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Efficacy of chlorine, chlorine dioxide and ultraviolet radiation as disinfectants against plant pathogens in irrigation water

Kelly Scarlett · Damian Collins · Len Tesoriero · Luke Jewell · Floris van Ogtrop · Rosalie Daniel

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Abstract A number of disinfection treatments are available to treat irrigation water to reduce the risk of plant disease. However, limited published studies had compared the efficacy of disinfection treatments on a range of plant pathogen species, on their various life stages and in different water qualities. In this study, propagules (spores, mycelium, cells) of eight plant pathogens including *Clavibacter michiganensis* subsp. *michiganensis*, *Alternaria alternata*, *Chalara elegans*, *Colletotrichum gloeosporioides*, *Calonectria pauciramosa*, *Fusarium oxysporum*, *Phytophthora cinnamomi* and *Pythium aphanidermatum* were exposed to chlorine (sodium hypochlorite), chlorine dioxide and ultraviolet radiation (UV) at a range of application rates and exposure times, in deionised water and dam water. The efficacy of treatments varied with exposure time, application rate, water type and the pathogen and propagule. *Cl. michiganensis* subsp. *michiganensis*, *Ph. cinnamomi* and *Py. aphanidermatum* propagules were most sensitive to all treatments, while propagules of *Ch. elegans*, *Ca. pauciramosa* and *F. oxysporum* required the highest rates and longest exposure times to chlorine, chlorine dioxide and UV to kill >99 % CFUs. Chlorine dioxide, applied as a "shock" treatment at a high rate for a limited time period, and UV radiation offered more effective biocidal activity than the chlorine levels tested in both water types. This study demonstrates that sensitivity to disinfection treatments and application rates varies between pathogens, and between propagules of the same pathogen.

Keywords Plant pathogen · Irrigation water · Disinfection · Nursery · Hydroponic · Plant disease

K. Scarlett · F. van Ogtrop
Faculty of Agriculture and Environment, Biomedical Building C81, The University of Sydney, Sydney, NSW 2006, Australia

D. Collins · L. Jewell · R. Daniel (✉)
Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Private Bag 4008, Narellan, NSW 2567, Australia
e-mail: rosalie.daniel@dpi.nsw.gov.au

L. Tesoriero
NSW Department of Primary Industries, Central Coast Primary Industries Centre, Locked Bag 26, Gosford, NSW 2250, Australia

Introduction

Water used for irrigation in plant nursery and horticultural production systems is frequently captured for reuse or sourced from open water systems. Reuse conserves water supplies, protects the environment from nutrient and pesticide run-off, and reduces production costs associated with purchasing water (Hong 2014; Stewart-Wade 2011). However, reusing water in production systems can increase the risk of plant disease (Hong and Moorman 2005). The reuse or recycling of such water may introduce plants pathogens into the water source, potentially providing a habitat, increasing inoculum pressure, the risk associated with infection, disease incidence and production loss.

Sampling in nurseries and intensive horticulture has revealed that a range of plant pathogens can occur and

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be spread in irrigation water. While *Phytophthora* spp. and *Pythium* spp. are commonly reported in irrigation water (Ali-Shtayeh and MacDonald 1991; Bush et al. 2003; Hong et al. 2003; Pittis and Colquhoun 1984; Thinggaard and Andersen 1995; Yang et al. 2013), several other pathogens including *Fusarium* spp., *Colletotrichum* spp. and a number of viruses have also been shown to occur (Fisher 2011; Jenkins and Averre 1983). These pathogens are responsible for diseases including damping off, root rots, shoot dieback and foliar blights. While some pathogens, such as *Alternaria* spp., are likely to be detected in irrigation water due to their prevalence in the environment, their epidemiological and economic significance in horticulture is not well understood (Hong 2014; Hong and Moorman 2005).

Plant pathogens found in irrigation water may originate from a number of sources (Hong and Moorman 2005). The pathogens may naturally occur in the water reservoir, or in the surrounding soil or plants, being washed into the reservoir following rainfall events. Alternatively, they may be introduced to the production system via externally-sourced propagation or potting materials, workers, visitors and equipment brought on to the site. Once in the nursery, the pathogen can be disseminated through the irrigation system and through surface run-off or drainage channels back to the storage reservoir. Pathogen levels in irrigation water may range from a few to many thousand propagules/L depending on a range of factors including the plant species present in the nursery, nursery hygiene, drainage infrastructure at the site and seasonality (Hong et al. 2003). However, even low pathogen numbers can cause disease when conditions are favourable. Oomycete plant pathogens such as *Phytophthora* spp. and *Pythium* spp. are commonly found in irrigation water and can cause root rots, damping off and a number of other symptoms in susceptible hosts (MacDonald et al. 1994; Thinggaard and Middelboe 1989; Taylor 1977). *Phytophthora* and *Pythium* species rely on the presence of free water for reproduction and spread. Both may release large numbers of motile zoospores, the primary infective propagule, as well as other survival structures that can be disseminated large distances in water. Following infection of a plant, *Phytophthora* spp. and many *Pythium* spp., can produce sporangia from which more zoospores are released back into the irrigation water with the potential to initiate more infections. *Phytophthora* spp. also produce chlamydospores and oospores, that enable them to survive adverse conditions for relatively long periods of time. Some *Pythium* spp. produce chlamydospores and oospores, as well as hyphal swellings. All of these pathogen propagules may be disseminated through water. Mycelium and a range of spore types from non-aquatic fungal and bacterial plant pathogens can also be disseminated through irrigation water (Hong and Moorman 2005). Several bacteria have also been reported to survive in, and be disseminated in irrigation water (Cappaert et al. 1988; Huang and Tu 2001). As a result, water reservoirs may become effective reproduction and dissemination systems for plant pathogens. Disinfection of recycled water for irrigation is therefore beneficial as a phytosanitary measure to reduce the risk of the development of plant disease.

A number of methods are available for disinfection of irrigation water including oxidising agents, filtration, ultraviolet radiation (UV) and heat treatment. Plant pathogens have been shown to differ in their sensitivity to disinfection treatments between different pathogen species and propagules types (Cayanan et al. 2009; Hong et al. 2003; Lang et al. 2008; Yamamoto et al. 1990; Zhang and Tu 2000). The efficacy of the specific disinfection treatment is influenced by factors such as inorganic and organic chemical characteristics, temperature and pH of the water as well as the presence of microbes and particulate matter (Stewart-Wade 2011). Consequently, it cannot be assumed that the same disinfectant application rate or contact time will be effective for all pathogens, propagule types and water qualities.

Chlorine, as either sodium hypochlorite or calcium hypochlorite, is commonly used to treat irrigation water as it is easy to apply, relatively persistent and inexpensive. The presence of chlorine and its residual can also be measured to ensure a biocidal dose is achieved. When chlorine is introduced to water, it reacts to form free chlorine species of hypochlorous acid (HOCl) and hypochlorite (OCl^-) ions, subject to the pH of the water, which oxidise organic materials, including any pathogens present in the water (Zheng et al. 2014a). The optimum level of chlorine to treat pathogens in water varies with the pathogen as well as the quality, pH and temperature of the water (Cayanan et al. 2009; Zheng et al. 2014a). The more organic matter present in the water, the greater the rate of quenching (deactivation) of free chlorine and hypochlorous acid in the water. The efficacy of chlorine disinfection is particularly limited by the pH of the water. Hypochlorous acid is the stronger, faster oxidiser, but is more prevalent when the pH of

the water is between 6.5 and 7. As the pH of water increases, the hypochlorous acid is converted to hypochlorite, which is a weaker oxidiser and disinfectant.

Chlorine dioxide (ClO_2) also acts as a disinfectant by oxidising organic matter, including pathogens. Chlorine dioxide exists as a dissolved gas in water and has a greater oxidising strength than hypochlorite salts. Newman (2004) reports that chlorine dioxide is at least 1.2 times more effective than sodium hypochlorite as a disinfectant. Like chlorine, chlorine dioxide is also affected by the presence of organic matter in water, but it is effective across a wider pH range (4–10). Chlorine and chlorine dioxide can be applied as a 'shock' treatment, at a high rate for a short period. Some commercial units also deliver chlorine dioxide continuously at lower rates (<1 ppm).

Ultraviolet radiation is applied at a wavelength of 254 nm to disinfect irrigation water. The energy discharged from the UV light reacts with the DNA and RNA of any microorganisms present, essentially eliminating the ability of vulnerable fungi, bacteria and viruses to be infectious. Effective disinfection depends on duration and intensity of exposure of the water, which are related to water flow. The efficacy of UV treatment is also dependent on the quality of the water, in particular turbidity and organic matter content (Zheng et al. 2014c). The UV energy is absorbed by, and reflected off, particles in the water. Therefore, the more organic and particulate matter present in the water, the less effective the UV treatment. In cases where plant pathogens are harboured inside organic matter or mucilage suspended in water, they may be protected from exposure to UV and other disinfectants, highlighting the advantage of filtration prior to treatment with disinfectants. The current recommended dosage rates for recycled irrigation water are 100 mJ/cm^2 for selective disinfection and 250 mJ/cm^2 for complete elimination of most fungal, bacterial and viral plant pathogens (Newman 2004; Runia 1995).

Pathogens present in nursery production systems and irrigation water also vary in their biology, and consequently, their sensitivity to disinfection treatments. Cayanan et al. (2009) showed that the free chlorine threshold and critical contact time for control of *Pythium aphanidermatum*, *Phytophthora cactorum*, *Ph. infestans*, *Fusarium oxysporum* and *Rhizoctonia solani* ranged between 0.3 and 12 mg/L for 3–10 min. Zoospores of *Phytophthora nicotianae* have been shown to be killed following exposure to 2 mg/L free chlorine for as little as 0.25 min while mycelial fragments required 8 min exposure to the same concentration, or exposure to 8 mg/L for 0.25 min for mortality (Zheng et al. 2008). At free chlorine concentrations of less than 8 mg/L mycelial fragments were still able to produce sporangia, from which zoospores are released and new infections may be initiated. The spores and hyphae of some pathogens, such as *Alternaria* sp. and *Calonectria* sp., have a melanised cell wall, which may be more difficult for certain disinfectant treatments to penetrate than spores of pathogens that lack pigmentation or a cell wall (e.g. *Phytophthora* spp.). Additionally, oomycete cell walls are composed of cellulose, while fungal cell walls are composed of chitin (Agrios 2005). Differences in cell wall components could result in a difference in treatment penetration and disinfection between fungi and oomycetes.

The chemical, physical and biological factors of both the water and the pathogen make it challenging to provide uniform guidelines on the required concentrations for chemical disinfectants to be effective against all pathogens. Although a number of plant pathogens are represented in the literature, data demonstrating the efficacy of common water disinfection treatments to enable comparison between pathogens and propagules is limited. The objective of this study was to assess the efficacy of a selection of application rates and times of chlorine, chlorine dioxide and UV radiation against different propagules of eight common plant pathogens in two water qualities.

Materials and methods

Pathogen and propagule culture

Plant pathogens and their propagules evaluated for sensitivity to chlorine, chlorine dioxide and UV radiation are listed in Table 1.

Clavibacter michiganensis subsp. *michiganensis* was cultured on Kings B medium (KB). Cell suspensions were prepared by transferring a single colony to 10 mL Luria-Bertani (LB) broth and shaken at 150 rpm at 25 °C in the dark for 16 h until the OD 600 reached 0.5–0.7 (Xu et al. 2010). To enumerate the bacterial cells, 10-fold dilutions were spread onto KB medium and incubated at 25 °C for 48 h in the dark.

To produce mycelial fragments all fungi and oomycetes cultures were grown on potato dextrose agar

Table 1 Pathogen name and propagule type for each pathogen tested

Pathogen	Propagule
Clavibacter michiganensis subsp. michiganensis	Bacterial cells
Alternaria alternata	Conidia Mycelium
Chalara elegans (*Thielaviopsis basicola*)	Chlamydospores Endoconidia Mycelium
Colletotrichum gloeosporioides	Conidia Mycelium
Calonectria pauciramosa (*Cylindrocladium pauciramosum*)	Conidia Chlamydospores Mycelium
Fusarium oxysporum	Conidia Chlamydospores Mycelium
Phytophthora cinnamomi	Zoospores Cysts Oospores Sporangia Mycelium
Pythium aphanidermatum	Zoospores Oospores Mycelium

(PDA) at 22 °C in the dark for 5–7 days. Mycelium was harvested aseptically and homogenized in a blender (Phillips Electronics Australia Limited) for 30 s with sterile deionised water to obtain mycelial fragments.

Conidia of *A. alternata*, *Ch. elegans*, *Co. gloeosporioides*, *Ca. pauciramosa* and *F. oxysporum* (macro- and micro-conidia) were prepared by growing cultures on ¼ strength PDA (¼PDA) at 22 °C, under a 12 h light:dark regime, for 14 days. Plates were flooded with sterile distilled water and scraped with a sterile rod to detach spores. The spore suspension was filtered through multiple layers of Kimwipes™ (Kimberly Clark USA) or sterile muslin cloth to remove mycelial fragments.

Sporangia and zoospores of *Py. aphanidermatum* and *Ph. cinnamomi* were produced according to the method of (Raftoyannis and Dick 2002). Cysts were produced by incubating zoospore suspensions on the bench for between 30 and 60 min until zoospores had encysted. Encystment was confirmed microscopically. Oospores of *Ph. cinnamomi* were produced according to the method of Saul-Maora (2008). Oospores of *Py. aphanidermatum* were produced by flooding 10–14 day old cultures, grown on 10 % V8 medium, with sterile water, scraping hyphae off and filtering the suspension through sterile muslin cloth to separate the oospores.

Chlamydospores of *Ch. elegans*, *Ca. pauciramosa* and *Fu. oxysporum* were produced according to Bennett and Davis (2013).

Suspensions of all fungal and oomycete propagules were quantified using a haemocytometer and diluted with sterile deionised water or autoclaved dam water to obtain 1500 propagules/mL prior to testing of chlorine and chlorine dioxide treatments. Due to the experimental system, applied propagule concentrations for the UV trial varied for some pathogens.

Water sources and water analysis

Deionised water and dam water were used in the experiments. Dam water was sourced from a commercial nursery at Kemps Creek, NSW, Australia. Water was sent for analysis to the NSW Department of Primary Industries (DPI) Diagnostic and Analytical Services Wollongbar, NSW, at each collection time. Water was analysed for pH, electrical conductivity (EC), total dissolved solids (TDS), $CaCO_3$, and key elements including N, P, K, Fe, Mg, Mn, Na, Cl and Cu. Suspended solids and turbidity were also determined. The pH of the dam water ranged between 7.7 and 8.0. The deionised water had a pH of 6.5 and a turbidity of 0.32 NTU.

The turbidity of the dam water ranged between 20 and 87 Nephelometric Turbidity Units (NTU), with a pH between 7.7 and 8.0 at the different sampling times. Dam water was diluted with deionised water and measured using a UVT meter (Real UV254 P200, Real Tech Inc., Canada) to achieve 50 % UVT prior to use in the UV tests. The dam water used in the chlorine and chlorine dioxide tests had a turbidity of 20 NTU and was not adjusted to 50 % UVT.

Chlorine and chlorine dioxide treatments

The efficacy of three disinfectant treatments, chlorine, chlorine dioxide and UV radiation, was tested against the 22 pathogen propagules according to the application rates and exposure times presented in Table 2.

Chlorine was applied to each water type as sodium hypochlorite (NaOCl) (Sigma-Aldrich, Australia). To neutralise the chlorine prior to plating the propagule suspension onto culture medium, 5 % sodium

Table 2 Exposure times and application rates for the disinfection treatments tested

Treatment	Time (min)	Rate/Concentration
Chlorine (sodium hypochlorite)	0, 10, 20, 30	0, 1, 2, 5 ppm
Chlorine dioxide	0, 4, 8, 10	0, 1, 3, 5 ppm
UV transmission (254 nm)	–	0, 113, 250 mJ/cm2

thiosulfate was added to a 1 mL aliquot of the propagule suspension (Hong et al. 2003).

Chlorine dioxide (Clean Oxide™, Natural Water Solutions, Perth, WA) was prepared to achieve the required concentrations according to manufacturer's directions. Free chlorine was measured at the start of the treatments and at the final sampling time using a photometer (ProMinent Fluid Controls Pty Ltd., Australia).

Appropriate quantities of chlorine or chlorine dioxide were added to each propagule suspension to achieve required concentrations in a total volume of 100 mL.

UV radiation treatment

A UV system was constructed in the laboratory to test the effectiveness of UV radiation. A UV lamp and control system (UV-Guard Service Australia Pty Ltd., Castle Hill NSW) was connected to a water storage tub via plastic irrigation tubing (25 mm diameter) with a 32 mm diameter outlet. A flow meter was connected to the system to monitor flow rate. UV exposure was calculated using the flow rate and dose calculation model supplied by the distributor. The calculator was set with a fixed variable of 50 % ultraviolet transmittance (UVT). Dam water was mixed with deionised water to achieve 50 % UVT prior to use in the experiments, while deionised water (UVT 100 %) was used as the control. Five litres of a known concentration of each propagule suspension was passed through the UV system at the calculated flow rate to achieve the required UV exposure (113 mJ/cm^2 and 250 mJ/cm^2). Pathogen propagules were added to the water storage source at the inlet.

Sampling and microbial counts

To determine the effect of exposure to each treatment on propagule survival, 100 μL of propagule suspension was sampled at required times (Table 2). Fungal and oomycete propagule samples were spread onto ¼PDA amended with 100 mg/L streptomycin and bacterial samples were spread onto KB medium. Three replicate tests of each propagule were conducted for each disinfection treatment, exposure time, application rate and water combination and three replicate samples were plated out. Culture plates were incubated at 22 °C for 1–5 days and checked daily for pathogen growth. The number of viable propagules was determined by comparing the number of growing colonies from treated samples with those in the untreated (control) samples (Cayanan et al. 2009; Hong et al. 2003).

Statistical analysis

The viable colony forming units (CFU) per mL for each replicate were calculated as an average across the three replicate samples. Viable CFUs were determined by comparing treated with untreated samples. All analyses were performed in the R statistical software environment (R Core Team 2014). For chlorine and chlorine dioxide, separate analyses were conducted for each pathogen/propagule type by treatment and water type. Each analysis was a factorial plus control ANOVA with effects of treatment (1 DF), time (3 DF), treatment by time (2 DF), rate within treatment (2 DF), and time by rate within treatment (4 DF). For the UV treatments, separate analyses were conducted for each pathogen/propagule, with ANOVA for combined water types to identify rate by water type interaction effects, as well as separate ANOVA to test rate effects within water type. The minimum rate to achieve >99 % kill was determined as follows: for each rate and time, the ratio of the mean propagules/mL to zero rate for each rate (and time) was calculated along with an approximate SE using the delta method, giving an approximate 95 % confidence interval as ratio +/− SE. Time was not tested for UV so not included in the analyses for these data. An upper 95 % confidence interval, of less than 0.01, indicated that the given rate and time killed >99 % of the pathogen (with 95 % confidence). The lowest rate and time required to achieve this outcome was calculated.

Results

The efficacy of the three disinfection treatments tested varied between pathogens and propagules types with application rate, time, water quality characteristics (pH and turbidity) (Tables 3 and 4). All three disinfection treatments applied significantly ($P < 0.001$) affected propagule survival. In some cases, there was no significant effect of time, rate, or rate by time for chlorine and chlorine dioxide treatments. This invariably corresponded to cases where the lowest rate of treatment at the shortest contact time resulted in complete mortality of the pathogen. A significant interaction of rate and time corresponded to treatments in which there was an effect of contact time at the lower exposure rate, but little or no effect of contact time at the higher application rates.

Chlorine dioxide, chlorine and UV killed >99 % of *Cl. michiganensis* subsp. *michiganensis* CFUs at all rates and times tested in both deionised water and dam water.

Chlorine dioxide was more effective than chlorine against *A. alternata*. Exposure to at least 5 ppm chlorine for 30 min was required to kill >99 % of conidia and mycelial fragments of *A. alternata*. Chlorine dioxide killed >99 % of conidia and mycelial fragments of *A. alternata* in both deionised and dam water when exposed for 4 min or more at a concentration of 5 ppm. The UV treatment killed *A. alternata* conidia and mycelium in deionised water at both 113 and 250 mJ/cm^2. In dam water, at both 113 and 250 mJ/

Table 3 Calculated minimum application rate and exposure time required to kill 99 % of propagules tested following exposure to chlorine and chlorine dioxide. A '–' indicates that propagules were not killed at the rates tested

Pathogen	Propagule	Chlorine (NaClO)				Chlorine dioxide			
		Deionised water		Dam water		Deionised water		Dam water	
		Rate (ppm)	Time (min)	Rate (ppm)	Time (min)	Rate (ppm)	Time (min)	Rate (ppm)	Time (min)
Clavibacter michiganensis	Bacterial cells	1	10	1	10	1	4	1	4
Alternaria alternata	Conidia	5	20	–	–	5	4	5	4
	Mycelium	5	20	5	30	3	4	5	4
Chalara elegans	Chlamydospores	2	20	5	20	3	4	5	4
	Endoconidia	5	30	–	–	5	4	1	10
	Mycelium	5	30	–	–	5	4	3	8
Colletotrichum gloeosporioides	Conidia	1	10	5	10	1	4	1	4
	Mycelium	5	10	–	–	1	4	3	4
Calnectria pauciramosa	Conidia	2	20	5	30	1	4	3	10
	Chlamydospores	2	20	5	30	3	4	5	10
	Mycelium	1	30	5	30	3	4	3	10
Fusarium oxysporum	Conidia	1	10	5	10	1	4	1	4
	Chlamydospores	5	20	–	–	1	4	5	4
	Mycelium	5	10	–	–	1	4	3	4
Phytophthora cinnamomi	Zoospores	1	10	1	10	1	4	1	4
	Cysts	1	10	1	10	1	4	1	4
	Oospores	2	10	1	30	3	4	3	4
	Sporangia	2	10	1	20	3	4	3	4
	Mycelium	5	10	2	30	3	4	3	4
Pythium aphanidermatum	Zoospores	1	10	5	10	1	4	3	4
	Chlamydospores	5	20	–	–	1	4	3	4
	Mycelium	5	20	–	–	1	4	3	4

Table 4 Calculated minimum exposure required to kill 99 % of propagules tested following treatment with UV radiation. A '–' indicates that propagules were not killed at the rates tested

Pathogen	Propagule	Deionised water (mJ/cm^2)	Dam water (mJ/cm^2)
Clavibacter michiganensis	Bacterial cells	113	113
Alternaria alternata	Conidia	250	–
	Mycelium	250	–
Chalara elegans	Chlamydospores	113	113
	Endoconidia	113	250
	Mycelium	113	113
Colletotrichum gloeosporioides	Conidia	113	113
	Mycelium	113	113
Calonectria pauciramosa	Conidia	250	–
	Chlamydospores	–	–
	Mycelium	250	–
Fusarium oxysporum	Conidia	250	–
	Chlamydospores	250	–
	Mycelium	250	–
Phytophthora cinnamomi	Zoospores	113	113
	Cysts	113	113
	Oospores	113	113
	Sporangia	113	113
	Mycelium	113	113
Pythium aphanidermatum	Zoospores	113	113
	Oospores	113	113
	Mycelium	113	113

cm^2, 94.5 % of mycelia were killed and 93 % of conidia were killed.

Exposure to chlorine dioxide was the most effective treatment against propagules of *Ch. elegans* in both deionised and dam water. In deionised water, effective kill (>99 %) of each *Ch. elegans* propagule by chlorine exposure was achieved only at the highest rate and contact time of 5 ppm for 30 min. In dam water, only 34 % of endoconidia were killed after exposure to 5 ppm chlorine for 30 min. Endoconidia did not survive 3 ppm chlorine dioxide when exposed for at least 8 or 10 min in deionised and dam water, respectively. Chlamydospores were sensitive to chlorine dioxide when exposed to 3 ppm in deionised water, or 5 ppm in dam water, for at least 4 min. UV exposure killed >99 % all propagules of *Ch. elegans* in deionised water and dam water at both 113 and 250 mJ/cm^2 with the exception of endoconidia in dam water, where only 61 % were killed.

In dam water, chlorine was ineffective at killing >99 % of *Co. gloeosporioides* mycelial fragments at any of the time or rates tested, while chlorine treatment with 5 ppm for 10 min killed >99 % of *Co. gloeosporioides* conidia. Chlorine treatment was required at 2 ppm for 20 min for >99 % kill of *Co. gloeosporioides* mycelium in deionised water. Chlorine dioxide and UV killed >99 % of *Co. gloeosporioides* mycelia and conidia in deionised water at the lowest application rates and exposure times tested. Chlorine dioxide was less effective at killing mycelial fragments in dam water, requiring exposure to more than 3 ppm for 4 min for effective disinfection.

Chlorine killed >99 % conidia, chlamydospores and mycelial fragments of *Ca. pauciramosa* in deionised water when applied at 1 ppm for 30 min. In dam water, chlorine treatment for more than 30 min at 5 ppm was required to kill >99 % of propagules of *Ca. pauciramosa*. When treated for 4 min or more at rates of 3 ppm or greater chlorine dioxide killed >99 % of all propagules of *Ca. pauciramosa* tested. UV treatment did not kill >99 % of *Ca. pauciramosa* conidia at any of the application rates tested in either deionised or dam water. Chlamydospores and mycelium were effectively

killed by exposure to UV at 250 mJ/cm^2 in deionised water, however in dam water only >99 % of mycelial fragments were killed at 250 mJ/cm^2.

Chlorine treatment was less efficient against *F. oxysporum* in dam water than in deionised water. In deionised water, exposure to 5 ppm of chlorine for at least 10 min was required to kill >99 % of conidia and mycelium and 20 min was required to kill >99 % of chlamydospores. In dam water, exposure to 5 ppm of chlorine for 10 and 20 min was required to kill conidia and chlamydospores, respectively. In dam water, chlorine was ineffective at killing *F. oxysporum* mycelium at any of the tested rates or times. When exposed to chlorine dioxide for at least 4 min at 1 ppm in deionised water >99 % of *F. oxysporum* propagules tested were killed. Chlorine dioxide also killed >99 % of all *F. oxysporum* propagules in dam water at 3 ppm for 8 min exposure, while exposure to 1 ppm chlorine dioxide for 4 min was required to kill >99 % chlamydospores and mycelium in deionised water. UV applied at 250 mJ/cm^2 killed 99 % of all *F. oxysporum* propagules tested in deionised water, however in dam water, neither of the UV rates tested killed the propagules.

Zoospores and cysts of *Ph. cinnamomi* were sensitive to all three disinfection treatments tested in both deionised and dam water and were killed (>99 %) at the lowest treatment, rates and times. A longer exposure time of 30 min in 1 ppm chlorine was required to killed >99 % of *Ph. cinnamomi* oospores and sporangia in deionised water. In dam water, an exposure time to chlorine of at least 20 min was required to kill >99 % of oospores and sporangia. Mycelial fragments were killed (>99 %) by chlorine at 1 ppm for 30 min in both deionised and dam water. Mycelial fragments were killed (>99 %) when exposed to chlorine dioxide for at least 4 min at 1 ppm, in deionised water, or 3 ppm, in dam water. UV radiation killed >99 % of all propagules of *Ph. cinnamomi* tested at 113 mJ/cm^2 in both deionised and dam water.

Pythium aphanidermatum zoospores were killed (>99 %) following exposure to all disinfection treatments, rates and times tested, in both deionised and distilled water. In deionised water, >99 % of oospores and mycelial fragments of *Py. aphanidermatum* were killed by chlorine at rates greater than 1 ppm for 30 min and 2 ppm for 30 min respectively. For >99 % kill in dam water, *Py. aphanidermatum* oospores and mycelia required exposure to 5 ppm chlorine for 30 min and 1 ppm chlorine at for 20 min, respectively. Chlorine dioxide killed >99 % oospores and mycelial fragments when applied for 4 min or longer at 1 ppm in deionised water, or 3 ppm in dam water. All propagules tested of *Py. aphanidermatum* were killed (>99 %) following exposure to 113 mJ/cm^2 UV radiation in deionised and dam water.

The application rates and times required to kill (>99 %) pathogen propagules in dam water were often greater than those required in deionised water. Dam water had a higher pH (7.8–8.0) than the deionized water (pH = 6.5). Turbidity of dam water was also greater (20–87 NTU) than that of the deionised water (0.32 NTU).

Discussion

Previous studies have investigated the effect of disinfection treatments on common pathogens such as *Phytophthora*, *Fusarium* and *Pythium* spp. (Cayanan et al. 2009; Copes et al. 2004; Hong et al. 2003). However, limited studies have shown a difference in efficacy of disinfection treatments between pathogens and propagule types (Hong et al. 2003). The current study demonstrated that the efficacy (>99 % kill of CFU) of chlorine, chlorine dioxide and UV radiation varies with exposure time, application rate, water quality, the pathogen and propagule type present.

In this study, *Cl. michiganensis* subsp. *michiganensis* was killed by the minimum application rates and exposure times of chlorine (1 ppm for 10 min), chlorine dioxide (1 ppm for 4 min) and UV (113 mJ/cm^2) in both dam and deionised water. Several bacteria have been reported to survive in, and be disseminated in irrigation water (Cappaert et al. 1988; Huang and Tu 2001). Critical levels of chlorine required to kill bacterial plant pathogens, including *Erwinia carotovora* and *Agrobacterium tumefaciens*, range between 1.0 and 4.0 ppm for 2 and 30 min, respectively (Zheng et al. 2014a). Other bacteria, including *Ralstonia* sp. (0.1–1.3 ppm) and *Xanthomonas campestris* (0.21 ppm) are killed by chlorine dioxide (Zheng et al. 2014b).

Phytophthora and *Pythium* spp. are well adapted to aquatic environments, requiring the presence of water for reproduction and spread. Zoospores are the primary infective propagule of *Phytophthora* and *Pythium* and can spread rapidly through irrigation water. This makes it critical that these propagules are killed by the disinfection treatment. Zoospores lack a cell wall, potentially

making them more susceptible to free chlorine treatment compared with other propagules such as mycelium and conidia of true fungi that do have cell walls (Harnik and Garbelotto 2007; Stein and Kirk 2003). This characteristic may explain why zoospores of both *Ph. cinnamomi* and *Py. aphanidermatum* were sensitive to the lowest rates and exposure times of all treatments tested. Zoospores have been reported to survive in water for several days (Granke and Hausbeck 2010; Porter and Johnson 2004) so a 'shock treatment' with chlorine or chlorine dioxide, or treatment with UV, may be more effective in eliminating these propagules from the irrigation system than the continuous application of low rate of chlorine compounds. The results of this study are consistent with those of Cayanan et al. (2009) who report that sporangia and zoospores of the Pythiaceae are more sensitive to chemical treatment than hyphae or chlamydospores and that the initial free chlorine concentrations required to kill *Ph. cinnamomi* and *Py. aphanidermatum* propagules were much lower than those required to kill true fungi such as *F. oxysporum*.

A number of fungal plant pathogens have been recovered from irrigation water including *Fusarium* spp. and *Colletotrichum* spp. (Hong and Moorman 2005; Shokes and McCarter 1979; Wick 2014).

Of the disinfection treatments tested against fungal pathogens and propagules in this study, chlorine dioxide killed >99 % of *Ch. elegans*, *Ca. pauciramosa* and *F. oxysporum*. UV killed *Ch. elegans* and *Co. gloeosporioides* in both deionised and dam water at 113 mJ/cm^2 against, although in dam water exposure to 250 mJ/cm^2 was required to kill >99 % of *Ch. elegans* endoconidia. The higher exposure rate of 250 mJ/cm^2 was required to kill >99 % *Ca. pauciramosa* mycelium in dam water, while conidia and chlamydospores in dam water were not killed at either UV rate tested. *Fusarium oxysporum* propagules in dam water were not killed by either of the UV rates tested. In other studies, UV radiation has been shown to kill conidia of *F. oxysporum* at 70 mJ/cm^2 in dam water (50 % dam water, 50 % rainwater; Runia 1995). Fungi such as *Chalara*, *Calonectria* and *Colletotrichum* spp. are pigmented, which may make them more tolerant of disinfection treatments, such as UV radiation. However, the lower sensitivity to UV radiation observed in *F. oxysporum* observed is difficult to explain, and may be due to thick cell walls of this fungus, as it does not have pigmentation. Conidia of *F. oxysporum* have been reported to be insensitive to chlorination at rates less than 14 ppm for 6 min (Cayanan et al. 2009).

Other studies report the need to use high concentrations of free chlorine to kill *F. oxysporum*, and suggest that the use of chlorine disinfection will only reduce infection but not provide complete control of this pathogen (Cayanan et al. 2009; Reuveni et al. 2002). However, our study demonstrates the efficacy of chlorine dioxide in dam water to effectively kill *F. oxysporum* conidia when applied as a one-off 'shock' treatment at 1 ppm for 4 min, mycelium at 3 ppm for 4 min, and chlamydospores at 5 ppm for 4 min. In deionised water, the lowest rate and exposure time of 1 ppm for 4 min effectively killed all *F. oxysporum* propagules. To effectively use high concentration chlorine dioxide 'shock' treatments to manage fungal pathogens in irrigation water, further research needs to investigate potential phytotoxicity associated with residual chlorine concentrations in irrigation water, and the effect of residual chlorine levels on beneficial microbial organisms in the plant rhizosphere.

Water quality is one of the factors affecting the efficacy of water disinfection treatments (Stewart-Wade 2011; Copes et al. 2004). Water stored in ponds and dams typically has a higher organic and microbial load than rainwater stored in a tank, or town water (Stewart-Wade 2011). Runoff from unlined drainage channels can also contribute to the sediment in water storage reservoirs, increasing organic and particulate matter in the water. The efficacy of the three disinfection treatments varied with the two water types tested. Longer exposure times or higher exposure rates were generally required to kill propagules in dam water compared with deionised water. This may be attributed to the higher pH and greater organic matter content and turbidity of the dam water, although more detailed studies would need to be conducted to eliminate other variables. Chlorine is most effective in irrigation water with a pH between 6.0 and 7.5 (Stewart-Wade 2011; Zheng et al. 2008 cited in Zheng et al. 2014a). Chlorine dioxide is effective at a wider pH range of 4.0–10.0 (Zheng et al. 2014b). The dam water used in this study had a pH of 7.8 to 8.0 compared to the deionised water, with a pH of 6.5. This may explain why chlorine treatment was frequently more effective in deionised water than in dam water, although other variables, such as turbidity of dam water, are also likely to be involved.

High organic matter, particles and turbidity can reduce the contact between the treatment and the pathogen cells (Zheng et al. 2008). This was apparent in the current study as treatments applied to propagules in deionised water were generally more effective at a lower rate than that required to kill propagules in dam water. Free chlorine species will be consumed by any oxidisable material in water, therefore a greater amount of chlorine species are required in water with a higher organic and inorganic content (Hong et al. 2003). Similarly, particulate matter in dam water can reduce the contact between the UV radiation and the pathogen cells (Zheng et al. 2014c). In cases where pathogen propagules are harboured inside organic matter, the treatment may not make contact with the pathogen, rendering it ineffective. Increased turbidity can also lead to the development of films or debris being deposited on the UV lamp reducing its efficiency. This highlights the importance of ensuring that the disinfection treatment selected is suitable for the water quality available. In many situations, appropriate pre-filtration is required prior to disinfection treatment to remove sediments and organic matter that may harbour pathogens from turbid water.

The recommended turbidity for optimum UV treatment is <2 NTU (Zheng et al. 2014c). The most widely used measure of water quality in relation to UV efficacy is ultra-violet transmittance (UVT). The Australian Nursery Industry Best Practice Water Management Guidelines (NGIA 2010) state that, "Ultra violet radiation can be recommended as best practice for nurseries which have recycled water with UV transmission greater than 50 % at a wavelength of 254 nm because of its environmentally friendly operation and low cost. Water with a UV transmission less than this can be disinfested with UV radiation, however, the dose needed increases greatly as UV transmission falls." Research on the relationship between NTU and UVT clearly shows that increased NTU causes significant reductions in UVT, with water of 20 NTU reducing UVT to 59 % (Hofmann et al. 2004). Therefore, both pH and turbidity may have affected the efficacy of the chlorine treatments tested, and turbidity of the dam water reduced the efficacy of the UV treatment for some propagules, such that higher rates or exposure times were required to kill many of the pathogen propagules, when compared with those required for deionised water.

Conclusion

This study has begun to address the gaps that exist in the available data testing the efficacy of disinfection treatments on different life stages, or propagules, of a given pathogen, and the role of water quality characteristics. Water quality can vary significantly between locations and the efficacy of disinfection treatments will vary with the quality of the water. The deionised water used in this study provided a uniform comparison across the three treatments, and against the dam water, which is more applicable to what may be present in a production nursery situation. The efficacy of disinfection treatments varied between pathogens, and between pathogen propagules. We have provided evidence supporting those treatments that are more effective against selected pathogens, and which propagules of those pathogens are more sensitive to each treatment. This is important when considering the epidemiology of each pathogen in the plant production system. Ultimately, the selection of a disinfection system for any given situation will depend on a number of factors including current hygiene practices in the nursery, water quality, volume of water to be treated, plant species grown in the nursery, pathogens present, targeted pathogens and propagules, and the resources available to the nursery or producer concerned (Pettitt 2003).

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