrations were determined using 2-chip disposable hemocytometers (Bulldog Bio). Oospores were stored at 4 °C in the dark.

2.5 Oospore viability staining

Fluorescein diacetate (FDA) came as a 10 mM stock solution in DMSO and was stored at 4 ºC. We used small 10 µL aliquots and limited freeze-thawing to three times. Working stocks were prepared by diluting the FDA stock to 200 µM in sterile water immediately prior to use. TOTO-3 iodide came as a 1 mM stock solution dissolved in DMSO. It was diluted to the working concentration of 20 µM in sterile water. Aliquots were stored at 4 ºC.

For viability staining, oospores were first stained with 10 µL of (20x) (FDA) and incubated at 37°C for 20 h. Then, 10 µL of 20 µM TOTO-3 iodide was added to sample and incubated for a further 4 h at 37 °C.

2.6 Fluorescence microscopy and image analysis

Stained oospores were imaged using a fluorescence microscope (Olympus BX63) at 4× magnification. Oospores were first imaged in brightfield illumination mode, then imaged using the green (FITC: excitation 475-495 nm, emission 515-545 nm) and red (Texas Red: excitation 540-580 nm, emission 590-630 nm) fluorescence filters. The exposure times used were as follows: bright field = 65 µs, FITC = 50 – 75 ms and Texas Red = 100 – 150 ms.

The images were analyzed and oospores counted using CellProfiler v3.1.8 (Laux et al, 2020). Oospores were counted as viable if they fluoresced in the green channel, counted as nonviable

if they fluoresced in the red channel, and counted as damaged if they fluoresced in both channels. Graphs were prepared using Prism Graphpad (Version 7.0).

2.7 Testing of oospore treatments

2.7.1 Kānuka fractionate testing

Kānuka fractions were re-suspended in 500 µL of 100% ethanol to obtain 10 mg/ml stock solutions. 10 µL of stock solution was pipetted into 990 µL of sterile water giving a final concentration of 10 µL/ml. Each diluted sample was passed through 0.22µm filters to clear it of plant debris. Ethanol was evaporated off Kānuka samples (1.5 mm tubes) by leaving them under a fume hood for 48 h. Extracts were then re-suspended in 500 µL sterile water and a working stock solution was made following the methods above.

For the viability assays, 10 µL of purified oospores were added to 90 µL of treatment. Viable and non-viable controls were included: each contained 90 µL of water instead of treatment. For the non-viable control, the oospores were heated at 98 °C for 1 h to kill all oospores.

The treated samples were incubated at 37 °C for 24 h then centrifuged at 1200 × g for 10 min. The supernatant was carefully removed from the oospore pellet. Angling the pipette above the oospore pellet near the corner of the tube helped avoid accidentally removing the pellet.

2.7.2 Disinfecting testing

Commercially available disinfectants were diluted in water to their recommended working concentrations (Table 2).

Table 2: Recommended disinfectant concentrations

Disinfectant	**Commercially recommended concentration**
Rely+On™ Virkon™	1% (w/v)
Napisan Vanish oxi gold™	0.5% (w/v)
Bleach	2% (v/v)

Denatured absolute alcohol P3 (ethanol)	70% (v/v)
Sterigene™	2% (v/v)
TriGene™	2% (v/v)

2.8 Mycelial growth inhibition assays

To assess mycelial inhibition, four sterile filter paper discs (Whatman Grade 1) were evenly spaced (using sterile tweezers) around the perimeter of a potato-dextrose agar plate 2 controls and 2 treatment discs. Accurate positioning of discs was achieved by placing a template underneath the agar plate. 5 µL of KK oil was pipetted onto treatment discs and sterile water onto control discs. Using aseptic technique, a plug was taken from the leading edge of mycelial growth and placed in the middle of the plate. The plates were incubated in the dark for 6 d at 22 °C. Plates observed on a light box. Mycelial growth was measured using a ruler and confirmed using Image J. Measurements were taken from the plug to the edge of mycelial growth.

Chapter 3
Testing commercial disinfectants against *P. agathidicida* oospores

3.1 Introduction

Cleaning footwear, vehicles, tools, equipment and machinery is the single most important management action available apart from avoiding infected areas entirely. Viable *P. agathidicida* present in mud and soil can be easily transferred on footwear, tires, hiking poles, *etc* to uninfected Kauri areas. At track entrances, wash station signage encourages visitors to scrub their shoes to rid them of soil or plant matter. Once clean, visitors are then supposed to spray shoes and any hiking equipment with 2% Sterigene or Trigene disinfectant. Sterigene and Trigene are both mixtures of halogenated tertiary amines. However, each has slightly different formulations. They are both considered broad-range, biodegradable disinfectants that are recommended by many regional councils. Sterigene/Trigene have also been used previously in the horticultural and agricultural sectors (Bellgard et al, 2018).

Unfortunately, Sterigene/Trigene is not effective against all of the life stages of *P. agathidicida*: it is known to be ineffective against oospores (Bellgard et al, 2010). Therefore, people travelling between Kauri stalls may unintentionally spread oospores, regardless of their compliance at wash stations.

Research into effective alternatives is therefore urgently required. The aim of the research in this chapter was to test a range of commercially available disinfectants against *P. agathidicida* oospores. The disinfectants were tested using oospore viability staining combined with fluorescence microscopy. The disinfectants chosen for testing were Sterigene, Trigene, Napisan Vanish Oxi, Virkon S, bleach and ethanol. Trigene was included for direct comparison with the previous study which used different methodologies. The composition and properties of these disinfectants are reviewed below.

3.1.1 Disinfectants

Disinfectant information has been sourced from the material safety data sheets (MSDSs) and primary literature.

Sterigene/Trigene

Sterigene and Trigene are broad-range disinfectants that are effective against a variety of bacteria, fungi and viruses. They are both based on a mixture of halogenated tertiary amine and organic salts. Trigene is a reformulation of Sterigene: the primary active ingredients are the same, however, the concentrations of the active ingredients and overall formulation differ as shown in Table 3.

Table 3. A comparison of the chemical composition of Sterigene versus Trigene.

Sterigene	**Trigene**
Alkyl (C12-16) Dimethylbenzylammonium Chloride (CAS 68424-85-1) 1-10%	Alkyl (C12-16) Dimethylbenzylammonium Chloride (CAS no. 68424-85-1) < 15%
Polymeric Biguanide hydrochloride (CAS 27083-27-8) 1-10%	Polymeric Biguanide Hydrochloride (CAS no. 27083-27-8) < 2%
Didecyldimethylammonium chloride (CAS 7173-51-5) 1-10%	Didecyldimethylammonium chloride (Cas no. 7173-51-5) < 5%
C9-C11 Alcohol Ethoxylate (CAS 68439-46-3) 1-10%	Sulphamic Acid (Cas no. 5329-14-6) < 5%
Propan-2-OL (CAS 67-63-0) 1-10%	Nonoxinol (Cas no. 26571-11-9) < 10%
	Demineralized water, solubilizes balance

Sterigene/Trigene are non-flammable and soluble in water. They are slightly acidic with a pH range of 4.5-6.5 at the recommended working concentration.

Trigene has been previously tested as a potential disinfectant for use against *P. agathidicida* due to its broad anti-microbial activity and minimal harm to equipment when used as directed. This previous study conducted by Bellgard et al (2010) reported that Trigene significantly inhibited *P. agathidicida* mycelial growth. Trigene also proved lethal against zoospores (Bellgard et al, 2010). Trigene had little effect on oospores with ~ 90% still viable after 10 days of incubation (Bellgard et al, 2010).

Ecological information and toxicity

Sterigene and Trigene are considered relatively non-toxic, with low risk to eye, inhalation and ingestion. They are also biodegradable with limited bioaccumulation potential. However, they can be harmful to aquatic organisms with long-term adverse effects in the aquatic environment.

Virkon

Virkon is a broad range disinfectant that is effective against viruses, bacteria and fungi. The primary active ingredient in Virkon is potassium peroxymonosulfate. Virkon is recommended for use as a hard surface disinfectant and is commonly used in laboratories, on farms, and in veterinary hospitals. The recommended retail price is ~ $300 for 5 kg of powder. When used on non-porous surfaces such as wheels, boots, and agricultural equipment, soil debris must be removed before disinfection.

Previous studies have found that 0.25, 0.5, 1 and 2% Virkon were effective against zoospore viability of *P.capsisci* and *P.agathadicida* (Cerkauskas et al, 2015). Virkon also prevented root rot on ornamental plants caused by *P.nicotianae* (Oneil and Pye, 2000; Cerkaukas et al, 2015). Virkon (at 10 % v/v), significantly reduced *P.agathidicida* oospore viability (~75%) after 10 days of incubation (Bellgard et al, 2010). A potential limitation in the use of Virkon is that it is corrosive to metals if left to soak longer than 10 minutes. Another limitation is that once diluted the product lasts only seven days.

Ecological and environmental toxicity

1% Virkon solution in water has a pH of ~2.4 – 2.7. It is classified as hazardous and harmful if swallowed or inhaled. It causes skin irritation, serious eye damage and should not be allowed to enter drains, watercourses or the soil as it is toxic to aquatic fish. According to available MSDS, Virkon is biodegradable.

The full composition of Virkon:

- 45-55 % Potassium hydrogen monopersulfate (CAS no. 70693-62-8)
- 10-12% Sodium C10-13-alkylbenzenesulfonate (CAS no. 68411-30-3)
- 7-10% Malic acid (CAS no. 6915-15-7)
- 4-6 % Sulphamidic acid (CAS no. 5329-14-6)

Virkon also contains the following ingredients at <5%:

- 1-5% Sodium toluenesulphonate (CAS no. 12068-03-0)
- <3% Dipotassium peroxodisulphate (CAS no. 7727-21-1)
- <0.25% Dipentene (CAS no. 138-86-3)

Bleach

Chemical and physical properties

Bleach (sodium hypochlorite solution, CAS 7681-52-9) is a clear alkaline liquid disinfectant which is effective against most bacteria, viruses and fungi. Hospital-grade bleach costs ~ $60 per 20L, making it a very affordable and effective option for disinfection. However, it is not recommended for use on colored or sensitive garments as it causes streaking, fading and other deterioration. The previous study by Bellgard et al (2010) found that bleach (Janola) at 5 %, completely suppressed mycelia and zoospores, but only reduced (not eliminated) oospore viability after 10 days of incubation.

Toxicity and ecological information

If left on the skin for too long, concentrated bleach can cause deep chemical burns (Lutrell, 2001); it will also cause burns on the mouth, mucous membranes and can lead to death if ingested. Environmental precautions must be taken to avoid discharge into drains, watercourses or onto soil. Bleach is not environmentally friendly and may cause great harm to natural biota, flora and fauna.

Ethanol

Chemical and physical properties

Ethanol (ethyl alcohol, CAS no. 64-17-5) is a colorless, transparent, volatile liquid. It is miscible in water, has a pungent taste and highly flammable. Ethanol is slightly basic and almost neutral pH of 7.5. It is irritating to mucous membranes and the respiratory tract. Ethanol has not been previously tested as a disinfectant for *P. agathidicida*.

Ecological information and toxicity

May cause nausea, headache, dizziness and vomiting. May cause eye irritation. Ethanol is toxic to many aquatic organisms in high concentrations. However, it is readily biodegradable with a low probability of bioaccumulation.

Napisan Vanish Oxi Gold

Chemical and physical properties

The active ingredient in Napisan is sodium percarbonate. It is alkaline, with a pH 10 – 11.3. Napisan is sold as an 'oxygen bleach' and is safe to use on everyday fabrics such as cotton and polycotton, colors and whites. It costs ~ $10 for 1.8 kg. Napisan and/or other oxygen bleaches have not been previously tested as potential disinfectants for *P. agathidicida*.

Ecological information and toxicity

In general, based on available data there are no known significant effects or critical hazards to chronic health, except that it causes serious eye damage and eye irritation. Napisan is classified as biodegradable. However, environmental precautions should be taken to avoid dispersal of spilt material, runoff, and contact with soil, waterways, drains and sewers.

3.3 Results

3.3.1 Initial viability screening of disinfectant-treated oospores.

All disinfectants were tested at their recommended concentrations (per manufacturer instructions). Purified oospores were treated with disinfectants for 24 hours, dual-stained for viability, then assessed using fluorescence microscopy. For oospore viability staining, a combination of fluorescein diacetate (live cell stain) and TOTO-3 iodide (dead cell stain) was used. Fluorescein diacetate is a non-fluorescent esterase substrate that can permeate cell membranes. In viable cells, fluorescein diacetate is hydrolyzed by intracellular esterase's to produce the green fluorescent compound fluorescein. The product – fluorescein – is not cell permeable and therefore accumulates in viable cells causing them to fluoresce green. TOTO3 is cell-impermeant and non-fluorescent in the absence of nucleic acids. In dead cells with compromised membranes, it generates a very bright red fluorescent signal upon binding to DNA. Weakened oospores appear to be stained by both fluorescein diacetate and TOTO-3 iodide. Damaged oospores may have a weakened cell wall, allowing TOTO-3 iodide to stain them red. However, they are still metabolically active, allowing for fluorescein diacetate to stain them green. Two independently cultured and purified batches were tested (each also in technical triplicate): the first batch (TNA11) were purified in early February; the second batch (TNA11.1) were purified in June. Oospore images were analyzed using CellProfiler, and the percentages of live, dead or weakened oospores post-treatment are reported below.

The results from the first batch of oospores (TNA11) are shown in Figure 3.1. Of the disinfectants tested (Napisan, ethanol, bleach and Virkon) all appeared highly effective against oospores. Of these, the most effective were bleach and Virkon, with no viable or weakened oospores remaining post-treatment. Ethanol and Napisan also showed promising results, with the majority of oospores either weakened or non-viable. In comparison, Trigene was not effective against *P. agathidicida* oospores (66% remained viable, 24% weakened, only 10% non-viable). Overall, the results with Trigene were similar to the untreated negative control (water-only treatment; 72% viable, 12% weakened, 6% non-viable). A maximum of 50 oospore cells were counted over 3 microscopy slides.

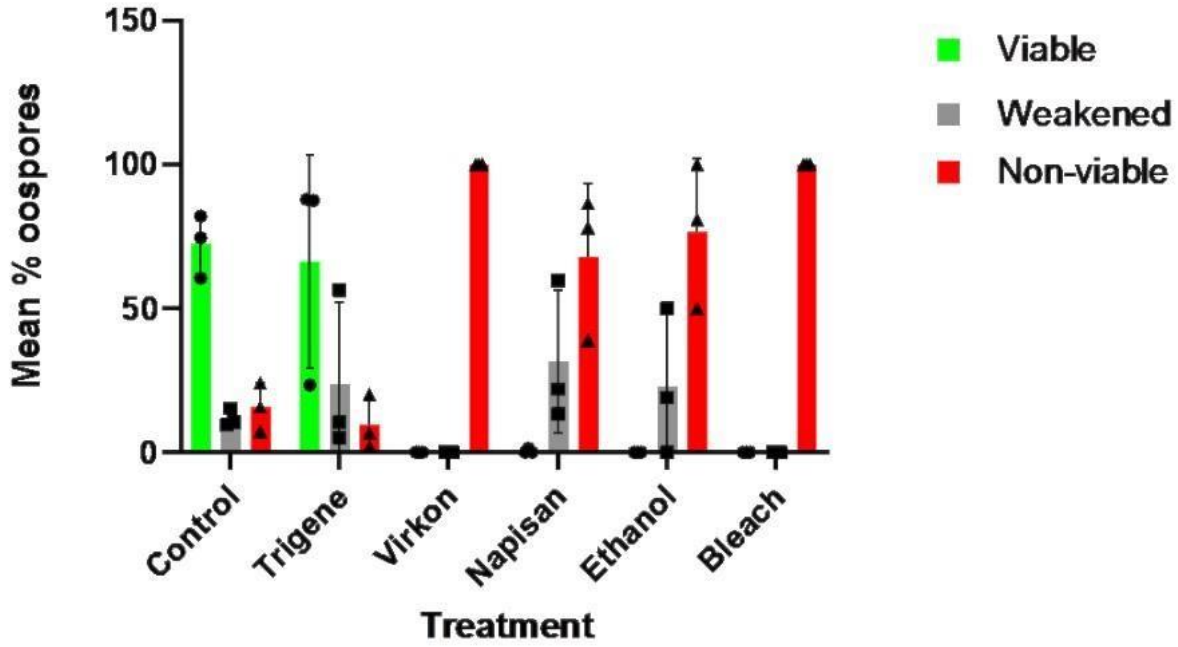


Figure 3.1. Viability *of P. agathidicida* oospores (TNA11) post-treatment with commercially available disinfectants. Each bar represents the percentage of oospores that were viable (green), non-viable (red) or weakened (grey) post-treatment with either a disinfectant or water (as the negative, untreated control. Symbols represent the individual technical replicates (3) for each disinfectant. Error bars show the range of variation between replicates.

Figure 3.1 appears to show that Napisan-treated oospores were predominantly non-viable and/or weakened. However, during the viability staining protocol, it was observed that Napisan appeared to interact with fluorescein diacetate. A rapid color change was observed upon addition of fluorescein diacetate that was not observed in the other samples (Figure 3.2). This potentially could lead to misestimation of viable cell counts, thus the Napisan results should be treated with caution.

Figure 3.2. The observed color-change in Napisan-treated oospores upon addition of fluorescein diacetate. Typically, transparent before the addition of fluorescein diacetate

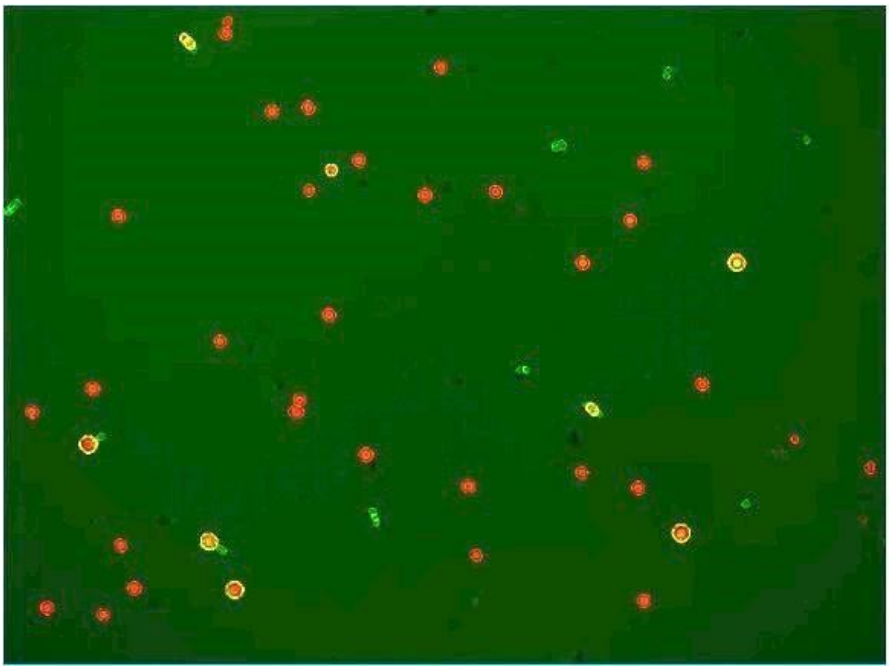


Figure 3.3 A merged CellProfiler image of representative TNA11 data. Green outlines around background debris were counted as viable oospores by CellProfiler. This highlights the need to cross-check the automated CellProfiler analyses.

The fluorescence microscopy images that were analyzed with CellProfiler were also hand counted and cross-checked with data outputs for each disinfectant. A small amount of background debris was noted as being miscounted as viable oospores, as shown in Figure 3.3. However, in general, the outputs generated by the cell counting software CellProfiler appeared accurate. Due to the uncertainty around the Napisan data, an extra wash step was included in subsequent trials to remove the disinfectants completely before the stains were added.

3.3.2 Viability screening of disinfectant-treated oospores (revised protocol). A second batch of oospores was independently grown and purified (TNA11.1). Overall, the viability of TNA11.1 oospore was comparable to the first batch of purified oospores (TNA11). The untreated (negative control) TNA11 oospores were 72% viable (Figure 3.1). In comparison, the TNA11.1 had 83% viable oospores (Figure 3.4).

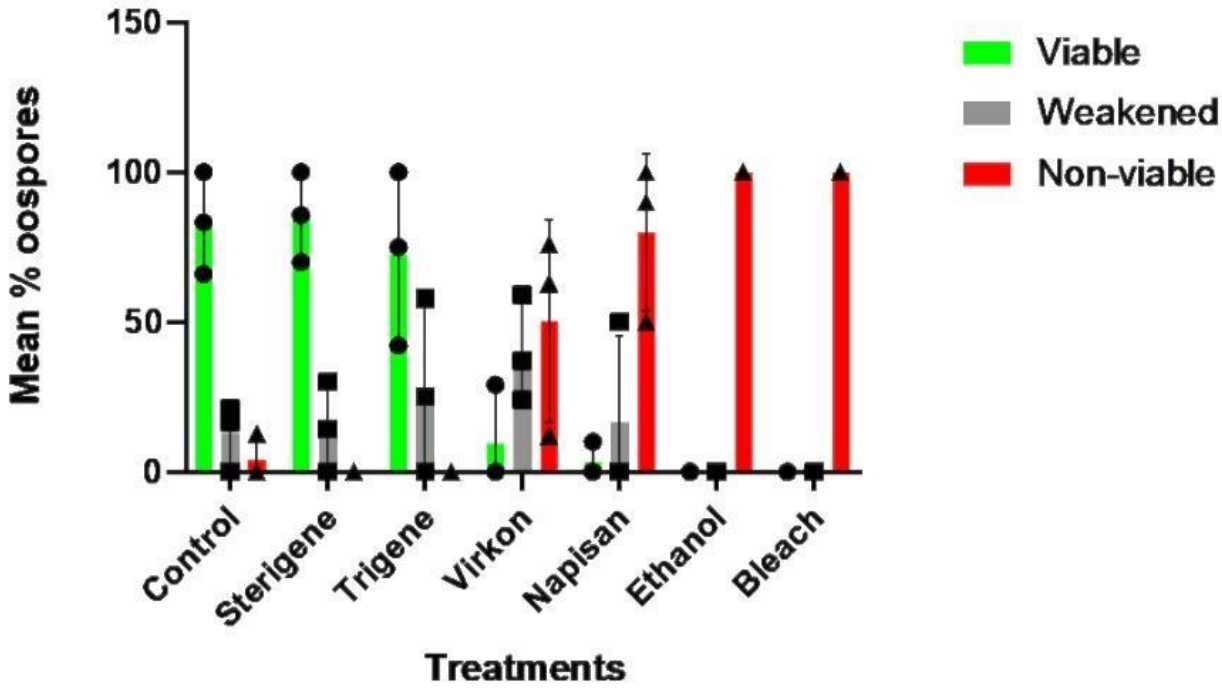


Figure 3.4. Viability *of P. agathidicida* oospores (TNA11.1) post-treatment with commercially available disinfectants, including Sterigene. Each bar represents the percentage of oospores that were viable (green), non-viable (red) or weakened (grey) post-treatment with either a disinfectant or water (as the negative, untreated control. Symbols represent the individual technical replicates (3) for each disinfectant. Error bars show the range of variation between replicates.

The new batch of purified oospores was treated with the disinfectants, dual-stained for viability, then assessed using fluorescence microscopy. In these experiments, a second wash step was added to ensure complete removal of disinfectants prior to staining. Sterigene was also included as a comparison with Trigene.

The results of disinfectant treatments with batch TNA11.1 are shown in Figure 3.4. Overall, similar results were obtained to TNA11. Again, ethanol and bleach were highly effective,

resulting in 100% non-viable oospores post-treatment. Trigene remained largely ineffective, with results similar to the untreated controls: Trigene 72% viable, 28% weakened, 0% nonviable; untreated control, 83% viable, 13% weakened, 4% non-viable). Despite the formulation differences, Sterigene was also largely ineffective: 85% viable, 15% weakened, 0% non-viable. With the additional wash step included in the protocol, the percentage viable oospores differed for Virkon and Napisan. With the revised protocol, 10% of Virkon-treated oospores remained viable, with 50 % non-viable and 40% weakened. Napisan had 80% nonviability and 3.3 % viability (16.7% weakened).

3.3 Discussion

Oospores are the most difficult lifecycle stage of *P. agathidicida* to deactivate due to their thick cell wall that shields them from chemical treatments and/or environmental stresses (Bradshaw et al, 2020). This chapter has explored the efficacy of a range of commercial disinfectants against *P. agathidicida* oospores. My results confirm that Trigene and Sterigene are ineffective against oospores. My results also show that 2% bleach, 1% Virkon, and 70% ethanol all reduce oospore viability. Napisan also reduced oospore viability, but also interacted with the viability stains, therefore further investigations are needed. The suitability and limitations of these disinfectants for Kauri Dieback are discussed below.

In relation to Kauri dieback, bleach is already recommended as a secondary option (after Sterigene/Trigene) for disinfecting *P. agathidicida* on hard surfaces. Internationally, bleach has been recommended for cleaning equipment (*e.g.,* bikes, footwear) potentially contaminated by other *Phytophthora* species (Chastagner, 2010). It also successfully eradicates *P. agathidicida* mycelial growth and zoospores on hard surfaces in greenhouse and nursery settings (Kimberley and Dick, 2013). Bleach (at 5% v/v) could not completely suppress the germination of oospores. In this chapter, the efficacy of bleach as a disinfectant for *P. agathidicida* oospores was confirmed. However, in the field, its applications are limited because it corrodes metal tools, destroys soft textiles and bleaches clothing (Bellgard et al, 2019). Despite these limitations, bleach may prove useful as a surface disinfectant for *P. agathidicida* in laboratories, nurseries and other controlled settings.

Virkon also had some efficacy against *P. agathidicida* oospores across both experiments. My results showed Virkon deactivated 100% oospores in TNA11 and 50% oospores in TNA11.1. This is consistent with previous results Bellgard et al (2010). However, similar to bleach,

Virkon has limited use in the field due to its corrosive nature, bleaching of clothing, and short active timeframe (*i.e.*, must be replaced every seven days).

Ethanol (at 70% v/v) deactivated >75% of oospores across both experiments. While not currently recommended as a disinfectant for Kauri Dieback, 70% ethanol is commonly recommended for sterilization procedures and is a commonly used disinfectant in laboratories. To be effective as a disinfectant, the concentration of ethanol is critical, with 70% being more effective than 100% ethanol (Haider, 2012). Alcohol-based hand sanitizers with at least 70% ethanol could serve as an option for pest trapping and conservation groups to disinfect hands after handling Kauri infested soils. However, the minimum exposure time of ethanol needed, and the activities across other lifecycle stages need to be established.

Napisan (at 0.5% w/v) also appeared effective against oospores, but the results were more variable, and more validation needs to be completed. Napisan is an oxygen-based bleach that is safe to use on clothes, soft textiles and is easily accessible by the general public. Future work should focus on validating these results, as well as testing the efficacy of Napisan against other life stages of *P. agathidicida*

Combined, my results with bleach and Napisan suggest that alkaline disinfectants may be a promising avenue for further research. These results are consistent with previous studies that have suggested that pH-based alkaline solution can deactivate *P. agathidicida* oospores (Kimberley and Dick, 2013). Kimberely and Dick (2013) found that oospore exposure to alkaline-based solutions pH 9 for 24 hours decreased the percentage of viable oospores considerably, and completely deactivated them after 48 hours (Kimberely and Dick 2013). Bleach has a pH of 12 and produced 100% non-viability of *P. agathidicida* oospores across both experiments. Napisan also has a pH of 10-11 and also decreased oospore viability.

3.4 Implications and future work

In summary, this chapter has explored the efficacy of a range of commercial disinfectants against *P. agathidicida* oospores. Currently, most wash stations in New Zealand are filled with Sterigene/Trigene. Standard operating procedures for soil sampling, tree pruning, event planning and best practice guidelines when operating in Kauri lands recommend the use of Trigene or Sterigene disinfectant. Hygiene procedures on the 'Keep Kauri standing' website also recommend the use of Trigene Advance II on shoes, equipment and machinery. However, the results of this study confirm that Sterigene and/or Trigene are not effective against *P. agathidicida* oospores. These results have significant practical implications. Many user groups continue to operate in infected Kauri lands including arborists, pest management

operators, private landowners, contracted pig hunters, park rangers and certified volunteer/community groups. The likelihood of accidentally spreading *P. agathidicida* by the movement of soil from one infected stall to another is therefore high amongst these groups. The Kauri Dieback Program supply Trigene vehicle kits to these groups and the general public. Results from this work highlight the need for a more efficient solution. Research into disinfectants that are effective against all life stages of *P. agathidicida* must be explored and better options recommended in Kauri Dieback standard operating procedures. Additional research is also required to test the efficacy of potential disinfectants on different surfaces *e.g.* clothing, hard surfaces, soil etc.

Chapter 4
Further investigations into the efficacy of Kānuka extracts against *P. agathidicida*

4.1 Introduction

4.1.1 Ko au ko te whenua, ko te whenua ko au

The essence behind this whakatauki (Māori proverb) is difficult to fully encompass in the English language, where ihi, wehi, wairua and mana are lost with translation. This is a famous whakatauki that states; I am the land and the land is me. Also explained as; the environment that revitalizes and brings sustenance to the people must also be maintained and sustained by the people. This whakatauki brings insight into a traditional Māori world view of kaitiakitanga. Descendants of the land here to cultivate and steward it.

Rongoā Māori (Māori medicine) extends far beyond plant extracts alone and branches deep into the metaphysical realms of Rongomatāne (guardian of peace and cultivation) (Nepe, 1991). Rongoā Māori is deeply embedded in the concepts of karakia, mauri and tapū. Therefore, in this thesis, the term rongoā denotes only to the natural plant medicine, referred and preferred by Māori. For generations, Tohunga (Māori healers) used native plants in their practices as they were well aware of their healing properties. Due to the Tohunga Suppression Act of 1907 much Mātauranga and rongoā Māori was lost (Aichle, 2016). In modern times, Mātauranga Māori is increasingly becoming acknowledged in health and environmental sectors (Aichle, 2016) and rongoā provide natural alternatives to processed chemical solutions. These alternatives are highly favored by Māori (K. Prime 2019, pers comms, 7/2). Some commonly used rongoā are Harakeke (*Phormium tenax*), Kawakawa (*Piper excelsum*), Mānuka (*Leptospermum species*) and Kānuka (*Kunzea species*). The leaves and roots of these plants can be used to treat bruises, wounds and sores on the skin whilst also treating diarrhea, dysentery, stomach aches, toothaches and inflammation (Aichle, 2016).

There is a growing interest worldwide in the use of plant extracts, herbal remedies and essential oils as biocontrol's against unwanted pathogens, bacteria, microbes, viruses and fungal communities (Wyatt et al, 2005). Plants are also a potential source of anti-*Phytophthora* compounds. For example, extracts from cocoa butter plants from the Mexican Chihuahuan desert were found to be active against *P. cinnamomi* and have been suggested as a potential tool to protect avocado crops (Castillo-Reyes et al, 2015). Similarly, a combination of essential oils (cinnamon, clove, and mustard and cassia oil) positively reduced 99.9% of *P. nicotinae* populations in soil after 21 days (Bowers and Locke, 2004). Lavender and rosemary plant extract also proved promising at reducing zoospore germination of several

Phytophthora species (Widmer and Laurent, 2006). In a recent Mātauranga-guided study, Kānuka root and leaf extracts successfully inhibited *P. agathidicida* mycelial growth, zoospore motility, and zoospore germination (Lawrence et al, 2019).

Kānuka is common in Kauri stalls and provides refugia for young Kauri (Lloyd, 1960). Kānuka form open canopies with ferns occupying the forest floor. Kānuka are widely dispersed from the coast to altitudes of up to ~ 900 m (Tipa, 2013). Kānuka readily colonizes land after the event of a forest fire and has a southern limit up to Dunedin on the east coast of Te Waipounamu. On average Kānuka grow to be 15 m tall with a diameter of 60 cm (Tipa, 2013). Though Kānuka, in comparison to Kauri, are quite small, Māori have benefited from its numerous uses. Kānuka leaves served as rongoā and its hardwood as tools (Morris, 2011). For example, under ideal conditions, Kānuka can grow long and straight, making it suitable for spears. These spears are significantly stronger than other native wood and is reported to split Kauri wood (K. Waititi 2020, pers comms, 25/9) *He iti Kahikatoa pakaru rikiriki te Totara*. This whakatauki (Māori proverb) states that although Kānuka is small compared to other natives, it is strong and can split a Totara tree (Morris, 2011).

Kānuka was often mistaken for Mānuka and up to 1983 was classified as *Leptospermum,* the same genus as Mānuka (*Leptospermum*) (Tipa, 2013). Kānuka is now classified in the genus *Kunzea* (Tipa, 2013). However, it is worth noting that native speakers of Ngāpuhi dialect refer to this plant as mānuka – as its flowers and timber are white or mā. Kānuka and Mānuka nectar are both favored by bees and produce distinctive amber-colored honeys (Tipa, 2013). Herbal and medicinal properties of Mānuka and Kānuka have been found to be very similar in one study (Porter and Wilkins, 1998). However, the biochemical differences or similarities between these two plants is an area that requires further investigation.

4.1.2 Bioactive properties of Kānuka

Traditionally Māori boiled Kānuka leaves and drank it as a tea to help with kidney and liver function (Aichle, 2016). It was also used to clear sinuses, hay fever and asthma (H. Ratana 2020, pers comms, 7/9). Resin produced from Kānuka bark helped relieve coughing and burns on skin (Aichle, 2016). Green fruits were chewed to relieve stomach-ache and seeds were made into a poultice to treat sores (Tipa, 2013). Kānuka contains ‘leptospermone’ a bioactive chemical that acts as an insecticide, effective against intestinal parasites (Tipa, 2013). Stem distillations of Kānuka essential oil have also been found to have antibacterial, antifungal, spasmogenic, spasmolytic and anti-*Phytophthora* properties (Wyatt et al, 2005).

Antiviral phloroglucinol derivatives have also been isolated from Kānuka foliage material (Bloor, 1992). Other parts of the plant were found to have insecticidal activities (Khambay et al, 2003). The flavanoid compounds from Kānuka are known to be highly bioactive against two key lifecycle stages of *Phytophthora*: mycelial growth and zoospores (Lawrence et al, 2019). The effect of Kānuka extract or purified compounds has not been tested against *P. agathidicida* oospores.

4.1.3 Mātauranga and Kauri Dieback solutions

Lawrence et al (2019), demonstrated that Mātauranga Māori is essential for the control and management of Kauri dieback. Matauranga Māori provided guidance for the screening of Kānuka for anti-*Phytophthora* compounds. This chapter builds on the research from Lawrence et al (2019), which explored anti-*Phytophthora* activities against zoospores and mycelial growth – but not oospores. In this current study, Kānuka samples for chemical extraction were collected from Waimā, New Zealand (September 2019) by matua Ian Mitchell (Te Uri Taniwha, Ngāpuhi). Kānuka leaves used for the preparation of KK oil were collected from North Hokianga on privately owned land and the oil distilled by matua Hone Rātana (Ngai Tūhoe). Overall, the aim of this study was to further explore the efficacy of different Kānuka preparations against various lifecycle stages of *P. agathidicida*. The specific aims of this study were to:

1. Compare the efficacy of leaf and root extracts from young (~4 yo) versus mature Kānuka (~40 yo).
2. Assess the efficacy of distilled rongoā Kānuka oil (KK oil) on *P. agathidicida* mycelial growth.

4.2 Results

4.2.1 Efficacy of extracts from mature Kānuka versus young Kānuka

Crude ethanol extracts (leaf and root) from young Kānuka (~4 yo; Sample 01A) and mature Kānuka (~40 yo; Sample 01B) were tested for their effect on oospore viability. All leaf and root extracts from mature or young plants were shown to reduce oospore viability. However, the oospore viability post-treatment did differ slightly across the two independent biological replicates (*i.e.,* different batch of cultured and purified oospores). In the first replicate

(TNA10, Figure 4.2.1) there was little difference between the young versus mature Kānuka samples. However, this batch of oospores also showed low overall viability (Figure 4.2.1, untreated control). Therefore, a second batch of oospores was grown a purified, and the ethanol extracts re-tested.

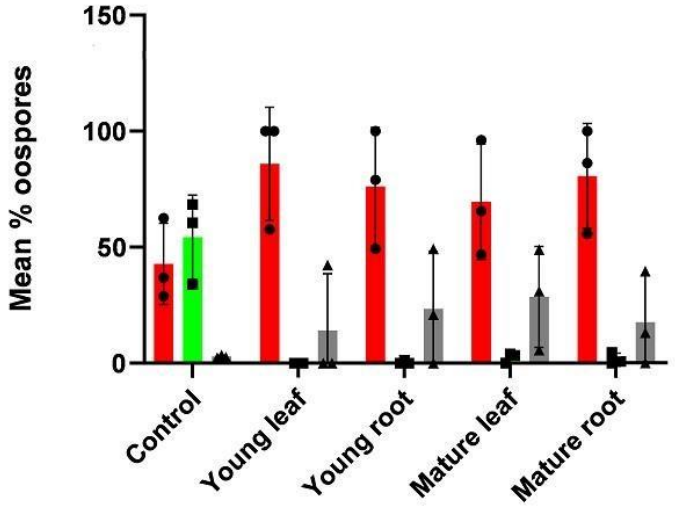


Figure 4.2.1: A comparison of young/mature and leaf/root Kānuka extracts (TNA10). A visual representation of data taken from Table 4.1. Bars show the average percentage of: viable (green), non-viable (red) and/or weakened (grey) oospores. Symbols represent the three technical replicates; bars represent the range.

In the second replicate treatment (TNA10.1, Figure 4.2.2), again all treatments dramatically reduced oospore viability. However, the extract of young Kānuka root was the only sample which resulted in completely non-viable (rather than weakened) oospores.

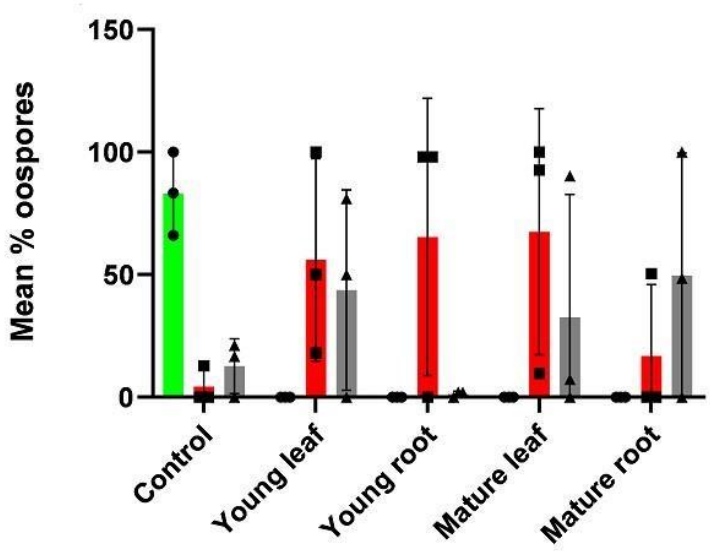


Figure 4.2.2: A comparison of young/mature and leaf/root Kānuka ethanolic extracts (TNA10.1). A visual representation of data taken from Table 4.2. Bars show the average percentage of: viable (green), non-viable (red) and/or weakened (grey) oospores. Symbols represent the three technical replicates; bars represent the range.

The Kānuka extracts tested in TNA10 and TNA10.1 contained 10% ethanol. In parallel to these assays, the results from my disinfectant testing showed that 70% ethanol treatment results in non-viable oospores (Chapter 3). This led to a suspicion that ethanol may have influenced the efficacy of Kānuka extracts in TNA10 and TNA10.1. Therefore, 10% ethanol was tested against *P. agathidicida* oospores to determine the effect of the solvent on oospore viability. The results of 10% ethanol treatment of oospores are shown in Figure 4.2.3. It was observed that 10% (v/v) ethanol also weakened oospores considerably, albeit less than the kānuka/ethanol extracts.

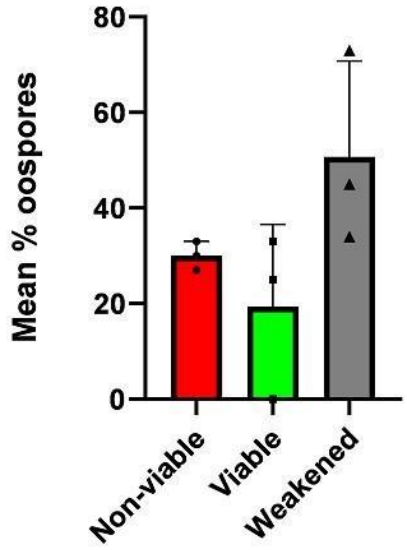


Figure 4.2.3 The effect of 10% ethanol on *P. agathidicida* oospore viability. The bars represent the average percentage of viable (green) non-viable (red) and weakened (grey) oospores after treatment with 10% ethanol. Graph represents one biological replicate and symbols represent each technical replicate (3). Error bars represent the range.

To eliminate any confounding effect of the solvent, Kānuka extracts were therefore dried down, re-suspended in water, and tested again in TNA10.2.

Overall, the water extracts were less effective at reducing oospore viability than the ethanol extracts. While the water extracted Kānuka samples both deactivated oospores to some

degree, the majority of oospores remained weakened or active (Figure 4.2.4). However, young Kānuka was again the most effective with 63% non-viable oospores post-treatment, whereas no non-viable oospores were observed in the mature Kānuka water extract treated samples (TNA10.2, Figure 4.2.4).

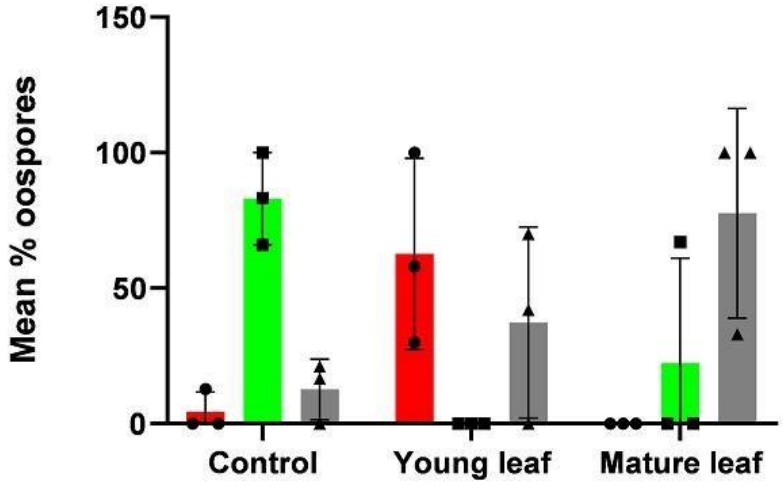


Figure 4.2.4: A comparison of young and mature Kānuka water extracts (TNA10.2). Bars show the average percentage of: viable (green), non-viable (red) and/or weakened (grey) oospores. Symbols represent the three technical replicates; bars represent the range.

4.2.2 Extract fractionation

Bioactivity-directed fractionation was then used to try to narrow down the bio-active fraction(s) and/or compounds. The crude ethanolic extracts were fractionated according to polarity using acetonitrile. The fractionation of TNA10 is shown in Table 4.1 and Figure 4.2.5. The fractionation of TNA10.1 is shown in Table 4.2 and Figure 4.2.6.

Table 4.1: Data of Kānuka extracts and fractionates (TNA10).

Plant code	Average % *P. agathidicida* oospores			Material	Fractionate
	Non-viable	Viable	Weakened		
01A	85.9	0.0	14.1	Young leaf	Parent fraction
01B	69.6	2.5	28.6	Mature leaf	Parent fraction
03A	99.2	0.0	0.8	Young leaf	20% ace
03B	63.0	2.2	36.6	Young leaf	40% ace
03C				Young leaf	60% ace
03D 03E	47.2	0.0	52.8	Young leaf	80% ace 20% ace
04A	59.8	1.7	38.5	Young leaf	
04B	85.7	0.9	13.4	Mature leaf	20% ace
04C	66.2	0.0	33.8	Mature leaf	40% ace
04D	59.9	0.0	40.1	Mature leaf	60% ace
04E				Mature leaf	80% ace
2210	83.6	0.5	15.9	Mature leaf	100% ace
2212 C+	84.0	1.4	14.6	Mature root	Parent fraction
	53.0	4.5	42.5	Young root n/a	Parent fraction n/a
	80.7	1.8	17.5		
	76.1	0.4	23.4		
	42.8	54.3	2.9		

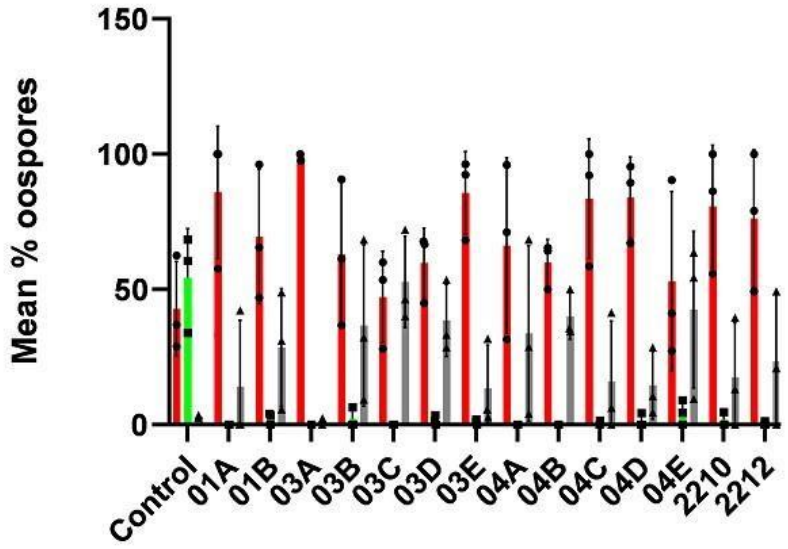


Figure 4.2.5: The viability of *P. agathidicida* oospores (mean %) treated with Kānuka extracts (TNA10). Oospores used in this experiment were purified in early February and viability was tested June (control = 24 h in sterile water). Crude Kānuka samples were re-suspended in 100% ethanol then diluted to 10% with sterile water. Parent (10% ethanol) extracts are 01A and 01B. Acetonitrile fractionate percentage increases from left to right of graph in 20% increments (only samples 03A to 03E and 04A to 04E). Mature Kānuka root (2210) and young Kānuka root (2212). Bars show mean % viable (green), non-viable (red) and weakened (grey) *P. agathidicida* oospores. Error bars represent the range of technical replicates n= 3 (symbols). Overall Kānuka extracts were highly effective against *P. agathidicida* oospore viability compared to negative untreated controls. All fractionates were resuspended in water before oospores were added.

Table 4.2: Data of Kānuka extracts and fractionates (TNA10.1).

	age % *P. agathidicida* oospores				
Plant code	**Non-viable**	**Viable**	**Weakened**	**Material**	**Fractionate**
01A	56.0	0.0	43.7	Young leaf	Parent fraction
01B	67.5	0.0	32.5	Mature leaf	Parent fraction
03A	49.6	0.0	50.4	Young leaf	20% ace
03B	49.0	0.0	51.0	Young leaf	40% ace
03C	66.4	0.0	33.6	Young leaf	60% ace
03D	73.2	1.9	24.9	Young leaf	80% ace
03E	53.8	4.3	41.9	Young leaf	20% ace

04A	48.5	0.0	51.5	Mature leaf	20% ace
04B	34.8	2.7	62.5	Mature leaf	40% ace
04C	42.5	0.0	57.5	Mature leaf	60% ace
04D	17.4	1.7	81.3	Mature leaf	80% ace
04E	41.0	3.3	55.7	Mature leaf	100% ace
2210	16.8	0	49.5	Mature root	Parent fraction
2212	65.3	0	1.3	Young root n/a	Parent fraction
C+	4.3	83.1	12.6		n/a

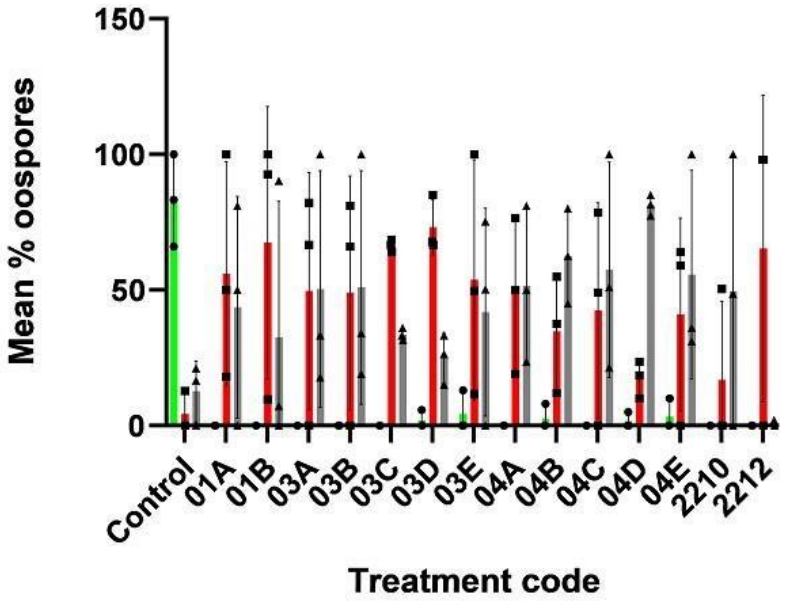


Figure 4.2.6: The viability of *P. agathidicida* oospores (mean %) treated with Kānuka (ethanol) extracts (TNA10.1). Control = 24 h in sterile water. Crude Kānuka samples were re-suspended in 100% ethanol then diluted to 10% with sterile water. Parent extracts are 01A and 01B. Acetonitrile fractionate percentage increases from left to right of graph in 20% increments (only samples 03A to 03E and 04A to 04E). Mature Kānuka root (2210) and young Kānuka root (2212). Bars show mean % viable (green), non-viable (red) and weakened (grey) *P. agathidicida* oospores. Error bars represent the range of technical replicates n= 3 (symbols). Overall Kānuka extracts were highly effective against *P. agathidicida* oospore viability compared to negative untreated controls.

All the Kānuka fractionates were highly effective against *P. agathidicida* oospores. Results were similar across the two biological replicates. However, from these experiments, no particular fractions were identified as potential leads to carry forward to compound purification and identification.

4.2.3 The effect of Kānuka rongoā oil on *P. agathidicida* mycelial growth

Kānuka was also extracted according to a rongoā method of distillation (referred to as KK oil by Hone Ratana) and tested for inhibition of mycelial growth using disc-diffusion assays. The KK oil inhibited the mycelial growth across the entire plate, irrespective of the position of the treatment discs. This is in comparison to (C), which shows normal growth when treated with a known negative control (water). These results are consistent with the known efficacy of Kānuka extracts towards *P. agathidicida* mycelial growth, but highlight the potential for alternative extraction methods. Observations were consistent across all three biological replicates.

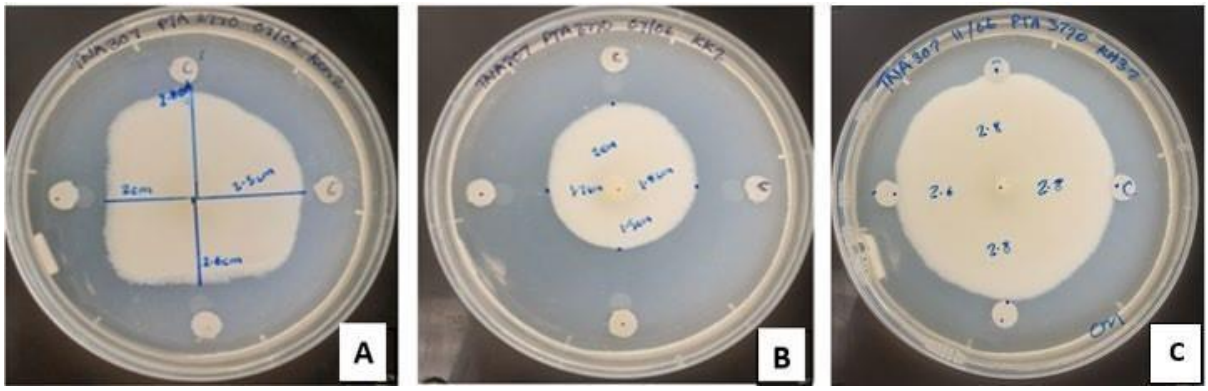


Figure 4.2.7 Disc diffusion assays for inhibition of mycelial growth. For each plate, two water-only control discs positioned were positioned at the top and right positions on plate (negative controls) and two treatment discs positioned were position at the bottom and left on the plate. (A) Kanamycin treatment. Kanamycin is a known inhibitor of *P. agathidicida* mycelial growth. (B) KK oil. In comparison to the kanamycin control, KK oil inhibited the mycelial growth across the entire plate, irrespective of the position of the treatment discs. This is in comparison to (C), which shows normal growth when treated with a known negative control (water). Representative data is shown; however, these observations were consistent across all three biological replicates.

4.3 Discussion

For centuries Māori have benefited from the many properties of Kānuka. Lawrence et al (2019) was the first to report the effects of Kānuka on *P. agathidicida* mycelia and zoospores. This chapter has further explored the efficacy of Kānuka extracts against oospores, while also exploring different preparation methods.

4.3.1 Plant age and material type

It is known that mature Kānuka are preferred in rongoā practices due to higher healing properties and to allow for young Kānuka to grow (H. Ratana 2020, pers comms, 18/9). However, the effect of plant age on the bioactivity of Kānuka extracts had not been previously explored. In this study, leaf and root extract from young Kānuka versus mature Kānuka varied between experiments. In TNA10.2 young Kānuka extracts were more effective against oospores (63% non-viable) than mature Kānuka (0% non-viable) However, in other experiments conducted as part of this thesis, oospore non-viability varied only slightly between young and mature Kānuka. Due to the small replicate size (two trees) and variable results it is difficult to make certain conclusions

There were no major differences between Kānuka leaf and root extracts against *P. agathidicida* oospores in both experiments (Figures 4.2.1 and 4.2.2). In a previous study, the greatest inhibition of mycelial growth of *P. agathidicida* was observed by Kānuka leaf extracts (40% inhibition) (Lawrence et al, 2019). Other studies have also found that leaf extracts from Kānuka were more bioactive than twig extracts in general, exhibiting higher antibacterial, anti-tumor and anti-inflammatory activity (Wyatt et al, 2005). Due to limited time frame replicate numbers were low in this study. Future research with increased replicates may provide more clarity regarding the effect of plant age and material type.

4.3.2 Traditional vs modern methods of preparation

There are various ways of preparing plant extracts from crude plant materials. Traditionally, plant compounds were extracted from Kānuka plant matter with boiling water alone (H. Ratana 2020, pers comms, 7/9; T. Parata 2020, pers comms, 12/2). Modern methods of extraction use other solvents and resins to extract chemicals (Habeeb, 2010). In another study, water extracts of Cassia *alata* leaves showed higher anti-bacterial activity than ethanol extracts (Somchit, 2002). Observations during preparation of another experiment - independent of this study - on Kawakawa (*Piper excelsum*) extracts, revealed obvious differences between the two methods. Natural ‘tea tonic’ extractions were pale brown in color and had a mild Kawakawa smell, whereas ethanol extracts were light green with a strong Kawakawa/alcohol smell. In this study, the Kānuka water extracts were overall less effective than ethanol extracts against *P. agathidicida* oospores (Fig 4.2.1 and 4.3.1).
However, it was also observed that 10% (v/v) ethanol also weakened oospores considerably.

Therefore, for the ethanol extracts, it was difficult to determine whether Kānuka compounds were acting alone or if the presence of ethanol was also having a synergistic effect.

In addition to chemical and water extracts, a Kānuka rongoā oil – prepared by rongoa Māori practitioner Hone Ratana – was also tested. There has been extensive research into the antimicrobial, antiviral and antifungal properties of essential oils on various plant diseases (Bi et al, 2012). However, in comparison to other plant disease sectors, few studies have explored the effects of essential oils on oomycetes. Essential oil research on *Phytophthora spp* is limited to *P.infestans* (Soylu et al, 2006), *P. Ramorum* (Manter, 2008; Kalantarzadeh, 2018), *P.capsici* (Bi et al, 2012) and *P.megakarya* (Nana et al, 2015). No studies have documented the effectiveness of essential oils on *P.agathidicida* and findings from this chapter are the first. Kānuka rongoā oil was highly effective against *P. agathidicida* mycelia. For other *Phytophthora* species, glasshouse trials have found that *Phytopthora* populations in soil were significantly reduced by oregano, palmarose, and red thyme (Bi et al, 2012). Future research should focus on potential field-applications of Kānuka extracts and KK oil as topical spray on plants and/or soil to control *P. agathidicida.*

4.3.3 Conclusions and future work

This research highlights many areas of future research. One limitation of this study was that the Kānuka water extracts were not prepared strictly according to traditional methods. These preparation differences could have resulted in lower efficacy. Similarly, these experiments only scratched the surface of the potential of KK oil to inhibit *P. agathidicida*. In particular, due to time constraints KK oil was not tested on oospores. In future research, alternative methods of preparation should be fully explored in anti-*Phytophthora* assays across all the lifecycle stages. Long-term feasibility should also be taken into consideration: for example, production of KK oil requires a considerable amount of plant matter and thus the sustainability of this rongoā is questionable.

Future research should also explore spatial and temporal variation of Kānuka extracts and essential oils. It is known that Kānuka from the East Cape have different chemotypes compared to Kānuka from the far north (Perry et al, 1997). Similarly, seasonality of harvest may also affect activity. During September to February months Kānuka are covered in clusters of white fragrant flowers less than 6 mm wide (Tipa, 2013). Therefore, harvesting leaves during flowering months may give variations in potency against *P. agathidicida.*

In addition to preparation methods, spatial variation and temporal variation of Kānuka, research into other native plant extracts and oils should also be explored. Kānuka was chosen for these studies based on Mātauranga from Ngā Puhi and a metaphysical understanding that they are an essential part of the natural process to establish and maintain Kauri forests (Lawrence et al, 2019). In addition to Kānuka, Mātauranga suggests other plants that could be explored. H. Ratana (Pers comms, 2020) rongoā Māori practitioner, expresses Mātauranga from Mataatua Te Arawa: that Tane Mahuta had 13 wives and the 13th wife was Tawakeketoro, who gave birth to Mānuka, Kānuka and Mingimingi. Future research should also focus on 'sibling' rongoā Mingimingi and Mānuka.

In summary, this chapter has explored the efficacy of Kānuka extracts and KK oil against *P. agathidicida.* These Mātauranga-guided solutions have been explored because processed chemical solutions for healing Kauri are not preferred by Māori (H. Ratana 2020, pers comms, 17/9). For example, the injection of phosphite in Kauri causes huge angst amongst Kaumātua as does Sterigene disinfectant stations located on tracks (K. Prime 2019, pers comms, 5/2). A lack of understanding and acknowledgement of tikanga Māori values is common in environmental science sectors. Our belief in te āo Māori is that the environment has the ability to heal itself, and this forms the basis of rāhui (temporary closures), karakia and rongoā Māori (K. Prime 2019, pers comms, 5/2; Waka Huia, 2019). Kānuka essential oils and extracts are promising rongoā for tīpuna Kauri as suggested by Mātauranaga Māori experts. More research into these – and other – natural solutions from native rongoā is essential for the future management of Kauri dieback.

Chapter 5
General discussion

5.1 Mātauranga Māori – *A holistic view*

The rāhui placed on the Waitākere ranges serves as a case study that outlines the significance of having kaupapa Māori based solutions. This thesis attempts to bridge the gap between matauranga Māori and western science. In the case of Kauri, both western and Māori parties persevere to achieve the ultimate goal: to protect Kauri from extinction. Mātauranga Māori is scarce in science literature and one of the aims of this thesis is to articulate mātauranga Māori in Kauri Dieback.

It is evident in literature that the Earth is declining at the hands of humans (Brundtland, 1987). Increased urban development has caused many communities to become isolated from direct interaction with nature and thus people have become disconnected from the environment (Oats, 2001). Environmental education is imperative now more than ever, however, education alone is not enough if beliefs, values, behaviors and attitudes remain unchanged. To heal the land, requires change in behaviors, attitudes and interaction with the environment around us (Ash, et al, 2010).-Embedded within Māori epistemology is the interconnectedness and inter-relatedness of people and nature. This is traced back through whakapapa (genealogy) to the beginning of time (Ka'ai, 2003; Williams, 2004) and is continually orated throughout Māoridom in many cultural forms (Williams, 2004). The word 'tangata whenua' directly translates to people of the land and signifies a deep understanding that we rely upon our natural systems to survive and thrive (Suzuki, 2007). This is a common understanding among many indigenous groups and Australian Aboriginals have demonstrated this over many thousands of years (McKay, 2012). This view is either forgotten or missing in western philosophies (Mehrotra, 2006). Suzuki (2006) even suggests that indigenous knowledge and understanding of the environment supersedes science knowledge as it has been trialed and "tested over time with the survival of those who possess the knowledge". Though the expectation of Māori roles as Kaitiaki has become unclear due to intense land degradation, social-economic pressures, language loss, land disputes and concentrated urban development, key values can still be learnt from the cultural norms of te āo Māori (Hartman et al, 2016)

Māori society has its own distinctive knowledge base accumulated by experiences through history (Kanwar et al, 2016). Kaupapa Māori knowledge encompasses beliefs, experiences, understandings and interpretations of the interactions of Māori people upon their environment (McKay, 2012). Māori experiences are generalized and often compared to other societies,

however, what is distinctly Māori lies within whakāro Māori (thinking Māori) and tikanga Māori (Māori protocol) (McKay, 2012). Thus, to grasp the full knowledge of Mātauranga Māori would mean writing this thesis in te reo Māori language. This is the means which gives expression to the conceptualization of Māori knowledge and so both work hand in hand (Nepe, 1991).

Māori children, emersed and raised in te āo Māori possess an innate awareness and sensitivity toward the environment, instilled as a type of 'common sense conservation ethic' (McKay, 2012). This is gained through everyday living and tutu (play). Hunting, fishing and growing kai (food), builds this environmental awareness and sensitivity to a level where one could literally 'read the environment' or understand 'te reo o te taiao' (voice of the land) through tohu (signs) (McKay, 2012). Therefore, a Māori view and way of living, forms the foundation of environmental education and environmental sustainability which are western concepts (McKay, 2012). Māori are natural orators, where Mātauranga is exchanged through wānanga, kanohi ki te kanohi (face to face) and sometimes passed on in the forms of parables, waiata, haka, whakairo, mahi-ā-toi, moteatea and poi. Expressed through many
Māori cultural forms is the significance of people and their relationship with the environment. Te āo Māori is embedded entirely within the metaphysical or te āo wairua. A holistic view and understanding that the environment is a living being that possesses mauri (life force). This mauri enhances wellbeing of tangata whenua (people of the land). Therefore, if the land is sick, the people are sick and vice versa, if the land is plentiful, so are the people. The role and responsibilities of tangata whenua are to tiaki, awhi me manaaki whenua (take care of and look after the land). Māori identify themselves by geographical features that raised them from childhood in pepeha. We identify ourselves with our maunga, awa, moana, marae, waka and tīpuna first before we even say our ingoā (birth names). This signifies the deep reverence, respect and connection Māori have to their turangawaewae (stomping grounds). In context of Kauri Dieback, behavioral change campaigns and educational awareness campaigns lack basic Māori Mātauranga. Māori knowledge is hugely under-represented in policy's and it is difficult to achieve equal collaboration between Treaty partners. However, it is not impossible if more Māori had a 'seat at the table'. This chapter not only summarizes the findings of this thesis, but it also seeks to provide understanding to the reader of a Māori world view. However, this chapter merely touches on Mātauranga Māori and further research is required.

Te tangi o te Kauri

Nā Te Amohaere Ngata-Aerengamate

Me muku ōu hū, muku ōu hū

Wairehu ōu hū, rehu ōu hū

Me muku ōu hū, muku ou hū, kia tū te Kauri hi! (Kia ū ki te ara tika!)

He aha te mea nui o te taiao

Ko te rākau Kauri

Ka tangi te ngāhere, ka hēmo te kīngi o te Waitākere.

The cry of the Kauri

(Written by Te Amohaere Ngata-Aerengamate)

Scrub your shoes

Spray your shoes

Scrub your shoes, the Kauri will stand (stay on the open tracks)

What is the greateast thing in nature

The Kauri tree the forest

is crying

the king of the Waitākere has fallen sick.

5.2 Te tangi o te Kauri

Though I whakapapa from Ngāti Porou, I was raised in West Auckland, near the Waitākere ranges and thus I am very much tangata whenua. During my time working at the Arataki visitor centre - located in the heart of the Waitākere ranges - I wrote a waiata on Kauri dieback that speaks of our roles as Kaitiaki to reduce the spread of the pathogen. Matua Kevin Prime even said the waiata could be a form of karakia. This waiata was produced as a part of an education program 'Puawai' for children all around Auckland, led by Joss Friend and Glenn Browne. A lot of whom were disconnected from their natural environment due to high urban development in Auckland. This program aimed to create environmental awareness and to re-connect them back to te taiao through tutu (mischievous play), waiata and pūrakau. Scrub your shoes, spray your shoes and stay on the open tracks are the main messages echoed in behavioral change campaigns and on Kauri dieback signage throughout the forest. These are also the lyrics that form the chorus of my waiata. The verse speaks of the cry of the forest (ka tangi te ngāhere) and personifies Kauri as the king of the Waitākere ranges who has fallen sick – ka hemo te kīngi o te Waitākere. This line was inspired by Apanui composer Rikirangi Gage, from the waiata-ā-ringa 'Taukiri e' (Matatini 2019). Lyrics for this waiata were cross checked with Rāwiri Waititi in the wharekai (house of eating) at Otūwhare marae. When I teach the waiata to children throughout Auckland, they often say 'why are the tree's crying' or 'how can Kauri be a king?' This provides an opportunity to teach mātauranga Māori and awaken environmental sensitivity and awareness within ngā tamariki mō āpōpō (children of the future). Children are encouraged to sing the waiata along tracks to remember they must muku (scrub), wairehu (spray) and kia ū ki te ara tika (stay on the right track). The waiata is available as an educational resource for tamariki (children) on YouTube, Apple music and Spotify. The name 'Te tangi o te Kauri' has a deeper meaning where tangi translates to cry, call and/or funeral of the Kauri. This indicates the past, present and future of Kauri if we as kaitiaki continue to mistreat te taiāo. Though this waiata was not a part of this master's thesis, it builds the body of work of mātauranga Māori in a Kauri dieback space.

5.3 Tohorā rāua ko Kauri (Whale and Kauri)

A famous Ngāti wai and Ngāti Hine pūrakau speaks of the inter-relatedness of Kauri and Tohorā (whale) (Waka Huia, H. Parata 2017). Both are tīpuna and are brothers. Their father, Tāne Mahuta (forest guardian), gifted Tohorā to his brother Tangaroa (ocean guardian). Tohorā explored the wonders of Tangaroa, returning to his brother to invite him on his endeavors. Kauri refused, preferring to stand staunch and tall within the realms of Tāne. Thus Tohorā gave his coat to safeguard Kauri as a protective skin and said that one day they would meet again. Tangaroa drew the line between land and sea, so Tohorā could never return to his brother again (Waka Huia, T. Ashby 2019). Māori made magnificent waka out of Kauri and thus, Kauri was reunited with his brother Tohorā in the realm of Tangaroa (Waka Huia, H. Parata 2017). Kaumatua have observed significant similarities in the whale coat and Kauri bark. Especially when they are shedding flakes (Waka Huia, H. Parata 2017).

This ancient pūraka has provided Segway into potential cures for Kauri dieback. Rongoā practitioner Tohe Ashby is using teachings within these stories to create a cure for Kauri. He most recently reported that Kauri were showing signs of recovery. He creates whale ointment made from ground whale bone and spermaceti and applies it to lesions on the tree (Stuff, T. Ashby 2020). Continuous monitoring of Northland Kauri, has shown less gum leakage on trunks and improved bark growth (Stuff, T. Ashby 2020). It is believed that Tohorā has heard the cries of his brother and the dieback illness inflicted on him through vibrations and frequencies in the ground (WakaHuia, H. Parata 2019). Increased numbers of Tohorā stranding's have occurred throughout Ngā Puhi and matua Tohe attributes these beaching's to Tohorā returning inland to save his brother Kauri. Pre-European settlement, Kauri stood right against the shoreline, all along the coast. Forest clearance has shifted them so far inland, Tohorā are dying, trying to reach Kauri (Stuff, T. Ashby 2019).

5.4 The significance of this work

5.4.1 The efficacy of disinfectants against *P. agathidicida* oospores.

One of the first challenges was finding alternative solutions to Trigene/Sterigene disinfectant, as it is known to be ineffective against oospores. Oospores are the main survival propagule of *Phytophthora spp* and the most problematic to eliminate. Disinfectant solutions have been trialed before on other *Phytophthora spp.* Similar data for *P. agathidicida* oospore was

lacking. My results demonstrate that Virkon, ethanol and bleach successfully deactivate *P. agathidicida* oospores; this is similar to findings from another *Phytophthora spp.* This study was the first to report the potential effectiveness of Napisan Vanish oxi on *P. agathidicida* oospores. Compared to bleach, Napisan was less effective against oospores. However, it was still very promising and deactivated most oospores at its recommended concentration 0.5% (w/v). Unlike bleach or Trigene, Napisan is safe to use on clothes, wool and soft textiles. Napisan therefore could be a promising alternative to Trigene at wash stations. It is also more affordable (RRP $8 per kg), and available to the general public. Future research will be needed to validate these results, and also test the effects of Napisan on mycelia and zoospores. These results also need to be compared across different isolates of *P. agathidicida*. Furthermore, there is also no data on the efficacy of Napisan against *P. agathidicida* oospores that reside on clothes, roots or in the soil of infested Kauri, thus more research is required.

5.4.2 Ahakoa he iti te Kānuka, ka whakaora tonu te Kauri.

In a Kauri dieback context this whakatauki implies that, although Kānuka is smaller than tīpuna Kauri, it has the strength to heal it.

Mātauranga Māori has provided promising rongoā for this research. The efficacy of Kānuka extracts and KK oil against *P. agathidicida* oospores and mycelia was trialed and tested in chapter 4. Kānuka extracts were highly effective against oospores, however, it was difficult to determine whether methods of extraction had influenced these results. This led to 'traditional vs modern extraction' tests. Overall ethanol extracts had higher non-viability of *P. agathidicida* oospores than water extracts. Results from Chapter 3, revealed 10% ethanol significantly weakened or deactivated oospores. Results from ethanol extracts were most likely attributed to the presence of ethanol rather than pure Kānuka extracts alone. Because of these findings, water extracts were prepared to test the efficacy of pure Kānuka and was considered the 'traditional method of extraction'. Water extracts mostly weakened oospores but could not deactivate them entirely and was least effective than ethanol extracts. This research did not test Kānuka extracts on other life stages of *P. agathidicida.* Though Lawrence et al (2019) was the first to report anti-*phytophthora* activity of Kānuka. They isolated 3 isoflavones' from crude plant material that successfully inhibited *P. agathidicida* mycelia and significantly reduced zoospore motility and germination. KK essential oil produced by matua

Hone Ratana significantly reduced *P. agathidicida* mycelia and validates findings from Lawrence et al (2019). Essential oils have shown anti-*Phytophthora* activity against other *Phytophthora spp.* Essential oils have been tested on *Phytophthora* that reside in soils near roots and were successful at inhibiting growth. Thus, future studies should focus on in-vivo and greenhouse trials, testing KK oil and Kānuka extracts as a topical spray on *P.agathidicida* infested soil. Though the findings in this thesis are part of academic research, the Mātauranga and rongoā are tāonga belonging to tangata whenua, the indigenous people of Aotearoa.

5.5 Future directions

Research shows that Kānuka has the ability to reduce zoospoore motility and germination, inhibit mycelia and now deactivate and weaken oospores. Future directions should test Kānuka water extracts and KK oil in greenhouse trials on Kauri inoculated with *P.agathidicida.* This can be done using methods from Bi et al (2012), creating a topical spray and spraying treatment near roots of infected Kauri and measuring *P.agathidicida* inhibition. The efficacy of Napisan on other life stages of *P.agathidicida* is uncertain and future trials should test Napisan on mycelia, sporangia and zoospores. Lastly, Napisan trials on clothes and hard surfaces contaminated with *P.agathidicida* could produce promising results and if successful, replace the use of Trigene/Sterigene disinfectant at wash stations.

5.6 Conclusion

P. agathidicda is a relatively new pathogen compared to other *Phytophthora spp* and therefore much is yet to be explored. A holistic Māori approach that views the system in its entirety as mentioned at the beginning of this chapter along with Western science technologies, methodologies and literature on other *Phytophthora spp* have helped shape this thesis. I believe my research has bridged (to some degree) the gap between Mātauranga Māori and western science and has provided insights into rongoā Māori and chemical solutions that may help reduce the spread of Kauri dieback disease.

Kuputaka – glossary

Aroha: love

Awa: river

Āwhina: to assist or support

Haka: war dance

Hapū: sub-tribe

Hinemāhou: one of the 13/14 wives of Tāne Māhuta

Ihi: essential force

Iwi: tribe

Kahikatoa: another name for Mānuka

Kaitiakitanga: guardianship

Kānuka: *Kunzea ericoides,* Brother of Mingimingi and Mānuka, Son of Tawakeketoro (13th wife of Tāne)

Karakia: prayer

Kaumātua: elderly

Kaupapa: subject/topic

Kauri: *Agathis australis,* brother to Tohorā (Ngāti Hine, Ngāti Wai), son of Hinemahou and brother of Tānekaha (Tūhoe, Matātua).

Kotahitanga: unity

Mahi-ā-toi: art

Mana whenua: territorial rights

Manaakitanga/ manaaki: hospitality

Mānuka: *Leptospermum scoparium*

Marae ātea: front of a marae

Marae: house of peace

Mātauranga: knowledge

Matua: respected elder (male)

Maunga: mountain

Mauri: life force

Mingimingi: *Coprosma propinqua*

Moana: ocean

Moteatea: chant

Papatuānuku: earth mother

Poi: dance with poi

Puawai: to blossom

Pūrākau: story

Rāhui: temporary closure

Ranginui: sky father

Reo: language

Rongoā Māori: Māori healing

Rongoā: medicine

Rongomatāne: guardian of peace and cultivation

Taiao: environment

Tāne Māhuta: forest guardian

Tangaroa: ocean guardian

Tangata rangatira: people of high status

Tangata whenua: people of the land

Tāonga: treasure

Taukiri e: oh dear!

Te Āo Māori: The Māori world

Tiaki: take care of

Tino rangatiratanga: sovereignty

Tīpuna: ancestor

Tohorā: whale

Tōtara: *Podocarpus totara*

Turangawaewae: place of standing

Waiata: song

Wairua: spirit

Waka: boat

Wānanga: to meet and discuss

Wehi: afraid, fear, something awesome

Whaikorero: speeches

Whakairo: carving

Whakapapa: genealogy

Whakapono: to believe

Whakāro: thoughts

Whakatauki: proverb

Wakatauaki: proverb said by someone

Whanau: family

Wharekai: house of eating

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