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Literature review: *Phytophthora agathidicida* inoculum deactivation

Williams N

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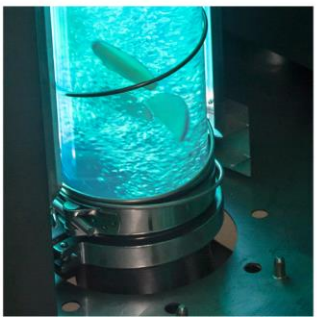


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Report prepared by:

Nari Williams
Scientist, Pathogen Ecology and Control, Havelock North
June 2020

Report approved by:

Beccy Ganley
Science Group Leader, Pathology - Bioprotection
June 2020

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Executive summary

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Disinfectants are key tools in mitigating against the spread of *Phytophthora* species in operational and/or recreational activities within nurseries, forests and natural areas. Protocols for disinfection are important components of integrated management aimed at limiting the spread of soil and water-borne pathogens through human activity. In the context of kauri dieback management, a range of surfaces and substrates need to be considered for disinfection including surfaces, equipment and bulk volumes of contaminated water, soil and plant debris.

The efficacy of any disinfectant is influenced by a range of factors including concentration, contact time, chemical/mode of action, and presence of organic matter and the biology of the target organism.

The Ministry for Primary Industry/ Kauri Dieback Programme have requested research on "Oospore Deactivation" for *P. agathidicida* given that tools are needed to deactivate *P. agathidicida* inoculum in human-mediated pathways. This project specifically requested investigation into the limitations and key considerations of using chlorine, steriGENE, methylated spirits, steam and boiling for disinfecting surfaces and water. Existing published research and grey literature (e.g. unpublished or published in non-commercial form) on the use of these are reviewed with reference to *P. agathidicida*.

Each of the disinfection methods has the potential for use in the management of *P. agathidicida.* Despite considerable amounts of work investigating the response of *Phytophthora* pathogens to various disinfection protocols, the specific conditions in which such methods are used in practice remain largely untested. In each case, a key consideration must be the target material being treated. Such information is critical in ensuring that the appropriate approach is taken to disinfection for the material being treated. The establishment of treatment dose and response curves for natural inoculum of *P. agathidicida* across key disinfection treatments will enable management prescriptions to be established for a range of applications without the need for repeated optimisation for specific treatment scenarios.

The potential environmental impacts and risks of using some chemicals also needs careful consideration for their routine application within natural ecosystems. In the case of chlorine and SteriGENE consideration should be used to minimise the volumes being applied and potentially discharged into sensitive natural ecosystems.

For further information please contact:

Nari Williams
Plant & Food Research Hawke's Bay
Private Bag 1401
Havelock North 4157
NEW ZEALAND

Tel: +64 6 975 8880
DDI: +64 6 975 8977
Email: Nari.williams@plantandfood.co.nz

1 *Phytophthora agathidicida* inoculum deactivation

Disinfectants are key tools in mitigating against the spread of *Phytophthora* species in operational and/or recreational activities within nurseries, forests and natural areas. Protocols for disinfection are important components of integrated management aimed at limiting the spread of soil and water-borne pathogens through human mediated activity (Colquhoun and Hardy 2000; Gehr et al. 2003; Tomasino 2005; Cheah et al. 2009; Fichtner et al. 2009).
In the context of kauri dieback management, a range of surfaces and substrates need to be considered for disinfection including surfaces, equipment and bulk volumes of contaminated water, soil and plant debris (James et al. 2012; Scarlett et al. 2016; Gómez-Gálvez et al. 2018).

The efficacy of any disinfectant is influenced by a range of factors including concentration, contact time, chemical/mode of action, presence of organic matter and the biology of the target organism (Best et al. 1990).

In the case of *Phytophthora* pathogens, disinfection needs to consider the mode of action, longevity and risk associated with key inoculum sources/substrates in which the pathogen lives and persists on surfaces or within the plant, soil or water substrates of concern (Russell 1983; Hong et al. 2003; James et al. 2012). For *Phytophthora agathidicida,* causal agent of kauri dieback, chlorine, steriGENE, methylated spirits and steam and boiling have all been identified as feasible disinfectants in various situations (Bellgard et al. 2011a). The Ministry for Primary Industry/ Kauri Dieback Programme have requested research on “Oospore Deactivation” for *P. agathidicida* given that tools are needed to deactivate *P. agathidicida* inoculum in human-mediated pathways (Williams and Arnet 2020). This project specifically requested investigation into the limitations and key considerations of using chlorine, steriGENE, methylated spirits, steam and boiling for disinfecting surfaces and water. Existing published research and grey literature (e.g. unpublished or published in non-commercial form) on the use of these are reviewed here with reference to *P. agathidicida*.

2 Chlorine

Chlorine is a commonly used to disinfect in a broad range of applications including recycled irrigation water (Hong et al. 2003; Cayanan et al. 2008), surface water (Lewis Ivey and Miller 2013) and on surfaces. Chlorine is an effective disinfectant against a range of plant pathogens including fungi and oomycetes (Datnoff et al. 1987; Hong et al. 2003; Scarlett et al. 2016; Lee et al. 2019). Chlorine can be used as a disinfectant in a range of formulations, including industrial use of chlorine gas (Cl_2) and chlorine dioxide (ClO_2) for disinfecting bulk water or as liquid bleach (sodium hypochlorite, NaOCl) commonly used in domestic applications (Lewis Ivey and Miller 2013). Bleach formulations inhibit the growth of *Phytophthora* mycelia and kill zoospores on contact (Hong et al. 2003; Cayanan et al. 2009; Lee et al. 2019), however chlorine sensitivity can differ with pathogen species and type of propagules of the same pathogens (Hong et al. 2003).

Hong et al. (2003) exposed zoospores of seven species and eight isolates of *Phytophthora* to free available chlorine at 0.25, 0.5, 1.0, 2.0, and 4.0 mg/L for 2 min with none surviving at concentrations of above 2 mg/L free chlorine. In the same study they also exposed zoospores, mycelial fragments and culture plugs of *Phytophthora nicotianae* to chlorine concentrations ranging from 0.25 to 8.0 mg/L for periods ranging up to 8 min in which it was shown that *P. nicotianae* mycelial plugs were able to survive and produce sporangia at concentrations of 0.77 mg/L active chlorine or lower. Zoospore mortality was observed to increase with chlorine concentration from 0.25 to 1 mg/L irrespective of contact time with the shortest period measured being 15 s. Mycelial fragments were found to be more recalcitrant but also showed increased mortality with time at concentrations above 0.5 mg/L (Hong et al. 2003). In nursery applications, *Phytophthora* species were recovered in irrigation water containing up to 0.77 mg/L of active chlorine from which the authors recommended this as a minimum concentration for chlorination protocols within nurseries (Hong et al. 2003) .

Bellgard et al. (2011) showed that a 5% solution of bleach (Janola) was lethal to *P. agathidicida* zoospores, significantly reduced oospore viability and completely suppressed mycelial growth beyond 8 days exposure. These observations on *P. agathidicida* were consistent with those of Hong et al. (2011) showing that mycelium is less sensitive to chlorine treatment than zoospores. Both studies highlight the importance of considering the propagule being targeted by disinfection and the need to consider the substrate it is contained within. Janola was found to be effective in suppressing *P. agathidicida* in soil and in surface application on contaminated footwear (Bellgard et al. 2011b). While Bellgard et al., (2011a) tested Janola at an active concentration of 5%, standard working concentrations range from 1–5% depending on application and warrant further investigation.

While each of the studies discussed in the previous paragraph considered the treatment of material over time, neither considered investigated the period of exposure of the disinfectant solution and associated degradation of chlorine over time. This is an important consideration as bleach/chlorine will become less effective over time with exposure to the air, soil and organic material (Cayanan et al. 2009) . In water, the active constituents of chlorination are chlorine (Cl_2), hypochlorous acid (HClO) and hypochlorite anion (ClO^-). As a contained liquid, these remain in equilibrium. However, upon exposure to air the degradation kinetics are driven by the loss of chlorine gas and the pH of the solution (Haskell et al. 2011). Ultraviolet radiation accelerates the dechlorination of water stored in the light. In large industrial applications the concentration of active chlorine within water is managed through chlorine injection, however in more direct applications chlorine solutions need to be change periodically. Little is known about the rate of degradation of chlorine solutions and efficacy of *Phytophthora* species disinfection in field applications.

Bleach has numerous benefits as a disinfectant as it is readily available, cost effective, can easily be stored and transported (Cayanan et al. 2009). It is often available as a concentrate or ready to use formulations where dosage is generally simple for direct use and application from small to large scale (Scarlett et al. 2016; Lee et al. 2019). Chlorination is often the most economical method of large volume water decontamination. High volume water treatment is most commonly applied with chlorine gas, but lower volumes can be treated with calcium or sodium hypochlorite. Chlorine is very effective in eliminating the risk of *Phytophthora* species contamination in water containing minimal organic and soil particulates where dosage is controlled (Hong et al. 2003). This means that chlorinated municipal water supplies are not a risk for the spread of *Phytophthora* pathogens, but the same is not necessarily true for unfiltered

surface water in which the presence of soil and organic particulates contribute to the loss of chlorine from solutions (Ivey and Miller 2013; Loyd et al. 2014).

There are several human health considerations needed for the practical use of chlorine based disinfectants. As a bleaching agent sodium hypochlorite usually contains between 1–5% sodium hypochlorite, has a pH of around 11 and is a skin, eye and respiratory irritant. Higher concentrates are commercially available; these contain 10–15% sodium hypochlorite, have a pH of 13, will burn the skin and eyes, is corrosive and can produce chlorine volatiles. Therefore, safety measures need to be taken when using chlorine based disinfectants to protect workers and the environment. Sodium hypochlorite is unstable and the active chlorine evaporates from solutions, disintegrates with heat or when in contact with acids, sunlight, certain metals and organic matter (Hong et al. 2003; Cayanan et al. 2009; Scarlett et al. 2016). This means chlorine will becoming less effective over time with dose and exposure periods dependent on contact time, storage and exposure to organic and metal compounds. As chlorine is a non-selective disinfectant, care must be taken to minimise environmental harm, especially in natural ecosystems. Appropriate application and disposal are essential.

3 SteriGENE

SteriGENE (TriGene Advanced) is a high level disinfectant used in a range of applications to kill viruses, mycobacteria, fungi and bacterial organisms (International 2020). It is used routinely in veterinary, health, commercial and agricultural settings to manage pathogen contamination (International 2020). SteriGENE is a mixture of halogenated tertiary amine and organic salts (<15%), polymeric biguanide, hydrochloride surface active agents, corrosion inhibitor, chelating agents, stabilising agents and demineralised water (International 2020) . Spray application of an earlier branded formulation of TriGene has been shown to effectively kill *P. agathidicida* zoospores on contact, mycelium with 8 days exposure and was associated with a significant reduction in oospore viability (Bellgard et al. 2011b). In these studies, no *P. agathidicida* was recovered in soils or surfaces directly treated and sprayed with TriGene, respectively (Bellgard et al. 2011b). However, the efficacy of TriGene over time and with ongoing accumulation of soil and organic material in solution, such as is found in kauri dieback cleaning stations and footbaths, has not yet been tested. Given the general activity of SteriGENE as a non-selective disinfectant, care must be taken to minimise their environmental impact with appropriate application and disposal to minimise environmental harm, especially in natural ecosystems.

4 Methylated Spirits

Alcohols have long been used as disinfectants for a range of microbial species and widely used for centuries in surgical applications to control infection (Harrington and Walker 1903; PRICE 1939). Ethanol and methylated spirits are commonly used in plant pathology laboratories and field work at concentrations of 70% v/v with exposure times ranging from 10 s to 5 min (Waller et al. 2002). Ethyl alcohol (ethanol) and Isopropyl alcohol (isopropanol, 2-propanol, or propan-2-ol) are commonly used at concentrations of 70% v/v to sanitize hands, tools, shoes, gloves and hard surfaces. Recent guidelines recommend the use of 60–90% alcohol for antisepsis, and disinfection is largely based on early studies dating back to the 1890's that focus on the bactericidal effects of alcohols. Alcohols have numerous benefits as disinfectants as they are relatively cheap and easy to obtain, are simple to use and achieve thorough surface contact on application to hard surfaces with no need for post treatment washing (Waller et al. 2002). While at low concentrations ethanol attracts but does not kill *Phytophthora* zoospores, at higher concentrations of 70–90% it is lethal (Allen and Newhook 1973).

Ethanol is most commonly available in the form of denatured alcohol (methylated spirits), which consists of a mixture of ethanol and methanol. Methylated spirits is often recommended as a disinfectant in the management of *Phytophthora* pathogens as it is cheap, readily available, portable and environmentally friendly (Suddaby and Liew 2008). In New Zealand, methylated spirits is formulated with either >95 % or 70% ethanol and other solvents including denatured benzoate, fluroscein and methyl violet to discourage consumption (Horn 2014). Ethyl alcohol and isopropanol are also available as rubbing alcohol in concentrations of 70–90% (in water) that can be used directly without further dilution.

Although ethanol and methylated spirits are commonly used in plant pathology laboratories and during field work, few systematic studies on the concentration by time effect of ethanol or methylated spirits exposure to *Phytophthora* pathogens have been carried out at the time-scales commonly used. James et al. (2012) found that ethanol at 70 and 90% concentrations prevented growth of *Phytophthora ramorum* zoospores and chlamydospores at exposure times of 5 and 10 min, respectively, but showed variable responses in mycelial growth. In this study, 7 mm mycelial plugs were exposed to ethanol and then plated onto agar to assess radial growth. This assay presumed diffusion of the disinfectant into the agar and did not replicate disinfection of naturally contaminated surfaces with soil or organic material (James et al. 2012). Our investigation of the literature found the application of ethanol and methylated spirits for surface decontamination by spray application and exposures up to 30 s as is commonly practiced but remains largely untested on Phytophthora pathogens and has not been assessed for *P. agathidicida.*

5 Heat, steam and boiling

Phytophthora species and other water moulds are relatively sensitive to heat with a direct relationship between lethal temperature and time (Shlevin et al. 2003; Ramsfield et al. 2010b). The most common methods for applying heat in operational activities are via steam or aerated steam (steam/air mixtures), hot water, dry heat, hot compost or solarisation (Ramsfield et al. 2010a; Funahashi 2015; Funahashi and Parke 2016). Moisture plays a significant role in heat treatment with moist heat being substantially more effective than dry heat in killing *Phytophthora* propagules (Lu et al. 2010).

A considerable number of studies have investigated the response of *Phytophthora* species to heat under a range of conditions and targeting different substrates. Moist heating of materials to between 50–60°C or higher for at least 30 min will kill most plant pathogenic fungi and is sufficient to kill *Phytophthora* species (Bollen 1969; Browning et al. 2008; Linderman and Davis 2008; Lu et al. 2010). In a recent study, Horner and Arnet (2019) showed that *P. agathidicida* is similarly sensitive to heat with no growth observed after incubation at 40°C or higher for 24 h or more or at 45°C for 4 h of exposure. Standardised protocols for the heat treatment of bulk potting mix, contaminated water and wood over extended periods to eliminate plant pathogens are well documented. Each of these is reliant on the uniform distribution of heat and a target temperature being achieved throughout the material being treated (Runia and Amsing 2001a; Funahashi 2015; Funahashi and Parke 2016; Ramsfield et al. 2016).

While many studies have investigated extended periods of heat exposure for bulk treatment, fewer studies have investigated the temperature response at higher temperatures for short periods. As little as 15 s at 44°C is sufficient to kill *Phytophthora cryptogea* (Runia and Amsing 2001a). From this study, standard treatments of heating water to 95°C for 30 s and 85°C for 3 min have been recommended to encompass more recalcitrant viral and fungal pathogens (Runia and Amsing 2001b). Such protocols suggest that boiling and the direct application of high pressure steam present low-technology options for pathogen decontamination from a range of potentially contaminated surfaces. However, we found no studies that have specifically investigated very short exposures to direct applications of steam or boiling to eliminate *Phytophthora* species from surfaces or small quantities of contaminated materials. Such information would help inform protocols for steam cleaning and non-chemical treatment of contaminated equipment.

6 Conclusions

Each of the disinfection methods discussed here have the potential for use in the management of *P. agathidicida.* Despite considerable amounts of work investigating the response of *Phytophthora* pathogens to various disinfection protocols, the specific conditions in which such methods are used in practice remain largely untested. In each case, a key consideration must be the target material being treated. Such information is critical in ensuring that the appropriate approach is taken to disinfection for the material being treated. The establishment of treatment dose and response curves for natural inoculum of *P. agathidicida* across key disinfection treatments will enable management prescriptions to be established for a range of applications without the need for repeated optimisation for specific treatment scenarios. For *P. agathidicida* it is therefore important to consider the decontamination of infected root and organic material when testing the efficacy of treatments.

The potential environmental impacts and risks of using some chemicals also needs consideration for their routine application within natural ecosystems. In the case of chlorine and SteriGENE consideration should be used to minimising the volumes being applied and potentially discharged into sensitive natural ecosystems.

7 References

Allen R, Newhook F. 1973. Chemotaxis of zoospores of *Phytophthora cinnamomi* to ethanol in capillaries of soil pore dimensions. Transactions of the British Mycological Society. 61(2): 287–IN12.

Bellgard S, Paderes E, Beever R. 2011a. Comparative efficacy of disinfectants against Phytophthora Taxon Agathis (PTA). In: Research MWL ed.

Bellgard S, Paderes E, Beever R. 2011b. Comparative efficacy of disinfectants against Phytophthora Taxon Agathis (PTA). In: Research L ed http://wwwkauridiebackconz/home/publicationsaspx. Landcare Research, Private Bag 92170, Auckland 1142, New Zealand.

Best M, Sattar S, Springthorpe V, Kennedy M. 1990. Efficacies of selected disinfectants against *Mycobacterium tuberculosis*. Journal of Clinical Microbiology. 28(10): 2234–2239.

Bollen G. 1969. The selective effect of heat treatment on the microflora of a greenhouse soil. Netherlands Journal of Plant Pathology. 75(1-2): 157–163.

Browning M, Englander L, Tooley PW, Berner D. 2008. Survival of *Phytophthora ramorum* hyphae after exposure to temperature extremes and various humidities. Mycologia. 100(2): 236–245.

Cayanan DF, Zheng YB, Zhang P, Graham T, Dixon M, Chong C, Llewellyn J. 2008. Sensitivity of five container-grown nursery species to chlorine in overhead irrigation water. Hortscience. 43(6): 1882–1887.

Cayanan DF, Zhang P, Liu W, Dixon M, Zheng Y. 2009. Efficacy of chlorine in controlling five common plant pathogens. HortScience. 44(1): 157–163.

Cheah L, Marsh A, McNeill M, Hedderley D. 2009. Evaluation of disinfectant products for microbial decontamination of imported used footwear. New Zealand Plant Protection. 62: 130–135.

Colquhoun I, Hardy GSJ. 2000. Managing the risks of Phytophthora root and collar rot during bauxite mining in the *Eucalyptus marginata* (Jarrah) forest of Western Australia. Plant Disease. 84(2): 116–127.

Datnoff L, Kroll T, Lacy G. 1987. Efficacy of chlorine for decontaminating water infested with resting spores of *Plasmodiophora brassicae*. Plant disease. 71(8): 734–736.

Fichtner E, Rizzo D, Kirk S, Webber J. 2009. Epidemiology of *Phytophthora kernoviae* in UK woodlands and heathland and risk to North American forests. Phytopathology, vol 99. p. S35.

Funahashi F. 2015. Modeling Survival of Soilborne Phytophthora spp. and Characterizing Microbial Communities in Response to Soil Solarization and Biocontrol Amendment in Container Nursery Beds.

Funahashi F, Parke J. 2016. Effects of soil solarization and *Trichoderma asperellum* on Soilborne Inoculum of *Phytophthora ramorum* and *Phytophthora pini* in container nurseries. Plant Disease. 100(2): 438–443.

Gehr R, Wagner M, Veerasubramanian P, Payment P. 2003. Disinfection efficiency of peracetic acid, UV and ozone after enhanced primary treatment of municipal wastewater. Water Research. 37(19): 4573–4586.

Gómez-Gálvez F-J, Vargas-Osuna E, Rodríguez-Jurado D. 2018. Suppressive and preventive activity of chemical disinfectants against sclerotia of *Verticillium dahliae* in water. Crop protection. 108: 12–22.

Harrington C, Walker H. 1903. The Germicidal Action of Alcohol. The Boston Medical and Surgical Journal. 148(21): 548–552.

Haskell D, Huang J, Meador D, Fisher P, Mohammad-Pour G. 2011. Water sanitizing agents such as chlorine and chlorine dioxide interact with peat substrate and suspended solids. International Symposium on Growing Media, Composting and Substrate Analysis 1013. p. 279–284.

Hong CX, Richardson PA, Kong P, Bush EA. 2003. Efficacy of Chlorine on Multiple Species of Phytophthora in Recycled Nursery Irrigation Water. Plant Dis. 87(10): 1183–1189.

Horn B. 2014. Health risk assessment: methylated spirits New Zealand Institute of Environmental Science and Research Limited.

International EA. 2020. SteriGENE® Green Technical Data Sheet. [accessed June 2020]. https://www.hygienedirect.nz/wp-content/uploads/2016/10/STERIGENE-MDS-Info.pdf

Ivey ML, Miller S. 2013. Assessing the efficacy of pre-harvest, chlorine-based sanitizers against human pathogen indicator microorganisms and *Phytophthora capsici* in non-recycled surface irrigation water. Water research. 47(13): 4639–4651.

James D, Varga A, Becker E, Sumampong G, Bailey K, Elliott M, Masri S, Shamoun SF. 2012. Screening of several disinfectants to assess their efficacy in controlling mycelia growth, sporangia germination, and recovery of viable Phytophthora ramorum. Crop protection. 42: 186–192.

Lee SH, Shin H, Kim JH, Ryu KY, Kim HT, Cha B, Cha JS. 2019. Effect on Colony Growth Inhibition of Soil-Borne Fungal Pathogens by Available Chlorine Content in Sodium Hypochlorite (NaOCl). Plant Pathol J. 35(2): 156–163.

Lewis Ivey ML, Miller SA. 2013. Assessing the efficacy of pre-harvest, chlorine-based sanitizers against human pathogen indicator microorganisms and *Phytophthora capsici* in non-recycled surface irrigation water. Water Res. 47(13): 4639–51.

Linderman R, Davis E. 2008. Eradication of *Phytophthora ramorum* and other pathogens from potting medium or soil by treatment with aerated steam or fumigation with metam sodium. HortTechnology. 18(1): 106–110.

Loyd A, Benson D, Ivors K. 2014. Phytophthora populations in nursery irrigation water in relationship to pathogenicity and infection frequency of Rhododendron and Pieris. Plant Disease. 98(9): 1213–1220.

Lu P, Aimonino DR, Gilardi G, Gullino ML, Garibaldi A. 2010. Efficacy of different steam distribution systems against five soilborne pathogens under controlled laboratory conditions. Phytoparasitica. 38(2): 175–189.

PRICE PB. 1939. Ethyl alcohol as a germicide. Archives of Surgery. 38(3): 528–542.

Ramsfield T, Ball R, Gardner J, Dick M. 2010a. Temperature and time combinations required to cause mortality of a range of fungi colonizing wood. Canadian Journal of Plant Pathology. 32(3): 368–375.

Ramsfield T, Bentz B, Faccoli M, Jactel H, Brockerhoff E. 2016. Forest health in a changing world: effects of globalization and climate change on forest insect and pathogen impacts. Forestry. 89(3): 245–252.

Ramsfield TD, Ball RD, Gardner JF, Dick MA. 2010b. Temperature and time combinations required to cause mortality of a range of fungi colonizing wood. Canadian Journal of Forest Pathology. iFirst: 1–8.

Runia WT, Amsing J. 2001a. Lethal temperatures of soilborne pathogens in recirculation water from closed cultivation systems. Acta horticulturae.

Runia WT, Amsing JJ. 2001b. Disinfection of Recirculation Water from Closed Cultivation Systems by Heat Treatment. Acta Horticulturae(548): 215–222.

Russell A. 1983. Mechanisms of action of chemical sporicidal and sporistatic agents. International journal of pharmaceutics. 16(2): 127–140.

Scarlett K, Collins D, Tesoriero L, Jewell L, van Ogtrop F, Daniel R. 2016. Efficacy of chlorine, chlorine dioxide and ultraviolet radiation as disinfectants against plant pathogens in irrigation water. European journal of plant pathology. 145(1): 27–38.

Shlevin E, Saguy IS, Mahrer Y, Katan J. 2003. Modeling the survival of two soilborne pathogens under dry structural solarization. Phytopathology. 93(10): 1247–1257.

Suddaby T, Liew E. 2008. Best practice management guidelines for Phytophthora cinnamomi within the Sydney metropolitan catchment management authority area: Botanic Gardens Trust, Sydney.

Tomasino SF. 2005. Efficacy testing of disinfectants. Journal of AOAC International. 88(1): 355–358.

Waller J, Lenne J, Waller S. 2002. Detection and isolation of fungal and bacterial pathogens. Plant pathologist's pocketbook. 3: 208–215.

Williams N, Arnet M. 2020. Project Proposal: MPI PA Oospore Deactivation. Ministry for Primary Industry: Plant and Food Research.

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