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Executive summary

The Kauri Dieback Programme requested a focused desktop literature review to identify research opportunities for developing an effective pH-based alkaline solution that is practical and can be operationalised to deactivate oospores of *Phytophthora agathidicida*. This follows on from the research carried out by Dick and Kimberley (2013), which showed that exposure of *P. agathidicida* oospores to a pH level of 9 for 24 hours reduced viability to levels below all other treatments, and that there were no viable oospores after 48 hours' exposure to pH 9 or pH 10.

pH alkaline-based solutions are used for topical spray formulations in greenhouses and the decontamination of footwear and horticultural machinery around the world (although not in environmental contexts). Also, (calcium) hypochlorite is used for urban water treatment. However, the caustic nature of alkaline-based chemicals presents a challenge for off-label use of pH-based products in kauri forest. Interventions such as pH-based products are non-specific and would have non-target impacts in the kauri forest soil. Also, at high pH these chemicals pose a hazard to human health and have the potential to harm native vertebrates and invertebrates if used in forest.

As a result, there are certain barriers, uncertainties and gaps in knowledge relating to the use of pH-based alkaline solutions. There are potential concerns from a cultural /social acceptability standpoint, and there are human health implications, and concerns about environmental and non-target impacts. Significantly, there are no data on the efficacy of alkaline solutions against *P. agathidicida* oospores that reside in the roots or soil of diseased kauri. Research to investigate the application of pH-based alkaline solutions for the *in situ* disinfestation of oospores contained in water could provide a feasible operational protocol.

There is also potential for the use of lime (CaO) as a soil amendment to increase pH in nursery potting mixes, although not as a biocide to deactivate oospores, but as disease suppressant/retardant. The potential use of 'green bridges' utilising crushed limestone needs to be studied to quantify the pH of the water leachate to see if this is at a suitably high level to achieve deactivation of oospores of *P. agathidicida.*

1 Introduction and background

In chemistry, pH (potential of hydrogen) is a logarithmic scale used to specify the acidity or basicity of an aqueous solution. It is approximately the negative of the base 10 logarithm of the molar concentration, measured in units of moles per litre of hydrogen ions. In this scale, a pH of 7.0 is considered neutral, and those entities with a pH greater than 7 are considered basic or alkaline. Alkaline solutions are characterised by having a low concentration of hydrogen ions and being capable of neutralising acids (Blackman 2015).

The pH of artificial media is known to influence the growth rate of *Phytophthora* species, but this has not been studied for *P. agathidicida*. For example, for *P. clandestina* the optimal pH for growth was found to be between 5.0 and 7.7 for maximal mycelium growth at 25°C (Wong et al. 1986). In this study the extent of growth was reduced by 65% under acid (pH 4) and by 32% under alkaline (pH 9) conditions. The effect of pH on the formation of sporangia of *P. agathidicida* has not been studied, but for a range of other *Phytophthora* species the pH optimum is between 4.0 and 9.0 (Simpfendorfer et al. 2001).

The effect of pH on oospore formation of *P. agathidicida* has not been studied either, but studies on other species have identified oospores germinating at pH optima of between 4 and 8 (American Phytopathological Society 1983). The laboratory-based New Zealand research of Dick and Kimberley (2013) identified a reduction in the viability of *P. agathidicida* oospores at higher pH levels, although this only occurred for extended exposure times. pH levels of 9 and 10 for 24 and 48 hours, respectively, reduced viability to levels below all other treatments.

So there is *a priori* evidence that a pH level above 9 could contribute to a reduction in the growth rate, sporangial formation and potentially oospore germination of *P. agathidicida*. The following review provides the findings that address the nine key questions outlined in the work authorisation.

2 Materials and methods

The methodology for this project included:

- a desk-top review of the research literature relating to using pH-based alkaline solution to deactivate plant pathogens
- discussion with Professor G. Hardy and Associate Professor T. Burgess, from the Centre for *Phytophthora* Science and Management, Murdoch University, Perth, Western Australia, on the use of green bridges to prevent the movement of propagules of *Phytophthora*
- input from New Zealand scientists from the Ministry for Primary Industries on the mode of action of lime.

3 Results, discussion and recommendations

3.1 pH-based alkaline solutions used overseas to fight *Phytophthora* species

Greenhouse, footwear the and machinery sanitation

Sodium hypochlorite (NaOCl) at 1–5% is effective at killing exposed propagules of *Phytophthora* on hard work surfaces in nursery and greenhouse settings (Vincelli & Hershman 2005). Sodium hypochlorite is a liquid and has an active chlorine concentration of 10–15% with a pH of around 13. It is unstable, and when it comes into contact with air, light or high temperatures chlorine evaporates, and therefore its concentration in water, and its biocidal efficacy, decreases (Lee et al. 2010). Ten percent bleach solution[1] is also recommended for the sanitation of footwear and bicycles potentially contaminated with sudden oak death caused by *P. ramorum* (COMTF 2018). Decontamination using a 10% solution of bleach is also recommended for chippers used for mulching green waste in between sites, to manage sudden oak death (Lee et al. 2010).

Water sanitation and hydroponic applications

Known as chloride of lime, calcium hypochlorite ($Ca(OCl)_2$) is a solid, which can be used as a powder, or in granulated or tablet form. It is widely used at small water treatment plants for the production of potable water for human consumption (Gray 2014).

Recycled irrigation water is one of the major sources of inoculum and may spread plant pathogens throughout nursery or greenhouse operations. Free chlorine (14 mg/L for 6 minutes) has been found to be effective at neutralising *Phytophthora* propagules (mycelium and zoospores only) in irrigation tanks (Cayanan et al. 2009).

Disease-suppressive potting mixes

Over the past 2 years alternative treatments for *Phytophthora* control on *Pinus radiata* have been investigated in forest nursery trials conducted by Scion and Plant & Food Research. Calcium in the form of lime (CaO) has been shown to have some ability to suppress *Phytophthora* root diseases (Reglinski et al. 2009).

Green bridge preventive barrier

Professor G. Hardy and Associate Professor T. Burgess[2] provided the following insights into the use of 'green bridges' as a preventive barrier to prevent the movement of propagules of *Phytophthora.*

The best example of the use of green bridges comes from the bauxite mines of southwestern Australia. A green bridge utilises gravels sourced from *Phytophthora*-free areas, which are placed on top of soil contaminated with *Phytophthora* to enable the movement of vehicles around the mine site, avoiding the risk of moving propagules of *Phytophthora* (because the infected substrate is effectively buried below the clean gravel layer).

Associated with this approach are strategically placed wash-down bays for cross-roads that are not green bridged. However, because of the costs of sourcing and moving disease-free gravels, Alcoa Australia has now moved away from the use of green bridges, and at

[1] Bleach contains 3–6% NaOCl.
[2] Centre for Phytophthora Science and Management, Murdoch University, Perth, Western Australia.

completion of mining they ‘fallow’ the roads for 2–3 years (until the pathogen is no longer present) and then rehabilitate the roads back to jarrah forest.

Crushed limestone[3] has also been used as a green bridge, and this has been found to be effective against *P. cinnamomi* (which is heterothallic; i.e. does not produce oospores in single culture) but not for *P. multivora* (which is homothallic, like *P. agathidicida*). This has now raised issues due to the wide spread of *P. multivora* and the fact that its host range is substantially increasing. In the opinion of Hardy and Burgess, ‘the level of pH is just suppressive, probably enough so that other microorganisms can attack the propagules [of *P. cinnamomi*]’. They think it is probable that the crushed limestone itself is so harsh and hostile, and low in organic matter that if a propagule finds its way onto the surface there is nothing for it to use as a ‘host’.

The mode of action appears to be related to the calcium ion concentration rather than a direct pH effect. The drainage (‘alkaline leachate’) of the surface could also suppress the germination of propagules, but this has not been tested via experimentation, and no standardisation of the approach (i.e. size of the gravel particles, volume or thickness of the barrier layer and duration of the pH effect) has been published.

3.2 How effective are the tools in reaching a pH of 9 or 10 with a 48-hour exposure?

pH plays a key role in the efficacy of the range of oxidising biocides that can kill micro-organisms through the electrochemical process of oxidation. The oxidising agent (such as chlorine) gains electrons in the process, while the substance being oxidised loses electrons. Depending on the oxidiser used, a new compound is created, and in the case of micro-organisms some life function is changed that causes the organism to die or at least not proliferate (Hong et al. 2003).

The biocidal activity is linked to the reaction chemistry life-cycle of the compound in solution. When chemical equilibrium is achieved, both reactants and products are present in concentrations that have no further tendency to change with time, so there is no further observable biocidal activity (Cayanan et al. 2009).

Topical efficacy of sodium hypochlorite

The most important factor affecting hypochlorite ClO^- content in the sodium hypochlorite solution is pH. A decrease in pH increases the concentration of dissociated ClO^- (as ClOH, hypochlorous acid), and this increases its antimicrobial effectiveness (Fukuzaki et al. 2007). The highly reactive hypochlorous acid attacks amino acids that make up proteins, altering the proteins’ three-dimensional structure. Cell membranes and cell walls contain a lipid bilayer with embedded proteins, which are targeted by the activity of hypochlorous acid, explaining the biocidal efficacy of hypochlorite (Winter et al. 2008).

Maximum efficacy of sodium hypochlorite solution to achieve cellular alkali lysis occurs at a pH between 10.5 and 11.5. A slight excess of sodium hydroxide (NaOH) can help protect the hypochlorite solution from the harmful effects of light. There is no evidence that excess alkalinity greater than 0.5% by weight of solution using sodium hydroxide has a beneficial effect on the stability of hypochlorite solutions. Sodium hypochlorite stability is also adversely affected by increases in temperature, exposure to sunlight and some metals in

[3] No indication was given of the size of the limestone gravel used in the process.

solution, which means it needs to be made up fresh and stored carefully to preserve its efficacy (DeQueroiz & Day 2008).

The broad-spectrum effectiveness of most bleaches is due to their general chemical reactivity with organic compounds rather than selective inhibitory or toxic actions. The previous research of Bellgard et al. (2010) found that sodium hypochlorite is efficacious at reducing mycelium growth, and killed zoospores of *P. agathidicida* at a concentration of 5%. However, at this concentration sodium hypochlorite could not completely suppress the germination of oospores, but no measure of pH was reported in this study (Bellgard et al. 2010).

Water sanitation

Hypochlorite was the original form of chloride used for disinfecting drinking water, and it is still widely used today at smaller water treatment plants. The more familiar liquid sodium hypochlorite (NaOCl), which is commonly known as bleach, is also used at smaller installations, although it is now commonly generated on site for disinfection at larger water treatment plants (Gray 2014).

Kong et al. (2009) found that extreme pH conditions induced zoospore encystment and some deformed structures, but that most zoospores or cysts lysed at pH 11. Kong et al. (2009) did not study the effects of pH on oospore activity.

Lime applications

'Lime' is a term used for a broad range of chemical products containing either calcium (Ca) or magnesium (Mg). It is used to neutralise soil acidity resulting from inherent alkalinity. Augmentation of agricultural soils with lime has long been associated with disease suppression, especially for *Phytophthora* (Benson 2008). Research from the potato industry suggests that amending the soil with lime to increase soil pH and calcium content is one approach to suppress pink rot in potatoes caused by *P. erythroseptica* (Benson 2008).

Water is required to mediate any pH effect from the outset of a 48-hour contact period. Water must saturate lime to achieve any pH modification effect, because it must be in a soluble form to interact with the oospores. In soils the liming effect is secondary to some dissolution and mixing with soil pore water. The equilibrium pH for water–calcium carbonate is reached at pH 8.3. Lime (CaO) raises soil pH by an indirect buffering mechanism. The carbonate can react with hydrogen ions, and the calcium plays a cation charge-balancing role.

Alternatively, adding water to burnt lime (calcium oxide) produces slaked lime (calcium hydroxide), which has a pH in the optimal range (i.e. 8–9) for deactivating oospores, in fact higher than needed at pH 12.4. However, slaked lime is very corrosive and must be handled with care. Sodium hydroxide (soda lime) is less corrosive but still presents a risk to the operator, and potentially to the environment[4].

3.3 How are the products applied in the environment?

Topical applications

In overseas research 15% bleach solution was found to be an effective disinfectant, which prevented the recovery of *P. ramorum* from plastic and metal surfaces (James et al. 2012).

[4] Personal communication received from Nick Kim, Senior Policy Analyst, Land Policy & Resource Information, Ministry for Primary Industries, 22 February 2018

The spray application increased in efficacy with a contact time of 5 minutes. Ten-minute exposure to 15% bleach was effective in preventing sporangium germination.

Water treatment

Calcium hypochlorite powder contains between 30 and 35% w/w of chlorine. It is transported and stored in sealed drums lined with plastic or rubber. The material can be stored for considerable periods, but once opened it will begin to decay (Gray 2014). The hypochlorite solution is made up by adding a known weight of powder to water in a mixing tank, where the reaction occurs. The solid fraction settles, leaving a concentrated chlorine solution, which is injected into the contaminated water using a positive displacement pump. The chlorine solution will rapidly decay if exposed to sunlight or the atmosphere (oxygen), and must be made every 24–48 hours. Approximately 3 kg of calcium hypochlorite powder is sufficient to dose 1,000 m^3 of water at a concentration of 1.0 mg Cl_2/L (Gray 2014).

Soil amendments

Water is required for lime to react with soil, so the effects of lime will be slower in dry soil (Benson 2008). The reactivity time also depends on the type of lime used and the size of the particles. The materials include limestone (both calcitic and dolomitic), burnt lime, and slaked lime, and various by-products. Liming materials are carbonates, oxides or hydroxides of Ca and/or Mg (Table 1).

Table 1. Neutralising capacity of various forms of 'lime'

Material	Composition	Calcium carbonate equivalent (%)*
Calcium carbonate	$CaCO_3$ (pure)	100
Calcitic limestone	$CaCO_3$	80–100
Dolomitic limestone	$CaCO_3$ / $MgCO_3$	95–108
Burned or quick lime	CaO (calcium oxide)	150–175
Hydrated or slaked lime	$Ca(OH)_2$ (calcium hydroxide)	120–135
Ground oyster shells	$CaCO_3$	90–100
Cement kiln dusts	Ca oxides	40–100
Power plant ashes	Ca, Mg & K oxides	25–50
Wood ashes	Ca, Mg & K oxides	40–50
Gypsum	$CaSO_4$	None

Source Virginia Cooperative Extension Publications 2018, 452, 452–510.

* Calcium carbonate equivalent (CCE) is the acid-neutralising value for a liming material relative to pure calcium carbonate, which has a CCE value of 100. This is the standard method of measuring lime purity.

3.4 What are the potential non-target impacts that will need to be considered in a kauri ecosystem?

Hypochlorite solutions are very alkaline (pH 11, 12), which ensures the free chlorine is stored as the hypochlorite ion (OCl^-). These solutions are corrosive but relatively safe to handle with personal protection equipment (PPE). If the pH is lowered by the addition of an acidic chemical, the hypochlorite ion is converted to hypochlorous acid (HOCl), which can then be converted to chlorine gas via the following pathway:

$OCl^- + H^+ \rightarrow HOCl$ / $H^+ + HOCl + Cl^- \rightarrow Cl_2 + H_2O$

Chlorine gas is highly toxic, and so respirators need to be worn when handling large volumes. The biocidal impact of hypochlorite and other high pH chemicals like sodium hydroxide (lye or caustic soda) is not species-specific, and if applied to soil in a kauri forest ecosystem setting there is potential for elimination of more than just *Phytophthora* inoculum propagules. High pH can lyse any bacterial cell membranes.

The microbiological technique of alkali lysis utilises the ability of sodium hydroxide (NaOH) to denature DNA (Birnboim & Doly 1979). Sodium hydroxide helps to break down the cell wall of an organism, but more importantly it disrupts the hydrogen bonding between the DNA bases, converting the double-stranded DNA in the cell (including the genomic DNA and plasmids) to single-stranded DNA. This process is called denaturation, and it is a central part of the procedure, hence alkaline lysis.

The principal safeguard is that the alkaline solution is contained and fully bunded (a bund is a raised embankment to prevent the overflow from a holding pond), so that there is no accidental discharge of the reagent. The broad-spectrum biocidal activity of liquid formulations of alkaline solutions such as hypochlorite, if directly applied to the kauri forest ecosystem, would probably have non-target effects.

3.5 What are the potential human health impacts of using a pH-based alkaline solution?

Caustic soda (NaOH)

Alkaline substances like caustic soda can burn skin on contact because they are capable of hydrolysing proteins. A chemical burn can result from a brief exposure to strong alkaline materials. Routes of exposure include inhalation, skin and eye contact. Accidental inhalation can cause severe respiratory irritation, with the inhalation of mists or vapours producing upper airway oedema, wheezing, pulmonary oedema, pneumonitis and respiratory failure (NaOH, MSDS 2013). Ingestion may produce burns to the lips, oral cavity, upper airway, oesophagus, and possibly the digestive tract. Skin scarring is a common outcome, with contact resulting in serious eye damage. Prolonged skin exposure may produce dermatitis. Caustic soda can dissolve metals and reacts with certain metals like zinc and aluminium to produce hydrogen, which ignites in the presence of oxygen. This also poses a risk to operators.

Sodium hypochlorite (NaOCl)

Sodium hypochlorite (5%) is hazardous in the case of skin contact (irritant), eye contact (irritant), and if ingested. Hypochlorite is only slightly hazardous in the case of inhalation (lung sensitiser), as it is non-corrosive for lungs. Liquid or spray mist may produce tissue damage, particularly on mucous membranes of the eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of the respiratory tract, characterised by coughing, choking, or shortness of breath. Prolonged exposure may result in skin burns and ulcerations. Over-exposure by inhalation may cause respiratory irritation. Inflammation of the eye is characterised by redness, watering and itching. Skin inflammation is characterised by itching, scaling, reddening or, occasionally, blistering (NaOCl, MSDS 2013). To prevent the formation of chlorite and chlorate ions, care must be taken in the preparation and storage of the hypochlorite solution. For this reason, concentrated hypochlorite (>5%) should only be used by experienced operators (Gray 2014).

Calcium oxide (CaO) powder

Calcium oxide is usually made from the thermal decomposition of materials such as limestone and seashells (which contain calcium carbonate, $CaCO_3$, as mineral calcite) in a lime kiln. Calcium oxide is not acutely toxic via the oral, dermal or inhalation routes (CaO, MSDS 2013). The substance is classified as irritating to skin and the respiratory tract, and can cause serious damage to the eyes. It is highly reactive and is usually slaked (hydrated) to stabilise its formulation.

Calcium hydroxide ($Ca(OH)_2$) powder, calcium hydrate, slaked lime, hydrated lime

Slaked lime is harmful if swallowed or inhaled. It causes burns to skin and eyes, and severe irritation to the respiratory tract ($Ca(OH)_2$, MSDS 2013). When in contact with skin it is corrosive and may cause severe burns and blisters, depending upon duration of contact. Slaked lime is corrosive when in contact with the eyes, causing severe irritation and pain, and inducing ulceration of the corneal epithelium, which can result in blindness. When inhaled it causes severe irritation and can cause chemical bronchitis. Ingestion causes gastric irritation, followed by severe pain, vomiting, diarrhoea and collapse. If death does not occur in 24 hours, then oesophageal perforation may occur, which will result in permanent disability ($Ca(OH)_2$, MSDS 2013).

3.6 What is the social and cultural acceptability of introducing a pH-based tool to the environment?

Topical spray applications of hypochlorite have been deployed in greenhouses and semi-industrial applications, but in these contexts operators have access to PPE. There are challenges associated with introducing a pH-based tool for the environmental management of the risk posed by oospores of *P. agathidicida*. For example, transitioning from SteriGENE® (halogenated tertiary amine) to a 5% hypochlorite solution for footwear hygiene would see potential impacts on health, and also on end-users’ clothing, as the spray application will bleach most fabrics.

A pH-based alkaline solution could be considered for water treatment applications of wash-down or produce water contaminated with *P. agathidicida* propagules (including oospores) associated with road construction and/or culvert maintenance. Here, contained water treatment becomes an issue, as a result of the sanitary process. The alkaline reagents could be transported to the point of operations, where a fully bunded, fenced, temporary water-holding ‘pond area’ has been established. The active ingredient could be injected into the pond, and after the reaction, the supernatant could be siphoned off by a certified liquid waste disposal agent. With appropriate engagement, and provision of on-site staff to supervise the operation, along with traffic management, there should be no adverse social impacts. The major problem is that you would not be able to determine the final concentration of your active ingredient in the pond, as this is likely to be exposed to environmental factors such as rain, which would dilute the active ingredient.

Cultural imperatives and expectations relating to the use of topical spray applications must have robust scientific data for uptake, and highlight the potential side-effects. Application of bleaching for nursery sanitation would be acceptable if all side-effects and education around PPE are provided as part of chemical handling certification.

Contained water treatment may also achieve cultural approval if the intervention does not impinge upon tukutuku o te ora (the web of life) – the wider environment of interacting species. The necessary safeguards would need to include:

1. no risk of pond overflow, to contaminate downstream water resources
2. no risk of wildlife or operators falling into the pond
3. no non-target impacts on plants, soil microbes, or micro- and meso-fauna by accidental release of product
4. no risks to humans
5. no aesthetic damage to forest.

If efficacy and safety can be demonstrated, there should be no cultural barriers to the use of lime for nursery amendments as part of plant production potting media.

3.7 Are there any implications for or barriers to applying such tools in a natural ecosystem?

The following have been identified as barriers to applying a pH-based alkaline solution for the deactivation of oospores of *P. agathidicida* in a kauri forest context.

1. *Non-target impacts on a range of trophic levels in the kauri ecosystem:* a range of sensitive microbes, symbionts and endophytes would be affected by direct contact. There would also be flow-on risks to other plant species, wildlife (e.g. kauri snail) and ground-dwelling birds.
2. *Human health risks:* these would arise from the consequences of alkali lysis and protein denaturation associated with broad-scale deployment of alkaline solution.
3. *Social and cultural licence to operate:* public consensus would need to be achieved, because of non-target impacts and potential flow-on effects to waterways if there was an accidental discharge from a holding pond in a kauri forest.
4. *Licence to operate from central governance:* Environmental Protection Authority and Department of Conservation concessions would be needed for the operational use of pH-based alkaline solutions in an environmental management context.

3.8 Over what scale can a pH-based alkaline tool be used (e.g. landscape scale or spot treatment)?

There is no scientific basis on which to consider the landscape-scale deployment of a pH-based alkaline solution to deactivate oospores of *P. agathidicida*, as the necessary pH shift at the landscape scale for the appropriate duration to achieve biocidal impacts is not achievable. Also, the broad-spectrum, non-target impacts and risks to human and animal health make a pH-based alkaline solution inappropriate for the management of spot infestations in the kauri forest.

With appropriate planning, environmental and human safeguards, ‘spot’ spray-treatments could be suitable in greenhouse/semi-industrial settings (e.g. for decontaminating chippers). Also, with appropriate engagement and consultation, *in situ* water treatment in the forest using pond systems with calcium could be used for small-scale (up to 150 L) deactivation of

P. agathidicida propagules, after the technique has been researched for efficacy and a standard operating protocol has been developed.

3.9 What are the gaps in the knowledge around using a pH-based alkaline tool for the deactivation of *P. agathidicida* oospores?

The following list provides some of the fundamental knowledge gaps around using a pH-based alkaline solution for the deactivation of oospores of *P. agathidicida*. Attaining such knowledge would be the first step in establishing proof of concept for a broader consideration of pH-based chemical intervention.

Knowledge is lacking with regard to:

1. *in vitro* pH sensitivity of *P. agathidicida* mycelium growth, and sporangium, zoospore and oospore production
2. *in vitro* pH sensitivity of *P. agathidicida* oospore germination, and determination of the lethal dosage necessary to achieve deactivation
3. laboratory screening of calcium hypochlorite and other water-sanitation, pH-based alkaline solutions to deactivate oospores of *P. agathidicida*
4. nursery/greenhouse studies to examine pH amendments and kauri dieback disease suppression that can be achieved through augmentation of pH in a pot-based assay
5. laboratory studies of the alkalinity of the water leachate produced from different-size-classes of crushed limestone and other alkaline mineral substrates
6. laboratory studies on the effects of alkaline leachate from the above mineral substrates on oospore germination of *P. agathidicida.*
7. investigations into the effects of dissolved calcium products on the germination of oospores of *P. agathidicida.*

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