Efficacy of Chlorine in Controlling Five Common Plant Pathogens

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Abstract. Recycled irrigation water is one of the major sources of inoculum and may spread plant pathogens throughout the nursery or greenhouse operation. Chlorination is the most economical method of disinfecting water and has been adopted by some North American commercial growers. However, chlorine has not been assessed as a disinfectant for the common plant pathogens *Phytophthora infestans*, *Phytophthora cactorum*, *Pythium aphanidermatum*, *Fusarium oxysporum*, and *Rhizoctonia solani*. These pathogens were exposed to five different initially free chlorine solution concentrations ranging from 0.3 to 14 mg·L⁻¹ in combination with five contact times of 0.5, 1.5, 3, 6, and 10 min to determine the free chlorine threshold and critical contact time required to kill each pathogen. Results indicated that the free chlorine threshold and critical contact time for control of *P. infestans*, *P. cactorum*, *P. aphanidermatum*, *F. oxysporum*, and *R. solani* were 1, 0.3, 2, 14, and 12 mg·L⁻¹ for 3, 6, 3, 6, and 10 min, respectively.

With environmental regulations focused on water quality and reducing pollution discharge associated with nutrient and pesticide applications, recycling irrigation water has been increasingly adopted by commercial nursery and greenhouse growers (Bush, 2002; Bush et al., 2003; Ehret et al., 2001; Hong and Moorman, 2005; Hong et al., 2003; Richard et al., 2006; Ryu and Beuchat, 2005; Schnitzler, 2003; Schoene et al., 2006; Skimina, 1992). Recycling involves collecting excess irrigation water and leachate into a reservoir until the water is needed again for irrigation. However, the risk of spreading plant pathogens found in recycled irrigation water is a deterrent for many operations (Bush, 2002; Bush et al., 2003; Ehret et al., 2001; Hong and Moorman, 2005; Newman, 2004; Poncet et al., 2001). Richard et al. (2006) conducted a survey on the status of irrigation water recycling in Ontario, Canada, and found that 33% of growers who use recirculating systems list disease control as a major problem. Recycling irrigation solutions have both environmental and ecological benefits (Schnitzler, 2003) but if not disinfected, it can result in serious disease epidemics and crop losses (Hong et al., 2003).

Plant pathogens detected in water supplies and irrigation systems include fungi,

fungal-like organisms, nematodes, viruses, and bacteria (Faulkner and Bolander, 1966; Hong et al., 2003; Koenig, 1986; Schnitzler, 2003; Thomson and Allen, 1974). Hong and Moorman (2005) reported that 17 *Phytophthora* spp., 26 *Pythium* spp., 27 genera of fungi, eight species of bacteria, 10 viruses, and 13 species of plant parasitic nematodes have been detected from ponds, rivers, canals, streams, lakes, runoff water, watersheds, reservoirs, wells, holding tanks, effluents, ebb and flow systems, recirculating systems, and hydroponic systems. The most destructive pathogens are *Pythiaceae* fungal-like organisms such as *Pythium* and *Phytophthora* followed by viruses, bacteria, and nematodes (Schnitzler, 2003).

Traditionally, growers rely on preventive fungicides for management of diseases in greenhouses and nurseries such as fosetyl-Al and metalaxyl to control phytophthora and pythium diseases (Ben-Yephet and Nelson, 1999; Kuhajek et al., 2003); however, there are already metalaxy-resistant *Phytophthora* and *Pythium* spp. such as *Pythium aphanidermatum* (Sanders et al., 1985), *Phytophthora infestans*, *Phytophthora capsici*, *Phytophthora nicotianae*, and *Phytophthora citricola* (Kuhajek et al., 2003). These fungicides are expensive and there is concern for further development of fungicide resistance (Hong et al., 2003; Sanders et al., 1985). There is a concern regarding pesticide pollution in the environment and contamination of drinking water. There are areas in Europe with raw water that contain relatively high concentrations of pesticides, which has increased public concern regarding pesticide contamination of drinking water (Griffini et al., 1999). Even with fungicide use, there is no completely effective treatment of *Rhizoctonia solani* (Johnson and Leach, 2006)

and *Fusarium oxysporum* (Reuveni et al., 2002).

Several disinfection techniques have been studied for their efficacy in minimize the spread of plant pathogens in recycling systems, which include slow sandbed filtration, ultraviolet irradiation (Ehret et al., 2001; Hong et al., 2003; Igura et al., 2004; Schnitzler, 2003), heating (Lin et al., 1998), ozonation, nonionic surfactants, and chlorination (Ehret et al., 2001; Hong et al., 2003).

Chlorination is an economical method of disinfecting water and remains the primary method of treating municipal water (Havard, 2003; Hong et al., 2003). Chlorination technology has already been adopted by some growers to disinfect their irrigation systems and water. However, specific recommendations for use in nursery or greenhouse irrigation to control the spread of plant pathogens have not been fully assessed (Datnoff et al., 1987; Hong et al., 2003; Johnson et al., 1997; Le Dantec et al., 2002; Taylor et al., 2000; White, 1992).

Some research in assessing chlorine's efficacy in killing several pathogens has shown that sensitivity to chlorine is pathogen-dependent. Taylor et al. (2000) reported that to inactivate 99.9% of five different *Mycobacterium avium* strains, chlorine concentrations ranged from 51 to 204 mg·L⁻¹. *Erwinia carotovora* subsp. *Zee* Sabet was killed at 1 mg·L⁻¹ of chlorine (Thompson, 1965), and Hong et al. (2003) reported that zoospores of *Phytophthora nicotianae*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, and *P. megasperma* were killed with free chlorine ranging from 0.25 to 2 mg·L⁻¹; however, there are no studies for control of *P. infestans* and *P. cactorum*. There are also no studies regarding chlorine's efficacy in controlling *Pythium aphanidermatum*, *F. oxysporum*, and *R. solani*. Literature has indicated that sensitivity to chlorine varies with contact time (Copes et al., 2001; Datnoff et al., 1987; Johnson et al., 1997; Segall, 1968).

Chlorine is divided into three types of chlorine residual: 1) free chlorine, which is composed of dissolved chlorine gas (Cl₂), hypochlorous acid (HOCl), and hypochlorite ion (OCl⁻) in water after chlorination; 2) combined chlorine, which is chlorine combined with ammonia (NH₃) or organic nitrogen in water as chloramines or other chloroderivatives (Hong et al., 2003); and 3) total chlorine, which is the sum of the free and combined chlorine in water (Hong et al., 2003; Morganti, 2002; Rutala and Weber, 1997; White, 1992). Combined chlorine is more stable but is less biocidal than free chlorine. For this reason, we used free chlorine for our research.

The free chlorine threshold and critical contact time are defined in this study as the lowest free chlorine concentration and the shortest contact time for that chlorine threshold at which there was no longer detection of any colony-forming units (cfu). Using this definition, the objective of this study was to determine the free chlorine threshold and

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critical contact time that would kill the sporangia of *Phytophthora infestans*, zoospores of *Phytophthora cactorum* and *Pythium aphanidermatum*, conidia of *Fusarium oxysporum*, and mycelia of *Rhizoctonia solani*.

Materials and Methods

Propagule preparation. A pure isolate of *Phytophthora infestans* P1661 was obtained from Dr. Harold Platt at Agriculture and Agri-Food Canada (Charlottetown, P.E.I., Canada) and incubated on rye agar at 18 °C with 12 h of light and 12 h of dark to produce sporangia. After 13 d, 5 mL of sterile double distilled water (SDDW) was added to the *P. infestans* growing on rye agar and the agar surface was gently rubbed with a cell scraper to dislodge the mycelia. The SDDW with the dislodged mycelia was collected in a sterile beaker and incubated at 9 to 10 °C for 1 h and then at 20 °C for another 1 h to release the sporangia. The sporangia suspension was filtered through two layers of sterile cheesecloth to obtain a suspension free of hyphal fragments. The sporangia concentration was determined with a hemacytometer and diluted to $\approx 10^5$ sporangia/mL. After each chlorine treatment (described subsequently), surviving sporangia were incubated on rye agar at 18 °C with 12 h of light and 12 h of dark for 4 d.

Rye agar was prepared as follows. Two liters of SDDW and 120 g of organic rye kernels (Grain Process Enterprises Ltd., Scarborough, Ontario, Canada) were boiled until only 1 L remained. Rye kernels were removed by straining the boiled rye water through two layers of cheesecloth. The rye water was centrifuged at 650 g_n for 5 min with a Du Pont Instrument Sorvall RC-JB Refrigerated Superspeed Centrifuge and a Sorvall GSA Rotor (Mandel Scientific Co. Inc., Guelph, Ontario, Canada). The supernatant was collected and SDDW was added to the supernatant to make up a final volume of 2 L. Two grams of dextrose (Fisher Scientific, Ottawa, Ontario, Canada) and 27 g of granulated agar (Fisher Scientific) were added to the rye water. The agar was melted and then sterilized at 121 °C for 20 min.

A pure isolate of *Phytophthora cactorum* was obtained from Audra Stechyshyn-Nagasawa (University of Guelph, Guelph, Ontario, Canada) and the methodology developed by Harris (1986) was used to obtain a pure suspension of *P. cactorum* zoospores with some modifications. A circular mesh (Home Hardware, Guelph, Ontario, Canada) with 1-mm holes was placed in a 250-mL conical flask as a solid, nondegradable substrate so that the mycelium could adhere to it during the growth phase. V8 broth was prepared as follows. One gram of CaCO_3 was mixed into 100 mL of V8 100% vegetable juice (Campbell Soup Co., Etobicoke, Ontario, Canada) and then centrifuged at 7974 g_n for 10 min. Fifty milliliters of V8 supernatant was added to 950 mL of

SDDW to make the V8 broth. Clarified V8 agar was made by adding 15 g of granulated agar to 1 L of V8 broth and melted. V8 broth and agar were sterilized at 121 °C for 20 min. The zoospore concentration was determined with a hemacytometer and diluted to $\approx 10^5$ zoospores/mL. After each chlorine treatment, surviving zoospores were incubated on clarified V8 agar at 25 °C for 40 to 48 h.

Pythium aphanidermatum was obtained from an infected green bell pepper (*Capsicum annuum*). To collect the zoospores, a 1-cm long piece of infected green bell pepper root was transferred to P₅AR agar and incubated under 24 h of light at 25 °C for 48 h. P₅AR agar, which is selective for members of the family *Pythiaceae*, contained 20 g cornmeal agar (Fisher Scientific), 5 mg·L⁻¹ pimaricin, 250 mg·L⁻¹ ampicillin, and 10 mg·L⁻¹ rifampicin dissolved in 95% ethanol. A 0.5-cm diameter plug of *P. aphanidermatum* from the outer edge of the culture was transferred to another P₅AR plate and incubated under 24 h of light at 25 °C for another 48 h. This step was repeated twice to obtain a purified culture of *P. aphanidermatum*. From the outer edge of the culture, another 0.5-cm diameter plug of *P. aphanidermatum* was transferred to V8 agar and incubated under 24 h of light at 25 °C for 48 h. V8 agar media used for *P. aphanidermatum* (described subsequently) was prepared differently from V8 agar media used for *P. cactorum*. The V8 agar plate was cut into six equal strips and three alternating strips were placed in an empty sterile 100 × 15-mm petri dish. Twenty milliliters of SDDW was added to each petri dish and incubated under 24 h of light at 25 °C for 48 h. The SDDW was decanted from the petri dishes, and another 20 mL of SDDW was added to each petri dish. Petri dishes were then incubated at room temperature with light for 4 h. The water suspension containing the zoospores from each petri dish was filtered through a sterile metal strainer with a 1-mm mesh to remove large hyphal fragments. The zoospore concentration was determined with a hemacytometer and diluted to $\approx 10^4$ zoospores/mL. After each chlorine treatment, surviving zoospores were incubated on P₅AR agar at 25 °C with 24 h of light for 20 to 24 h.

V8 agar used for *P. aphanidermatum* was prepared as follows. Three grams of CaCO_3 was mixed into 200 mL of V8 100% vegetable juice (Campbell Soup Co.) and then added to 800 mL of SDDW with 12 g of granulated agar (Fisher Scientific). The agar was melted and then sterilized at 121 °C for 20 min.

A pure isolate of *Fusarium oxysporum* was incubated on potato dextrose agar (PDA) (Fisher Scientific) at 25 °C with 12 h of light and 12 h of dark for 10 d to collect conidia. The mycelia was then removed by adding 25 mL of SDDW and then dislodged with a cell scraper. Two milliliters of the *F. oxysporum* suspension was added to 100 mL of potato dextrose broth (PDB) (Fisher Scientific) in a 250-mL flask and incubated at 30 °C for 72 h.

Fifty milliliters of the 100 mL *F. oxysporum* culture was then subcultured into another 1 L of PDB in a 2800-mL flask and incubated at 30 °C for 72 h to produce and release the conidia. The *F. oxysporum* culture was filtered through two layers of cheesecloth to remove the mycelia. The filtrate was centrifuged at 1464 g_n with a temperature of 4 °C for 30 min. The supernatant was decanted and the pellet resuspended in 200 mL of SDDW. The conidia concentration was determined with a hemacytometer and diluted to $\approx 10^6$ conidia/mL. After each chlorine treatment, surviving conidia were incubated on PDA at 30 °C for 20 to 26 h.

A pure isolate of *Rhizoctonia solani* was obtained from Diana Mooij from the Department of Environmental Biology, University of Guelph (Guelph, Ontario, Canada) and incubated on PDA at room temperature for 7 d to collect the mycelia. Fifty milliliters of PDB in a 125-mL Erlenmeyer flask was inoculated with two 5-mm diameter plugs of 7-d-old *R. solani* grown on PDA and incubated at 30 °C for 7 d. The PDB was decanted and the two 5-mm plugs were removed from the mycelia mat. The mycelia mat was rinsed with 20 mL of SDDW and homogenized in a Westinghouse Model WST2024ZE 3 Speed Blender at speed 1 (Maxim-Toastmaster, Lake Forest, IL) for 1 min with 100 mL of SDDW. The mycelia concentration was determined with a hemacytometer and diluted to $\approx 10^4$ mycelia/mL. After each chlorine treatment, surviving mycelia were incubated on PDA at 30 °C for 7 d.

Chlorinated water. A fresh stock solution of free chlorine (69 mg·L⁻¹) was prepared from 4% to 6% sodium hypochlorite (Fisher Scientific) with SDDW and adjusted to pH 6.5 to 7 with hydrochloric acid because chlorine is reported to be the most effective at this pH range (Frink and Bugbee, 1987). The stock solution was diluted with SDDW to achieve the desired free chlorine treatment concentration (as given subsequently). Initial free chlorine concentrations were measured with a C201 Oakton Colorimeter using the protocol specific to this colorimeter (Oakton Instruments, Vermont Hills, IL).

Treatments. Sporangia, zoospores, conidia, or mycelia of the pathogens were exposed to different initial free chlorine concentrations with SDDW as an untreated control for five contact times (0.5, 1.5, 3, 6, and 10 min). *P. cactorum* and *P. aphanidermatum* were exposed to 0.3, 0.4, 0.5, 1, and 2 mg·L⁻¹ of free chlorine. *P. infestans* was exposed to 0.3, 0.4, 1, 2, and 3 mg·L⁻¹ of free chlorine. *F. oxysporum* was exposed to 4, 8, 10, 12, and 14 mg·L⁻¹ of free chlorine. *R. solani* was exposed to 5, 6, 7, 10, and 12 mg·L⁻¹ of free chlorine.

Each free chlorine treatment was achieved by mixing 90 mL of chlorinated water with 10 mL of pathogen suspension. At each contact time, a 1-mL sample was removed from the treatment to obtain a survival curve. The free chlorine reaction was stopped by adding the 1-mL sample to

9 mL of PDB to neutralize the free chlorine. Two more serial dilutions were made immediately into another two 9 mL of PDB and then an aliquot of 100 μ L of each dilution was spread onto a 100 $\times$ 15-mm petri dish containing the pathogen's growth media. Petri dishes were incubated under each pathogen's growth condition as described previously. The colony count method was used to determine the total number of developed survivor colonies under each contact time and was recorded. The survivors were represented by the number of cfu in a 1-mL cell suspension (cfu/mL). This was also done for the untreated control with SDDW. Ten milliliters of SDDW was also added to 90 mL of treatment chlorinated water as a control to monitor if the chlorine concentration would change over time when there were no pathogen propagules present (chlorine control). In addition, as the 1-mL sample was removed from the free chlorine treatment at each contact time, simultaneously another 10 mL of the free chlorine treatment was removed to determine the free chlorine concentration under each contact time using a colorimeter. Ten milliliters was also removed from the chlorine control at each contact time and measured with a colorimeter to determine the free chlorine concentration under each contact time when there were no pathogen propagules present. A C201 Oakton Colorimeter (Oakton Instruments) was used to measure the free chlorine concentration of chlorine treatment and chlorine control. This test was performed three times for each free chlorine concentration on each pathogen.

Statistical analysis. SAS software version 9.1 (SAS Institute, Cary, NC) was used to perform homogeneity testing to pool the data and to calculate the least squares means and SEs.

Results

As the initial free chlorine concentration increased, the number of inactivated sporangia of *P. infestans*, zoospores of *P. cactorum* and *P. aphanidermatum*, conidia of *F. oxysporum*, and mycelia of *R. solani* increased. The free chlorine threshold and critical contact time for *P. infestans* sporangia, *P. cactorum* and *P. aphanidermatum* zoospores, *F. oxysporum* conidia, and *R. solani* mycelia were 1, 0.3, 2, 14, and 12 mg·L⁻¹ of free chlorine for 3, 6, 3, 6, and 10 min in double distilled water, respectively (Figs. 1–5).

For each pathogen, the contact times in which the concentration of inactivated propagules became consistent were 0.5 min for all five initial free chlorine concentrations for *P. infestans* sporangia (Fig. 1); 1.5 min for 0.3 mg·L⁻¹ of free chlorine (Fig. 2A) and 0.5 min for 0.4, 0.5, 1, and 2 mg·L⁻¹ of free chlorine (Fig. 2B–E) for *P. cactorum* zoospores; 3 min for 0.3 mg·L⁻¹ of free chlorine (Fig. 3A) and 0.5 min for 0.4, 0.5, 1, and 2 mg·L⁻¹ of free chlorine (Fig. 3B–E) for *P. aphanidermatum* zoospores; and 1.5 min for 4, 12, and 14 mg·L⁻¹ of free chlorine (Fig. 4A, D, and E), 3 min for 8 mg·L⁻¹ of free chlorine (Fig. 4B),

and 6 min for 10 mg·L⁻¹ of free chlorine (Fig. 4C) for *F. oxysporum* conidia. Unlike *P. infestans*, *P. cactorum*, *P. aphanidermatum*, and *F. oxysporum*, the concentration of inactivated propagules of *R. solani* only became consistent at 12 mg·L⁻¹ of free chlorine after 6 min of contact time (Fig. 5E).

The free chlorine concentration in each treatment also decreased over time by contact with the propagules of all five pathogens (Figs. 1–5) compared with the chlorine control, which did not decrease in free chlorine concentration over time (data not shown). The free chlorine concentration, however, did not decrease as rapidly by contact with *P. cactorum* zoospores (Fig. 2), *F. oxysporum* conidia (Fig. 4), and *R. solani* mycelia (Fig. 5) as the free chlorine that came in contact with *P. infestans* or *P. aphanidermatum*.

Discussion

The sporangia of *Phytophthora infestans*, the zoospores of *Phytophthora cactorum* and *Pythium aphanidermatum*, the conidia of *Fusarium oxysporum*, and the mycelia of *Rhizoctonia solani* in this study were killed by free chlorine at different initial concentrations. Previous investigations demonstrate that chlorine sensitivity can differ with genus, species, pathovar, and/or propagules (Hong et al., 2003). Chauret et al. (2001) reported that bacterial spores of *Bacillus subtilis* and *Clostridium sporogenes* are more sensitive to chlorine than *Clostridium parvum* oocysts. Blaser et al. (1986) reported that three different strains of *Campylobacter jejuni* were killed with 0.1 mg·L⁻¹ of free chlorine at a contact time of 5 min. Thompson (1965) reported *Erwinia carotovora* subsp. *zeae* Sabet was killed with 1 mg·L⁻¹ of free chlorine, but *Erwinia chrysanthemi* Burkholder et al. and *E. carotovora* subsp. *carotovora* (Jones) Bergey were less sensitive to chlorine, which survived at 10 mg·L⁻¹ (Lacy et al., 1981). Differences in the sensitivity of different pathogens and propagules to chlorine support our findings that the pathogens and propagules in this study exhibited differences in sensitivity to chlorine.

The five pathogens in this study were killed at different critical contact times. Literature supports that pathogen sensitivity to chlorine varies with contact time (Copes et al., 2001; Datnoff et al., 1987; Johnson et al., 1997; Segall, 1968). Korich et al. (1990) reported that 80 mg·L⁻¹ applied for 90 min was required to inactivate 90% of *Cryptosporidium parvum* oocysts. Datnoff et al. (1987) reported that resting spores of *Plasmodiophora brassicae* were killed after treatment with 2 mg·L⁻¹ for at least 5 min, and Berman and Hoff (1984) reported that 0.5 mg·L⁻¹ of chlorine inactivated simian rotavirus SA11 within 4 min.

Initial free chlorine concentrations required to kill *P. infestans*, *P. cactorum*, and *P. aphanidermatum* were much lower than that of *F. oxysporum* and *R. solani*. Literature reports that the order of fungal

propagule sensitivity (most to least) to fungicides is cysts, zoospores, sporangia, hyphae, and conidia (Fernández-Torres et al., 2003; Harnik and Garbelotto, 2007; Stein and Kirk, 2003). These findings suggest that sporangia and zoospores of *Pythiaceae* pathogens are more sensitive to chemical treatment than hyphae and conidia.

Research by Hong et al. (2003) reported that free chlorine concentrations ranging from 0.25 to 2 mg·L⁻¹ were able to kill the zoospores of *Phytophthora nicotianae*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, and *P. megasperma*, which supports our results that low free chlorine concentrations can kill *P. infestans*, *P. cactorum*, and *P. aphanidermatum*.

Both chemical and nonchemical methods of control for *Fusarium* and *Rhizoctonia* only reduce infection and do not fully control the spread of these pathogens (Asaka and Shoda, 1996; Howell et al., 2000; Reuveni et al., 2002; Szczech and Shoda, 2006), which demonstrate how difficult it is to control these pathogens. This supports our results that *F. oxysporum* and *R. solani* are resilient to chlorine and require higher free chlorine concentrations to kill them. This is further supported by Igura et al. (2004) who reported that water containing ozone concentrations up to 1 mg·L⁻¹ was only able to reduce the conidia of *F. oxysporum* by a 10⁴ order from a concentration of 10⁸ cfu/mL. Ozone is considered to be a stronger oxidizer than chlorine (Graham, 1997; Tyrrell et al., 1995), but the inability of ozone to completely kill *F. oxysporum*, as shown by Igura et al. (2004), demonstrates the resilience of *F. oxysporum* to chemical treatment and supports the necessary use of a high initial free chlorine concentration to kill *F. oxysporum*.

As the initial free chlorine concentration increased, the number of propagules that were killed increased for all five plant pathogens. This may be because as the initial concentration of free chlorine increased, there was more free chlorine available to inactivate the pathogen, therefore inactivating more propagules. The same phenomenon was also observed by Hong et al. (2003) in which the number of inactivated sporangia, zoospores, and mycelia of *Phytophthora* spp. increased with increasing free chlorine concentration. Igura et al. (2004) also reported that the number of inactivated conidia increased with increasing ozone concentrations.

Free chlorine concentration rapidly decreased by contact with *P. infestans* and *P. aphanidermatum*. Because free chlorine is oxidized by organic matter and other oxidizable substances (White, 1992), free chlorine concentrations decreased as a result of the oxidation of pathogen propagules. This phenomenon was also observed by other researchers who have used chlorine or other oxidizing compound to disinfect pathogens (Hong et al., 2003; Igura et al., 2004); however, the free chlorine concentrations did not decrease as rapidly for *P. cactorum*, *F. oxysporum*, and *R. solani*. Because conidia

Fig. 1. Relationships between survivors of *Phytophthora infestans* sporangia (cfu/mL) and free chlorine concentration (mg·L⁻¹) after treatment with initial free chlorine concentrations 0.3 (A), 0.4 (B), 1.0 (C), 2.0 (D), and 3.0 (E) mg·L⁻¹. The solid and open symbols indicate the survivors (cfu/mL) and the free chlorine concentrations (mg·L⁻¹), respectively. Each datum point is the mean of three replicates $\pm$ the SE of the mean.

and mycelia have thicker cell walls than zoospores and sporangia (Alexopoulos et al., 1996; Carlile et al., 2004; Erwin et al., 1983; Fernández-Torres et al., 2003), it may require more time for free chlorine to oxidize conidia and mycelia. This would result in a slower decrease in free chlorine concentration over time. However, this does not explain why free chlorine concentrations did not decrease rapidly when it came into

contact with *P. cactorum* zoospores. Because *P. cactorum* zoospores have the lowest free chlorine threshold of 0.3 mg·L⁻¹, only a minimal concentration of free chlorine was required to kill the zoospores. Therefore, not as much free chlorine would be needed to kill *P. cactorum* zoospores and would not decrease as rapidly as free chlorine oxidized by *P. infestans* sporangia and *P. aphanidermatum* zoospores.

For *P. infestans*, *P. cactorum*, *P. aphanidermatum*, and *F. oxysporum*, the concentration of inactivated propagules eventually became consistent during chlorine treatment. The same phenomenon was also observed in research conducted by Hong et al. (2003) with chlorine and Igura et al. (2004) with ozone. However, this was only observed in *R. solani* treated with 12 mg·L⁻¹ of free chlorine (Fig. 5E). The concentration

Fig. 2. Relationships between survivors of *Phytophthora cactorum* zoospores (cfu/mL) and free chlorine concentration (mg·L⁻¹) after treatment with initial free chlorine concentrations 0.3 (A), 0.4 (B), 0.5 (C), 1.0 (D), and 2.0 (E) mg·L⁻¹. The solid and open symbols indicate the survivors (cfu/mL) and the free chlorine concentrations (mg·L⁻¹), respectively. Each datum point is the mean of three replicates $\pm$ the SE of the mean.

Fig. 3. Relationships between survivors of *Pythium aphanidermatum* zoospores (cfu/mL) and free chlorine concentration (mg·L⁻¹) after treatment with initial free chlorine concentrations 0.3 (A), 0.4 (B), 0.5 (C), 1.0 (D), and 2.0 (E) mg·L⁻¹. The solid and open symbols indicate the survivors (cfu/mL) and the free chlorine concentrations (mg·L⁻¹), respectively. Each datum point is the mean of three replicates $\pm$ the SE of the mean.

of inactivated mycelia hardly became consistent for free chlorine treatments at 5, 6, 7, and 10 mg·L⁻¹ (Fig. 5A–D). This may be because the free chlorine was constantly inactivating the mycelia, therefore never reaching a consistent concentration of inactivated mycelia within the 10-min interval the mycelia were exposed to the chlorine.

Because free chlorine is consumed as it is oxidizing the pathogen propagule over time, the free chlorine thresholds reported are the initial free chlorine concentrations in which pathogen propagules were treated and represent the maximum free chlorine concentrations at which pathogen propagules can be killed. The critical contact times are the times in which pathogen propagules were no longer

detected without keeping the free chlorine concentration constant at the initial free chlorine concentration. The free chlorine threshold and critical contact time of *P. infestans*, *P. cactorum*, *P. aphanidermatum*, *F. oxysporum*, and *R. solani* were 1, 0.3, 2, 14, and 12 mg·L⁻¹ for 3, 6, 3, 6, and 10 min in double distilled water, respectively. Because irrigation water is loaded with organic matter,

Fig. 4. Relationships between survivors of *Fusarium oxysporum* conidia (cfu/mL) and free chlorine concentration (mg·L⁻¹) after treatment with initial free chlorine concentrations 4.0 (A), 8.0 (B), 10.0 (C), 12.0 (D), and 14.0 (E) mg·L⁻¹. The solid and open symbols indicate the survivors (cfu/mL) and the free chlorine concentrations (mg·L⁻¹), respectively. Each datum point is the mean of three replicates $\pm$ the SE of the mean.

Fig. 5. Relationships between survivors of *Rhizoctonia solani* mycelia (cfu/mL) and free chlorine concentration (mg·L⁻¹) after treatment with initial free chlorine concentrations 5.0 (A), 6.0 (B), 7.0 (C), 10.0 (D), and 12.0 (E) mg·L⁻¹. The solid and open symbols indicate the survivors (cfu/mL) and the free chlorine concentrations (mg·L⁻¹), respectively. Each datum point is the mean of three replicates $\pm$ the SE of the mean.

the free chlorine thresholds that we have reported would be kept constant during the contact time required for treatment once the chlorine demand of the irrigation water has been satisfied. However, contact time may decrease at each pathogen's free chlorine threshold because the free chlorine concentration of the irrigation water would be kept constant, thus reducing the amount of time required to kill the pathogen.

If recycled irrigation water were maintained at a free chlorine concentration of 14 mg·L⁻¹ of free chlorine for 6 min, it may control the spread of the five common plant pathogens. However, our previous research (Cayanan et al., 2008) found that the threshold of chlorine concentration, which would not adversely affect the growth or appearance of plants, was less than 2.5 mg·L⁻¹. Recycled irrigation water with 14 mg·L⁻¹ of free chlorine is greater than the free chlorine threshold for the nursery species studied.

Because previous studies have indicated that *Pythiaceae* pathogens are the most common, destructive, and significant contaminants in irrigation systems to cause disease in crops (Alexopoulos et al., 1996; Bush, 2002; Errampalli et al., 2006; Erwin and Ribeiro, 1996; Schnitzler, 2003; Shokes and McCarter, 1979), it would be in the best interest of nursery and greenhouse growers to design their chlorinated irrigation systems to target *Pythiaceae* pathogens, which would only require irrigation water to maintain a free chlorine concentration of 2 mg·L⁻¹ to kill the pathogens. This would allow growers to control the spread of *Pythiaceae* pathogens and not cause any negative effects on plants if irrigated with chlorinated water. If growers

find that *F. oxysporum* and *R. solani* are a common problem in their nursery or greenhouse, then chlorinated irrigation systems may be designed to target these pathogens with a constant free chlorine concentration of 14 mg·L⁻¹, but the system must be designed to remove chlorine from the irrigation water before watering the plants. Removal of chlorine can be done by aerating, use of active carbon, or chemical treatment with sodium dioxide, sodium sulfite, or sodium metabisulfite (White, 1992). Another option for growers that need to target all five pathogens, but do not want to design their system to remove chlorine from the irrigation water, is to maintain irrigation water at a free chlorine concentration of 2 mg·L⁻¹ to kill *Pythiaceae* pathogens, but the contact time must be increased to allow a sufficient contact time to kill *F. oxysporum* and *R. solani*. However, further research is required to determine what contact time would be sufficient to kill *F. oxysporum* and *R. solani* at 2 mg·L⁻¹ of free chlorine.

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