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Control of Pathogens in Irrigation Water Using Chlorine Without Injury to Plants[®]

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INTRODUCTION

Issues surrounding water and fertilizer usage continue to be of critical importance to the nursery and greenhouse industries. Issues related to irrigation water discharge has become a major environmental concern. In 1996, Environment Canada listed the agriculture sector as one of the five main water users in Canada (Environment Canada, 2005a). This sector accounted for 9% of total water usage with 85% of it for irrigation (Environment Canada, 2005a). The nursery and greenhouse industries are major contributors to the release of irrigation water laden with residues of nutrients and pesticides, which may cause environmental damage including groundwater pollution. Legislative and economic pressures have lead to a gradual shift towards closed-loop irrigation systems. These systems collect and re-use irrigation water which lead to greater labour and energy savings as well as reducing the environmental impact. There are still several problematic issues which growers must face when adopting this technology. The first and foremost concern of growers is the potential for disease propagation and spread in these closed-loop systems. In a survey on the status of nutrient solution recirculation in Ontario, Richard et al. (2006) found that 33% of growers using recirculating systems listed disease management as a major problem.

Plant pathogens detected in water supplies and irrigation systems include fungi, nematodes, viruses, and bacteria (Faulkner and Bolander, 1966; Thomson and Allen, 1974; Koenig, 1986; Hong et al., 2003). Hong and Moorman (2005) reported that 17 *Phytophthora* spp., 26 *Pythium* spp., 27 genera of fungi, 8 species of bacteria, 10 viruses, and 13 species of plant parasitic nematodes have been isolated from ponds, rivers, canals, streams, lakes, runoff water, watersheds, reservoirs, wells, holding tanks, effluents, ebb and flow, and recirculating and hydroponic systems. The most destructive plant pathogens are of the Pythiaceae family, including *Pythium* and *Phytophthora* spp., followed by viruses, bacteria, and nematodes (Schnitzler, 2003).

Chlorination is an economical method of disinfecting water and remains the primary method of treating municipal water (Havard, 2003). Chlorination technology has already been adopted by some growers to disinfect their irrigation systems and water. However, specific recommendations (i.e., critical free chlorine concentrations and contact times for both plants and pathogens) for use in nursery irrigation to prevent the spread of plant pathogens have not been fully assessed. This paper will provide an overview of chlorine chemistry, measurement method, chlorine products, concentrations and contact times which are required to control certain pathogens but remain safe for plants, particularly ornamental nursery plants.

CHLORINE CHEMISTRY

Chlorine is comprised of three common chlorine species: free residual chlorine, combined residual chlorine, and total residual chlorine (White, 1992; Fig. 1). Free residual chlorine is composed of dissolved chlorine gas (Cl_2), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-) after chlorination of water. Hypochlorous acid is a weak acid that is the direct disinfectant agent, which partially dissociates in water to form OCl^- . Hypochlorite ion has an indirect disinfecting action due to the production of new HOCl from OCl^- once HOCl is consumed. Therefore, HOCl is a strong oxidizer whereas OCl^- is a weaker oxidizer with respect to HOCl (Newman, 2004).

The concentrations of HOCl and of OCl^- remaining in the water after chlorination depends on the pH and the total concentration of chlorine (White, 1992). At a $\text{pH} > 7.5$, very little HOCl exists ($< 50\%$) and chlorine is in its less active form OCl^- . At a $\text{pH} > 9$, there is almost no HOCl ($< 4\%$) and little disinfecting power. At a $\text{pH} < 4$, noxious chlorine gas (Cl_2) is formed. Hypochlorous acid is the form most readily transferred across a microbial cell wall to cause disinfection, so antimicrobial activity is best at $\text{pH} 6.0\text{--}7.5$ because HOCl is most abundant at this pH range ($\sim 90\%$ at $\text{pH} 6.5$). In this pH range, this ensures adequate disinfection activity without the formation of chlorine gas, which is irritating to workers and corrosive to equipment.

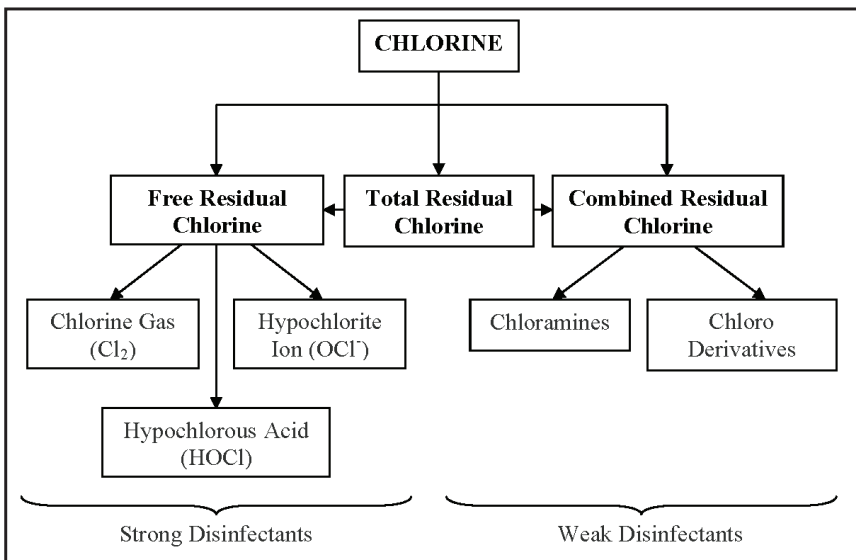


Figure 1. Chlorine species: free residual chlorine, combined residual chlorine, and total residual chlorine.

Combined residual chlorine is chlorine that is combined with ammonia (NH_3) or organic nitrogen in water as chloramines or other “chloro” derivatives. Combined residual chlorine is oxidizable and has disinfection action. Compounds of combined residual chlorine include monochloramine, dichloramine, and nitrogen trichloride.

Chloramines are unstable chlorine compounds formed by reaction of hypochlorous acid with organic nitrogen (ammonia, NH_3). The initial concentration depends on the pH of the solution and the total concentration of chlorine in the water. Concen-

tration decreases due to hydrolysis; increasing doses of chlorine causes chloramines to oxidize to nitrogen gas, while chlorine is reduced to chloride ion (White, 1992). Combined residual chlorine compounds are more stable, but are less biocidal than free residual chlorine forms and are weaker disinfectants with respect to free chlorine. Total residual chlorine is the sum of the free and combined residual chlorine.

Use of different chlorine species or unspecified species in literature has been a source of confusion leading to misinterpretations and misuse of research data because the same levels of different chlorine species have different biocidal properties. Because free chlorine is the most biocidal chlorine species, we will focus on free residual chlorine in this paper. For simplicity, henceforth, we will refer to free residual chlorine and combined residual chlorine as free chlorine and combined.

The chlorine demand of various water sources is an important consideration in chlorination protocols. Chlorine demand is the measure of the amount of chlorine that will be consumed by organic matter and other oxidizable substances in water before a chlorine residual can be established. Chlorine demand differs with the type and amount of organics existing in water (White, 1992). This must be taken into consideration when chlorinating recycled irrigation water as it tends to be loaded with organic matter. Thus, some chlorine will be consumed before a free chlorine status (concentration) is established.

CHLORINE MEASUREMENT

The most commonly used chlorine detection method is colorimetry. Free chlorine in water reacts with *N,N*-diethyl-*p*-phenylenediamine (DPD) to produce a pink colour. The intensity of the pink colour produced is proportional to the free chlorine concentration in the water. Direct light current at a 525 nm wavelength is emitted from a light emitting diode (LED) and passed through the pink sample. A photometric device that measures the properties of light is used to measure the amount of light that is absorbed by the pink colour produced by the reaction of DPD with free chlorine. This information is then converted into the free chlorine concentration by a colorimeter.

Other compounds in water samples that react with DPD may give a false reading. For example, DPD may oxidize forms of manganese giving a false chlorine concentration. Other compounds in water that may cause interference are CaCO_3 at concentration >250 ppm, monochloramine, oxidants (bromine, chlorine dioxide, iodine, and ozone), and oxidized manganese or oxidized chromium (Oakton Instruments and Eutech Instruments Pte Ltd., 2005). Therefore background readings of a water sample were subtracted from readings with chlorinated water to give a true reading.

COMMONLY USED CHLORINE COMPOUNDS

Chlorine Gas. Chlorine gas (Cl_2) is a poisonous, heavy gas that is soluble in water and is the cheapest form of chlorine to produce (Newman, 2004). It is a strong oxidizing agent that hydrolyzes readily in water, producing H^+ and Cl^- and free chlorine in the form of HOCl (White, 1992). Hypochlorous acid partially dissociates in water to form OCl^- and/or reacts with ammonia to produce combined chlorine.

Using Cl_2 risks the inadvertent release of the poisonous gas into the atmosphere when not dissolved in water. The gas affects skin, body tissues, and the respiratory

system, resulting in irritation, burning, and ultimately, fatality. A concentration as low as 5 ppm by volume provokes difficulties in breathing and 1000 ppm causes death within a few minutes (White, 1992). Special equipment and facilities are required for storage and use, and for prevention of corrosion. Chlorine gas is extremely dangerous to handle and government regulations must be followed for its use in North America.

Chlorine Dioxide. Chlorine dioxide (ClO_2) is generated from sodium chlorate (NaClO_3) or sodium chlorite (NaClO_2) (White, 1992). Chlorine dioxide is 25 times more effective than chlorine gas as a disinfectant, but due to its unstable explosive nature as a gas, it is only used in aqueous solution. Because chlorine dioxide is unstable, it easily breaks down, which makes storage not commercially viable. Thus, it must be manufactured on-site with special equipment (White, 1992). Recently there are some new developments in producing ClO_2 and hopefully we will see some easy and cost-effective ways in producing ClO_2 in the near future.

Calcium Hypochlorite. Calcium hypochlorite [$\text{Ca}(\text{OCl})_2$] is a salt of hypochlorous acid, which hydrolyzes in water to produce free chlorine in the form of HOCl (White, 1992). Hypochlorous acid partially dissociates in water to form OCl^- and/or reacts with ammonia to produce combined chlorine.

Calcium hypochlorite is sold in a dry form such as bleaching powder, granules, compressed tablets or pellets. However, $\text{Ca}(\text{OCl})_2$ sold in its dry form contains impurities, which can affect the composition of the water. In addition, the use of $\text{Ca}(\text{OCl})_2$ results in crystallization, which clogs metering pumps, piping, and valves. Calcium hypochlorite also loses its strength when not properly stored in a cool and dry location away from direct sunlight (White, 1992), and it requires to be stored in a dark glass or plastic containers with airtight caps. When added to water it produces OH^- , which causes the solution to become alkaline and raises the pH; thus, the pH must be adjusted with acid to 6.0–7.5 to maintain HOCl at its optimum level (White, 1992; Dehghanisani et al., 2005).

Sodium Hypochlorite. Sodium hypochlorite (NaOCl) is also a salt of hypochlorous acid, which also hydrolyzes in water to produce free chlorine in the form of HOCl . Hypochlorous acid partially dissociates in water to form OCl^- and/or reacts with ammonia to produce combined chlorine.

Sodium hypochlorite is the active ingredient in household bleach which is commercially available at different concentrations. Chlorine concentration ranges from 1% to 5% (wt) for common domestic use and 5% to 16% (wt) for industrial use. Sodium hypochlorite is inexpensive and is the most readily available form of chlorine (Tietjen et al., 2003).

Like $\text{Ca}(\text{OCl})_2$, when NaOCl is added to water it produces OH^- , which causes the solution to become alkaline and raises the pH; thus, the pH must be adjusted to 6.0–7.5 (White, 1992; Dehghanisani et al., 2005).

CHLORINE AND PATHOGEN CONTROL

Chlorine disinfects by altering the chemical structure of microorganisms (Newman, 2004). Oxidizing compounds such as HOCl “burn” pathogens as well as organic material in the water (Newman, 2004). During this process, the oxidizing compounds are reduced and their activity is lost, therefore, it is important to maintain the desired free chlorine concentration in the water to allow continued disinfection (Newman, 2004).

Chlorine has been widely used in municipal water treatment. The common chlorine concentrations used in treating municipal water are listed in Table 1.

Table 1. Summary of chlorine concentrations ($\text{mg}\cdot\text{L}^{-1}$) used to treat municipal water.

Chlorine concentration ($\text{mg}\cdot\text{L}^{-1}$)	Chlorine compound	Reference
1–10	NaOCl	White, 1992
≤ 1 , 5–10	NaOCl	Frink and Bugbee, 1987
0.5–1	NaOCl	Newman, 2004
2–3	NaOCl	Havard, 2003
1–2, 10	NaOCl	Nelson, 2003

Research assessing the efficacy of chlorine in killing pathogens has shown that chlorine sensitivity varies with each pathogen and the duration of exposure of each pathogen to chlorine (Table 2). Taylor et al. (2000) reported that chlorine concentration in the range of 51 to 204 $\text{mg}\cdot\text{L}^{-1}$ is required to inactivate 99.9% of five different *Mycobacterium avium* strains. Thompson (1965) reported that a concentration of 1 $\text{mg}\cdot\text{L}^{-1}$ killed *Erwinia chrysanthemi* pv. *zeae* (syn. *E. carotovora* subsp. *zeae* Sabet) and prevented the spread of bacterial stalk rot of corn (*Zea mays* L.), but *E. chrysanthemi* Burkholder et al. and *E. carotovora* subsp. *carotovora* (Jones) Bergey were less sensitive to chlorine and survived at 10 $\text{mg}\cdot\text{L}^{-1}$ (Lacy et al., 1981). Hong et al. (2003) reported that zoospores of *Phytophthora capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. megasperma*, and *P. nicotianae* were killed with free residual chlorine concentrations ranging from 0.25 to 2 $\text{mg}\cdot\text{L}^{-1}$. There were no data available in the literature specifically for the management of *P. ramorum* and *P. cactorum* until recently. Cayanan et al. (2008a) conducted an extensive study recently to determine chlorine thresholds and critical contact times for common plant pathogens *Phytophthora infestans*, *P. cactorum*, *Pythium aphanidermatum*, *Fusarium oxysporum*, and *Rhizoctonia solani*. These pathogens were exposed to five different initial free chlorine solution concentrations ranging from 0.3 to 14 $\text{mg}\cdot\text{L}^{-1}$ 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 $\text{mg}\cdot\text{L}^{-1}$ for 3, 6, 3, 6, and 10 min, respectively.

Table 2. Summary of the free chlorine thresholds ($\text{mg}\cdot\text{L}^{-1}$) and contact times (min) required to kill microorganisms.

Microorganism	Propagule	Chlorine threshold ($\text{mg}\cdot\text{L}^{-1}$)	Contact time (min)	Reference
<i>Agrobacterium tumefaciens</i>	Bacteria	4.0	30.0	Poncet et al., 2001a
<i>Campylobacter jejuni</i>	Bacteria	0.1	5.0	Blaser et al., 1986
<i>Erwinia chrysanthemi</i> subsp. <i>zoeae</i>	Bacteria	1.0	1.0	Thompson, 1965
<i>Escherichia coli</i>	Bacteria	2.0–3.0	45.0	Havard, 2003
<i>Fusarium oxysporum</i>	Conidia	14	6	Cayanan et al., 2008a
<i>Helicobacter pylori</i>	Bacteria	1.1	45.0	Johnson et al., 1997
<i>Legionella pneumophila</i>	Bacteria	3.3	0.0	Skaliy et al., 1980
<i>Mycobacterium tuberculosis</i>	Bacteria	1000.0	10.0	Rutala et al., 1991
<i>Pseudomonas aeruginosa</i>	Bacteria	100.0	10.0	Rutala et al., 1998
<i>Staphylococcus aureus</i>	Bacteria	100.0	10.0	Rutala et al., 1998
<i>Acanthamoeba castellanii</i>	Protozoa	1.02	30.0	Cursons et al., 1980
<i>A. culbertsoni</i>	Protozoa	1.25	30.0	Cursons et al., 1980
<i>Naegleria fowleri</i>	Protozoa	0.74	30.0	Cursons et al., 1980
<i>N. gruberi</i>	Protozoa	0.79	30.0	Cursons et al., 1980
<i>Bacillus subtilis</i>	Endospores	100.0	60.0	Babb et al., 1980
<i>B. anthracis</i>	Spores	2.2	120.0	Brazis et al., 1958
<i>Plasmodiophora brassicae</i>	Spores	2.0	5.0	Datnoff et al., 1987
<i>Streptomyces griseus</i>	Spores	0.79	1.5	Whitmore and Denny, 1992
<i>S. griseus</i>	Mycelia	0.96	2.5	Whitmore and Denny, 1992

Table 2. (Continued)

Microorganism	Propagule	Chlorine threshold (mg L ⁻¹)	Contact time (min)	Reference
<i>Phytophthora capsici</i>	Zoospores	1.0	2.0	Hong et al., 2003
<i>P. cinnamomi</i>	Zoospores	1.0	2.0	Hong et al., 2003
<i>P. citricola</i>	Zoospores	0.5	2.0	Hong et al., 2003
<i>P. citrophthora</i>	Zoospores	2.0	2.0	Hong et al., 2003
<i>P. cryptogea</i>	Zoospores	0.25	2.0	Hong et al., 2003
<i>P. megasperma</i>	Zoospores	1.0	2.0	Hong et al., 2003
<i>P. nicotianae</i>	Zoospores	0.5	0.5	Hong et al., 2003
<i>P. infestans</i>	Sporangia	1.0	3.0	Cayanan et al., 2008a
<i>P. cactorum</i>	Zoospore	0.3	6.0	Cayanan et al., 2008a
<i>Pythium aphanidermatum</i>	Zoospore	2.0	3.0	Cayanan et al., 2008a
Hepatitis B virus	Virus	500.0	10.0	Bond et al., 1983
Norwalk agent	Virus	10.0	30.0	Keswick et al., 1985
Poliovirus 1	Virus	3.75	30.0	Keswick et al., 1985
Rotavirus (human strain Wa)	Virus	3.75	30.0	Keswick et al., 1985
<i>Rhizoctonia solani</i>	Mycelia	12.0	10.0	Cayanan et al., 2008a
Simian rotavirus SA11	Virus	0.5	4.0	Berman and Hoff, 1984

CHLORINE PHYTOTOXICITY

The ability of chlorine to kill microorganisms risks causing phytotoxic damage to plants, especially if chlorine is used in excess. Symptoms include chlorosis (bleaching action of tissues), necrotic mottling (red and black dark spots on the leaf surface), foliar necrosis (death of cells and cell tissue) (Vijayan and Bedi, 1989, Schreuder and Brewer 2001a), premature abscission of foliage (Frink and Bugbee, 1987), decrease in plant growth (Brown, 1991; Carrillo et al., 1996; Schreuder and Brewer, 2001b; Karaivazoglou et al., 2005), leaf discoloration, curling of leaves (Brown 1991, Karaivazoglou et al. 2005), cuticular damage resulting in increased rates of cuticular transpiration and decreased photosynthesis (Schreuder and Brewer, 2001b), damage to chloroplast membranes in conifers and reduced photosynthetic leaf area (Vijayan and Bedi, 1989; Schreuder and Brewer, 2001a), and marginal burning of leaves (Vijayan and Bedi, 1989).

Research on the phytotoxic effects of chlorinated water on herbaceous ornamental and vegetable plants was sparse, and even more limited for woody ornamentals (Table 3). Frink and Bugbee (1987) reported that geranium and begonia receiving chlorinated water declined in growth and Brown (1991) also reported reduction in height, and flower and bud production, of marigold and impatiens. There has been no research on nursery crops irrigated daily with chlorinated water in typical commercial nursery operations until recently, even though chlorine continues to be used in this context.

Recently, Cayanan et al. (2008b,c) studied the responses of container-grown ornamental plants both in a university research setting and a commercial nursery operation setting. Firstly, phytotoxic responses of five container-grown nursery species (*Spiraea japonica*, *Hydrangea paniculata*, *Weigela florida*, *Physocarpus opulifolius*, and *Salix integra*) to chlorinated irrigation water and critical free chlorine thresholds were evaluated. Plants were overhead-irrigated with water containing 0, 2.5, 5, 10, and 20 mg·L⁻¹ of free chlorine for 6 weeks during the late growing season (fall). All species exhibited one or more signs of chlorine injury including foliar necrotic mottling, foliar necrosis and chlorosis, decreased plant height, and increased premature abscission of foliage with species varying in sensitivity to free chlorine concentrations of irrigation water. The results indicated that the free chlorine threshold of *Spiraea*, *Hydrangea*, *Weigela*, and *Salix* was 2.5 mg·L⁻¹, and 5 mg·L⁻¹ for *Physocarpus*. Based on these results and the results of common pathogens response to chlorine (Cayanan et al., 2008a), 17 species of container-grown nursery plants (including 10 deciduous shrubs, seven evergreen species) were overhead-irrigated with water that was treated with NaOCl (bleach) for 11 weeks in the early growing season (summer) in a commercial nursery and potential phytotoxic responses were evaluated (Cayanan et al., 2008c). Irrigation water was treated with 2.4 mg·L⁻¹ of free chlorine for 5 min before watering plants. No visual injuries or growth reduction on the evergreen shrubs were observed, but there were visual injuries and/or growth reduction on some of the deciduous shrubs. Interestingly, the authors also noticed that the extent of anthracnose infection on the leaves of chlorine-sprayed dogwood were much less than that on the leaves of the control. The reduction of anthracnose severity with chlorinated water may reduce the number of applications of fungicides. A DNA multiscan of the irrigation water samples indicated that the chlorine killed all fungi and oomycetes detected in the irrigation water. Although there were visible leaf injuries and growth reduction on

Table 3. Summary of free chlorine thresholds ($\text{mg}\cdot\text{L}^{-1}$) for plants.

Chlorine threshold ($\text{mg}\cdot\text{L}^{-1}$)	Plants	Reference
< 1	Vegetable seedling	Frink and Bugbee, 187
≤ 2	Vegetables	Brown, 1991
2.9	Ornamental crops	Skimina, 1992
4	Rose	Poncet et al., 2001b
4	Gerbera, rose	Poncet et al., 2001b
4	Gerbera, rose	Poncet et al., 2001b
< 5	Zinnia, chrysanthemum	Bridgen, 1986
10	Kentucky bluegrass sod, snapdragon	Brown, 1991
20-40	Tobacco	Karaivazoglou et al., 2005
> 0.1	Herbaceous crops	Brennan et al., 1996
> 0.4	Chrysanthemum and rose	Nelson, 2003
> 0.5	Weed	Brennan et al., 1996
> 1	Pine tree	Brennan et al., 1996
> 1	Tree	Coder, 2004
> 2	Begonia, geranium	Frink and Bugbee, 1987
> 5	Impatiens, marigold	Brown, 1991
> 5	Potted plants	Krone and Weinard, 1931
> 7.6	Zinnia seedling	Bridgen, 1986
> 8	Pepper, tomato	Frink and Bugbee, 1987
> 15.2	Mums	Bridgen, 1986
> 18	Kalanchoe, lettuce, tradescantia	Frink and Bugbee, 1987
> 37	Broccoli, petunia.	Frink and Bugbee, 1987
> 50	Sweet pepper	Ehret et al., 2001
> 77	English ivy, Madagascar palm, Swedish ivy	Frink and Bugbee, 1987

some of the deciduous plants, the plants were still salable. Results suggested that irrigation water treated with $2.4 \text{ mg}\cdot\text{L}^{-1}$ of free chlorine for 5 min can be used to prevent the spread of the common plant pathogens, *P. infestans*, *P. cactorum*, and *P. aphanidermatum*, without losing the market value of plants.

CONCLUSIONS

Chlorine is an economical and effective mean in controlling pathogens. For most of the chlorine products, maintaining certain level of free chlorine for a certain contact time in the subjected water is the key. To target only *Phytophthora* and *Pythium* pathogens, which are the most common plant pathogens in irrigation water, only $2 \text{ mg}\cdot\text{L}^{-1}$ of free chlorine is required. For *F. oxysporum*, *R. solani*, and some other chlorine-resistant pathogens higher free chlorine concentration is needed, but one can design a chlorination system to remove the free chlorine from the irrigation water

before watering the plants. To target the most common pathogens, a treatment with $2 \text{ mg}\cdot\text{L}^{-1}$ of free chlorine and use of 5 min. or longer contact times is recommended.

Future research needs to determine the contact time required to kill chlorine-resistant pathogens such as *F. oxysporum* and *R. solani* at a lower free chlorine concentration that is safe for plants. This will allow growers to not only kill *P. infestans*, *P. cactorum*, and *P. aphanidermatum*, but also chlorine-resistant pathogens such as *F. oxysporum* and *R. solani*, without harm to plants. Future research also needs to investigate the long-term effects of chlorine bi-products such as chloramines, which over a long period may build up. This is important as chloramines can cause severe plant damage. Currently, the safe threshold level of chlorine bi-products is unknown. This knowledge will be useful for growers to allow them to plan for the future when watering crops with chlorinated irrigation water.

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