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Phytotoxic Effects of Hypochlorous Acid, Chloramines, and Chlorine Dioxide in Irrigation Water Applied to Bedding and Vegetable Plants

Catherine M. Donovan*, Paul R. Fisher, and Jinsheng Huang

Environmental Horticulture Dept., University of Florida, IFAS, Gainesville, FL 32611-0670

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Many producers sanitize recycled irrigation water before reapplication to crops in order to avoid transmission of waterborne pathogens. Previous research and grower experience has shown the potential for phytotoxicity when sanitizing agents including hypochlorous acid, chlorine dioxide, and chloramines are applied in irrigation water. The objective of this study was to measure and photograph phytotoxic responses when 0 to 100 mg·L^{-1} of these three sanitizers were applied to 39 species of container-grown ornamental and vegetable plants. In one trial, 0, 1, 2, 4, 8, or 16 mg·L^{-1} was applied once daily as an overhead irrigation at 15 mL/plant to seed *Pelargonium ×hortorum* L.H. Bailey 'Ringo 2000 Deep Red', *Gomphrena* sp. 'Fireworks', and *Viola ×wittrockiana* Gams 'Panola XP Baby Boy Mixture' in 144-count plug trays over three weeks. No phytotoxic or growth suppression effects were observed. In a second trial, 39 species received either no sanitizing agent or 8 mg·L^{-1} of chloramine, hypochlorous acid and chlorine dioxide applied five times a week for 6 weeks in both seedling plug trays and after transplant into 10-cm-diameter (4-inch) pots. *Ocimum basilicum* L. 'Genovese' basil, *Begonia obliqua* L. 'Baby Wing White' begonia, *Dianthus chinensis ×barbatus* L. 'Floral Lace Purple' dianthus, *Lactuca sativa* L. 'Vulcan', and 'Green Star' lettuce and *Lobularia maritima* (L.) Desv. 'Clear Crystal White' alyssum showed phytotoxicity symptoms (leaf bronzing and chlorosis) from hypochlorous acid or chlorine dioxide. *Ocimum basilicum* L. 'Genovese' basil also showed some phytotoxic responses to chloramineas well. In a third trial, the 39 species received two applications of 0 or 100 mg·L^{-1} of the sanitizers, resulting in widespread damage on most (64%) plant species from chlorine dioxide, but no phytotoxicity from hypochlorous acid or chloramines except on *Angelonia angustifolia* Benth. 'Serena Purple' angelonia, *Ocimum basilicum* L. 'Genovese' basil, or *Salvia splendens* Sellow ex Nees 'Vista Red' salvia. Given that typical applied concentrations of hypochlorous acid or chlorine dioxide are below 2 mg·L^{-1}, results indicate that at this level phytotoxicity is not likely when solutions are applied to foliage once daily under rapid drying conditions. However, under other conditions, such as when chemical injector equipment malfunctions, or a mixing error occurs, sanitizers can rapidly cause phytotoxicity.

Greenhouse growers are under increasing pressure to conserve and re-use irrigation water given increased concern regarding water resources and quality. Capture and reuse (recycling) of irrigation water reduces runoff and conserves water, but increases potential for distribution of waterborne pathogens. Hong and Moorman (2005) reported 17 different species of *Phytophthora*, 26 species of *Pythium*, 27 species of fungi, 8 species of bacteria, 10 different viruses, and 13 species of plant parasitic nematodes present in surveyed bodies of irrigation water. Buildup of algae and biofilms in water tanks and irrigation lining can also occur (Dehghanisanij et al., 2005). Although growers historically use fungicides to control *Phytophthora* and *Pythium* diseases, increasing fungicide resistance is making control of these diseases more challenging (Cayanan et al., 2009). In order to control potential pathogens, diseases, and algae, many growers therefore add a sanitizing agent into recycled irrigation water such as chlorine, chlorine dioxide, ozone, hydrogen peroxide and activated peroxygens, copper, silver, or bromine. Physical treatments such as UV and heat as well as ecological treatments such as constructed wetlands are also used (Raudales et al., 2014).

The most widely used sanitizing agents are chlorine-based products because of their low economic cost, and known efficacy in drinking water disinfection (Cayanan et al., 2008). Chlorine can be applied to recycled water in several forms, including hypochlorous acid (HOCl) from sodium hypochlorite, chlorine gas, calcium hypochlorite and purified hypochlorous acid, and acts through both oxidation and chlorination of reagents (Raudales et al., 2014). Chlorine dioxide (ClO_2) remains as a dissolved gas, and acts as an oxidizer (Raudales et al., 2014). Chloramines are formed by a reaction of ammonia with hypochlorous acid, and the resulting solution can contain monochloramine (NH_2Cl), dichloramine ($NHCl_2$), or nitrogen trichloride (NCl_3) (EPA, 1999). Chloramines are more stable than hypochlorous acid and have longer residual effects but require longer contact time for sanitation (Raudales et al., 2014).

A potential issue when using these sanitizing agents is to quantify the dosage that will sanitize irrigation water without causing phytotoxic effects on the crop diseases (Cayanan et al., 2008 and 2009). The phytotoxic thresholds for most crops are unknown and many growers use a standard concentration of 2 mg/L or 0.25 mg/L of residual chlorine and chlorine dioxide, respectively, in their recycling systems (Fisher et al., 2008; Raudales et al., 2014).

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*Corresponding author. Phone: 707-529-3274; e-mail: catherine.donovan@ejgallo.com

Materials and Methods

From 12 Feb. 2015 to 25 Mar. 2015, at the University of Florida in Gainesville, FL, chloramine, hypochlorous acid, and chlorine dioxide were applied to a range of bedding and vegetable plants in four experiments. A solution of 100 mg·L^{-1} hypochlorous acid was generated by combining 5.1 mL of sodium hypochlorite (7.85% active chlorine assay, Clorox, Clorox Company, Oakland, CA) with 4 L of deionized water into a 4-L brown glass bottle. The concentration of the new stock solution was tested using a DPD colormetric meter for both free and total chlorine levels. In order for the solution to be within the measurable range, 1mL of stock solution was drawn from the glass bottle and added to a 100 mL graduated cylinder and the cylinder was filled to 100 mL with deionized water for a 100% dilution. The solution was mixed well before taking a 10 mL sample for the reading using an Orion AQ4000 DPD colormetric meter. In order to dilute the 100 mg·L^{-1} stock hypochlorous acid solution into various experimental levels, the following equation was used to calculate the correct dosage: 100 mg·L^{-1} x A = target mg·L^{-1} x target volume in milliters, where A = volume of 100 mg·L^{-1} stock to be added in milliters.

The 100 mg·L^{-1} chloramine sanitizing agent was generated by combining 5.1 mL of regular Clorox brand bleach (7.85% active chlorine assay) with 4 L of deionized water and 1mL of ammonium hydroxide (assay of 28.89%) into a 4-L brown glass bottle. The resulting solution was tested as described for the hypochlorous acid solution, with the added step whereby the free chlorine reading was subtracted from total chlorine to provide a combined chlorine measurement which indicates the chloramine level of the solution. The resulting chloramine concentration was only used if measured concentration was within 0.15 mg·L^{-1} of the expected 1.0 mg·L^{-1}.

To generate the chlorine dioxide solution, a "Z-series Solution sachet" sodium chlorite-precursor to chlorine dioxide solution pack was provided by ICA-TriNova LLC (Newnan, GA). Once received, in a well ventilated area under a fume hood, the Precursor Poly-Pack was opened and the reactor sachet (containing sodium chlorite) and activator sachet were removed. The Reactor Sachet was opened, and the contents of the Activator (which contained the sulfuric acid activator for the chlorine dioxide solution pack) were placed into the Reactor Sachet. The empty activator pack was then discarded. The reactor sachet was sealed along the zip-lock seal and shaken to mix the materials now inside that reactor sachet. The reactor sachet was then placed into a 5-L water bath with the clear side of the bag facing up. The bag was kept in the water bath for three days at room temperature allowing as much chlorine dioxide to form in the solution as possible. The sachet was removed and the resulting solution was transferred into 4-L brown glass bottles and tested for a concentration.

To test the concentration of the resulting chlorine dioxide stock solution, an iodometric titration method recommended by the TriNova manufacturer was used. Distilled water (10 mL) was placed into a 250-mL beaker and 10 mL of buffered 10 wt% potassium iodide solution was added to the solution. The chlorine dioxide stock solution (5 mL) was drawn into a pipette and this volume was recoded as VS. The chlorine dioxide solution was released into the sub-surface of the solution in a 250-mL beaker. The pH was measured, and in this trial always remained within the required range of 6.5 to 7.5 without addition of a buffer. Eight to ten drops of starch indicator were added to the solution and the potassium iodide solution was titrated to a colorless end point using 0.01 N sodium thiosulfate ($Na_2S_2O_3$). The volume of sodium thiosulfate used for the titration was recorded as VN (neutral titration). Using the pH probe to stir the potassium iodide solution, 2 N H_2SO_4 was added slowly to a pH of < 2.0. The solution was allowed to stand for 5 min. in the dark. The potassium iodide solution was titrated to a colorless end point using the 0.01 N sodium thiosulfate and the titrated volume of sodium thiosulfate was recorded as VA (acid titration).The solution was discarded. To calculate the resulting chlorine dioxide concentration, the following equation was used along with the values recoded from the resulting titration as described above:

If VA/VN < 4, the solution contained chlorine dioxide and hypochlorous acid and the following equation was used to quantify ClO_2 concentration, where N = normality of sodium thiosulfate:

$$ClO_2, (mg/L) = \frac{V_A x N x 67{,}500}{4 x V_S}$$

If VA/VN > 4, the solution contained chlorine dioxide and chlorite ions, and the following equation was used:

$$ClO_2, (mg/L) = \frac{V_N x N x 67{,}500}{V_S}$$

Trial One

Seedlings of *Pelargonium xhortorum* L.H. Bailey 'Ringo 2000 Deep Red', *Gomphrena* sp. 'Fireworks', and *Viola xwittrockiana* Gams 'Panola XP Baby Boy Mixture' were received in 144-count trays. Trays were cut into portions with each section containing six cells, where each six-cell section represented one replicate for the trial. Each six-cell group was assigned a sanitizer treatment at a specific level. For the first trial, stock solutions of six different levels of each sanitizing agent (0, 1, 2, 4, 8, or 16 mg·L^{-1}) were prepared and tested every few days to ensure the solution stayed within plus or minus 0.05 mg·L^{-1} of the target dosage. The experimental design consisted of four blocks with one replicate per species in each block for each sanitizing agent at each dosage level and three sets per block of additional control replicates. The replicates were randomly assigned positions on the greenhouse bench using a random number generator. A measured volume of 30 mL of sanitizing agent was applied per replicate per day as a foliar spray for the first five applications, increasing to 90 mL for later applications. The sanitizer was applied five days per week, with two days of fertilizer application per week of 100 mg·L^{-1} of N from a modified Hoagland's solution with 100% of N as NO_3-N to avoid possible reaction with the hypochlorous acid. After 15 applications, average chlorophyll content was measured using a Minolta SPAD SO2 Plus Chlorophyll meter from five representative leaves from each plant. Three plants from each replicate were harvested to calculate dry weight.

Trial Two

A second trial was run in parallel to the first trial which included the 36 species shown for Trial Two in Table 1. The plants were received in 144-count plug trays and cut into 20-cell portions. There is one replicate per treatment per species and each replicate was a 20-cell portion of a 144-count tray. Each replicate received one of four possible treatments: 8 mg·L^{-1} chloramine, 8 mg·L^{-1} chlorine dioxide, 8 mg·L^{-1} chlorine, and 0 mg·L^{-1} control. For the first five applications, 60 mL per replicate was applied, followed by 180 mL for the following 10 applications. Treatments were

Table 1. Species and cultivars evaluated in the three trials.

Included in Trials	Species Common Name	Species Scientific Name and Cultivar
One and Three	Geranium	*Pelargonium* x*hortorum* L. H. Bailey 'Ringo 2000 Deep Red'
	Gomphrena	*Gomphrena sp*. 'Fireworks'
	Pansy	*Viola* x*wittrockiana* Gams 'Panola XP Baby Boy Mixture'
Two and Three	African marigold	*Tagetes erecta* L. 'Taishan Orange'
	Alyssum	*Lobularia maritima* (L.) Desv. 'Clear Crystal White'
	Angelonia	*Angelonia angustifolia* Benth. 'Serena Purple'
	Arugula	*Eruca vesicaria* (L.) Cav. subsp. *sativa* (Mill.) Thell.
	Basil	*Ocimum basilicum* L. 'Genovese'
	Begonia	*Begonia obliqua* L. 'Baby Wing White'
	Blueberry	*Vaccinium corymbosum* L. 'Misty'
	Celery	*Apium graveolens* L. 'Conquistador'
	Cilantro	*Coriandrum sativum* L.
	Coleus	*Solenostemon scutellarioides* (L.) R. Br. 'Premium Sun Chocolate Covered Cherry'
	Cosmos	*Cosmos bipinnatus* Cav. 'Sonata Premium Pink'
	Cucumber	*Cucumis sativus* L. 'Marketmore 76'
	Dianthus	*Dianthus chinensis* x*barbatus* L. 'Floral Lace Purple'
	Dusty miller	*Senecio cineraria* DC. 'Silverdust'
	Eggplant	*Solanum melongena* L. 'Galine'
	French marigold	*Tagetes patula* L. 'Durango Bolero'
	Gerbera	*Gerbera jamesonii* Adlam 'Mega Revolution Yellow'
	Impatiens	*Impatiens walleriana* Hook. f. 'Super Elfin'
	Lavender	*Lavandula angustifolia* Mill. 'Ellagance Purple'
	Lettuce	*Lactuca sativa* L. 'Green Star'
	Lettuce	*Lactuca sativa* L. 'Romaine Green Forest'
	Lettuce	*Lactuca sativa* L. 'Vulcan'
	Lisianthus	*Eustoma grandiflorum* L. 'Florida Blue Sky'
	Lobelia	*Lobelia erinus* L. 'Regatta Blue Sky'
	New Guinea impatiens	*Impatiens hawkeri* W. Bull 'Florific White Blush'
	Pansy	*Viola* x*wittrockiana Gams* 'Matrix Yellow Clear'
	Pentas	*Pentas lanceolata* (Forssk.) Deflers 'Red Butterfly'
	Pepper	*Capsicum annuum* L. 'Bell King Arthur'
	Petunia	*Petunia* x*hybrida* hort. ex E. Vilm. 'Dreams Red'
	Salvia	*Salvia splendens* Sellow ex Nees 'Vista Red'
	snapdragon	*Antirrhinum majus* L. 'Snapshot Yellow'
	Spinach	*Spinacia oleracea* L. 'Savoyed'
	Tomato	*Lycopersicon esculentum* Mill. 'Big Beef'
	Verbena	*Verbena* x*hybrida* Groenland & Rümpler 'Quartz XP White'
	Vinca	*Catharanthus roseus* (L.) G. Don 'Titan Dark Red'
	Zinnia	*Zinnia hybrida* 'Profusion Double Fire'

applied once a day five times a week, with fertilizer solution twice a week. After 15 treatments, phytotoxicity symptoms were photographed and average chlorophyll content was measured using a SPAD SO2 Plus Chlorophyll meter from five representative leaves per replicate. Four plants from each replicate were harvested to calculate dry weight.

Trial Three

Control plants from the 39 species (Table 1) from Trials One and Two were transplanted into 10-cm-diameter containers, with four replicate plants per species receiving either no sanitizing agent (the control), or three applications of 100 $mg \cdot L^{-1}$ of hypochlorous acid, chlorine dioxide, or chloramines once a day for three days, beginning 8 days after transplant. The number of damaged leaves (showing bronzing or chlorosis) per plant (or leaflets per plant for African and French marigold) was recorded and photographs were taken.

All sanitizing agent solutions were prepared using deionized water with final electrical conductivity (EC) less than 0.47 mS/cm. For Trials One and Two, pH was corrected to 6 for all solutions using either 2N sulfuric acid (H_2SO_4) for the basic hypochlorous acid and chloramine solutions or 1N sodium hydroxide (NaOH) for the acid chlorine dioxide solution. For Trial Three, the high concentrations (100 $mg \cdot L^{-1}$) of hypochlorous acid and chloramine solutions were highly buffered, and were adjusted to pH 8. The acidic chlorine dioxide solution was less buffered, and was adjusted to pH 6. Substrate-pH and substrate-EC were measured during all trials using the plug squeeze method (Scoggins et al., 2002). Solutions did not affect substrate-pH or substrate-EC, and averaged pH 6.2 and and EC of 0.4 mS/cm.

Statistical Analysis

Trials One and Three were analyzed as complete randomized designs with factors of species and chemical treatment and four replicates. Data were analyzed using ANOVA with SAS PRO GLM. Means were separated using Tukey HSD test at α=0.05 by species. Trial Two did not have independent replicates, but observations and photos were recorded.

Results

Trial One: Three species at six levels of chlorine, chloramine and chlorine dioxide

There was no significant difference between chemical applications in either dry mass or SPAD chlorophyll index. No phytotoxic symptoms were observed in *Pelargonium xhortorum* L. H. Bailey 'Ringo 2000 Deep Red', *Gomphrena* sp. 'Fireworks', and *Viola xwittrockiana* Gams 'Panola XP Baby Boy Mixture', even at 16 $mg \cdot L^{-1}$ of the three sanitizers.

Trial Two: 36 species at either 0 $mg \cdot L^{-1}$ or 8 $mg \cdot L^{-1}$ of chlorine, chloramine, and chlorine dioxide

Phytotoxic symptoms were observed in certain cultivars at 8 $mg \cdot L^{-1}$ of either hypochlorous acid or chlorine dioxide, but no obvious damage was noted for the chloramine treatment in any species except for basil. Bronzing or chlorosis were observed in the chlorine and chlorine dioxide treatments on the following cultivars: *Ocimum basilicum* L. 'Genovese' basil, *Begonia obliqua* L. 'Baby Wing White' begonia, *Dianthus chinensis xbarbatus* L. 'Floral Lace Purple' dianthus, *Lactuca sativa* L. 'Vulcan' and 'Green Star' lettuce and *Lobularia maritima* (L.) Desv. 'Clear Crystal White' alyssum (Fig. 1).

Trial Three: 39 species at 100 $mg \cdot L^{-1}$ of chlorine, chloramine and chlorine dioxide

This experiment investigated the phytotoxic responses in terms of number of damaged leaves per plant in 39 species to three foliar applications at 100 $mg \cdot L^{-1}$ of hypochlorous acid, chloramine or chlorine dioxide. There were significant main and interaction effects between species and sanitizer type ($p < 0.0001$). Application of 100 $mg \cdot L^{-1}$ hypochlorous acid caused bronzing on *Angelonia angustifolia* Benth. 'Serena Purple' angelonia (4.0 damaged leaves per plant) and *Ocimum basilicum* L. 'Genovese' basil (9.1 damaged leaves per plant) but other species were not affected by hypochlorous acid. Chloramines caused slight bronzing on basil (8.9 damaged leaves per plant) and chlorosis on salvia, but not affect other species.

Chlorine dioxide caused by far the greatest phytotoxicity, including spotting or bronzing on 25 species (64% of those tested, Fig. 2). Fourteen species (36% of those tested) showed no damage from any chemical. Damage from chlorine dioxide (Fig. 2) ranged from high levels of over twenty leaflets per plant in French marigold to no leaves showing damage in species such as blueberry, cilantro, and tomato.

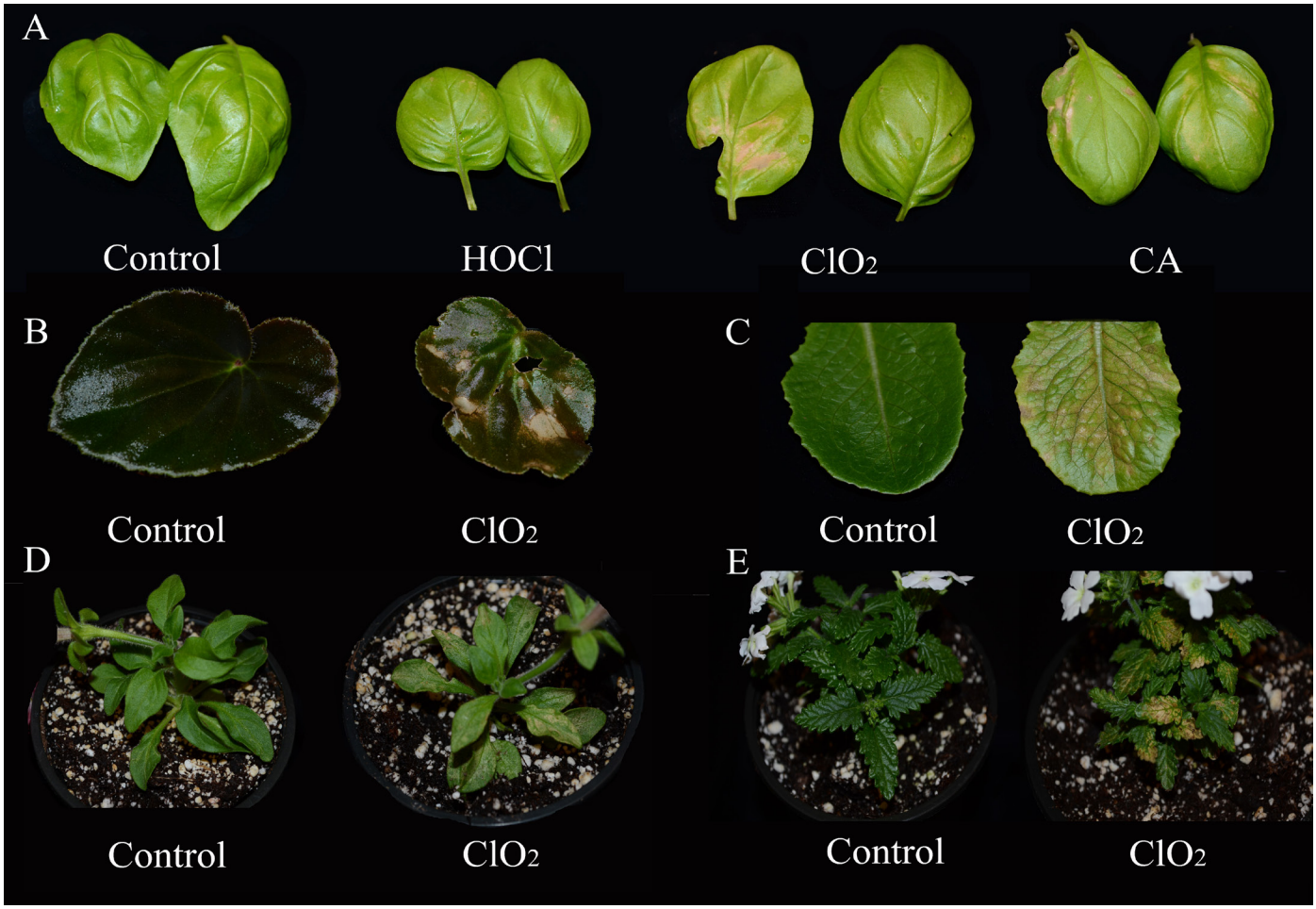

Fig. 1. Phytotoxic symptoms for (**A**) *Ocimum basilicum* L. 'Genovese' from 8 $mg \cdot L^{-1}$ of either hypochlorous acid (HOCl), chlorine dioxide (ClO_2) or chloramines (CA) in Trial Two. Subsequent photos from Trial Three show response to 100 $mg \cdot L^{-1}$ of chlorine dioxide (ClO_2) to (**B**) *Begonia obliqua* L. 'Baby Wing White', (**C**) *Lactuca sativa* L. 'Romaine Green Forest', (**D**) *Petunia* x*hybrida* hort. ex E. Vilm. 'Dreams Red', and (**E**) *Verbena* x*hybrida* Groenland & Rümpler 'Quartz XP White'.

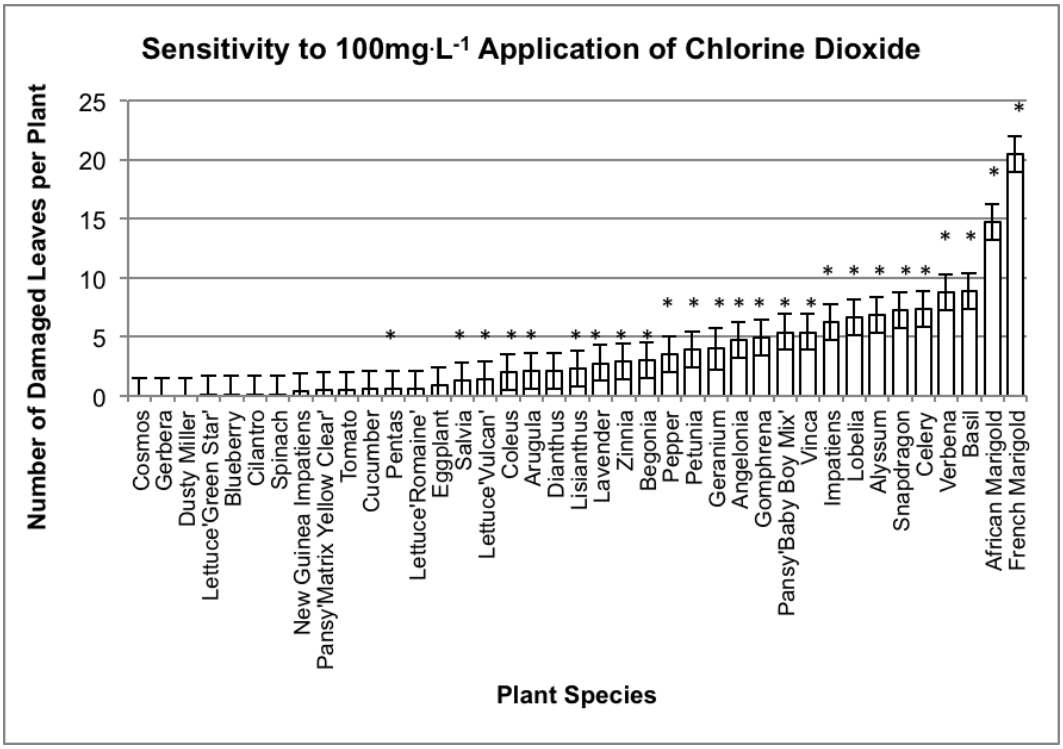

Fig.2. Phytotoxicity from foliar application of chlorine dioxide at 100 mg·L^{-1}, quantified as the number of leaves per plant exhibiting damage (spotting, bronzing, or chlorosis) for 39 species. Error bars represent 95% confidence intervals. Asterisks above the bars indicate significantly different from the control plants (zero chlorine dioxide).

Conclusion

In conclusion, a very high rate (100 mg·L^{-1}) of hypochlorous acid or chlorine dioxide caused phytotoxicity in the majority of bedding and vegetable plants tested. Although at lower dosages up to 16 mg·L^{-1} minimal phytotoxicity was noted, previous research indicates that container-grown ornamental crops can be susceptible to phytotoxicity due to free chlorine with dosages as low as 2.4 mg·L^{-1} (Cayanan et al., 2009). Therefore, there are risks associated with the use of chlorine and chlorine dioxide sanitizing agents and there is a need for growers to be able to minimize these risks. We may have observed low levels of phytotoxicity in our trial at 1–16 mg·L^{-1} because of applying solutions only once daily, and because of rapid drying on foliage. It was found that high and regular rates of chlorine dioxide (50 or more mg·L^{-1}) as well as hydrogen dioxide when sprayed five times at three day intervals did result in phytotoxic damage at the highest dosages (Copes et al., 2003). Other conditions, such as mist application with high humidity and high applied water volumes, may cause greater damage.

Some suggestions for avoiding such examples of phytotoxicity are to increase contact time while reducing the chlorine dosage (Raudales et al., 2014). Also the use of activated carbon filters to remove the active portion of the chlorine sanitizing agent before reapplication has been suggested (Suidan et al., 1980). Avoidance of dosage miscalculations and errors as well as frequent monitoring of dosage machinery for malfunctions are practical solutions as well, because simple miscalculations and mechanical malfunctions could lead to overdoses of a sanitizing agent and phytotoxic damage to crop plants.

Literature Cited

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

Cayanan, D.F., Y. Zheng, P. Zhang, C. Chong, and J. Llewellyn. 2008. Sensitivity of five container-grown nursery species to chlorine in overhead irrigation water. HortScience 43(6):1882–1887.

Copes, W.E., Chastagner, G.A., and Hummel, R.L. 2003. Toxicity responses of herbaceous and woody ornamental plants to chlorine and hydrogen dioxides. Online. Plant Health Progress doi:10.1094/PHP-2003-0311-01-RS. <http://www.plantmanagementnetwork.org/pub/php/research/2003/disinfect/>.

Dehghanisanij, H., T. Yamamoto, B. Ould Ahmad, H. Fujiyama, and K. Miyamoto. 2005. The effect of chlorine on emitter clogging induced by algae and protozoa and the performance of drip irrigation. Trans. ASAE. 48(2):519–527.

Fisher, P., W.R. Argo, J. Huang, P. Konjoian, J. Majka, L. Marohn, and R. Yates. 2008. Water treatment series: Chlorine dioxide can treat water. Greenhouse Management and Production. 28(9):14–17.

Hong, C.X. and G. W. Moorman. 2005. Plant pathogens in irrigation water: challenges and opportunities. Crit. Rev. Plant Sci. 24(3):189–208.

Raudales, R., J. Parke, C. Guy, and P. Fisher. 2014. Control of waterborne microbes in irrigation: A review. Agricultural Water Management. 143:9–28.

Scoggins, H.L., D.A. Bailey, and P.V. Nelson. 2002. Efficacy of the press extraction method for bedding plant plug nutrient monitoring. HortScience 37(1):108–112.

Suidan, M., W. Cross, and K. Chacey. 1980. Extended dechlorination studies with granular activated carbon filters. J. Water Pollution Control Federation. 52(11):2634–2646.

U.S. Environmental Protection Agency. 1999. Chloramines. In EPA Guidance Manual: Alternative Disinfectants and Oxidants. U.S. Environmental Protection Agency, Washington, DC. <http://www.epa.gov/ogwdw/mdbp/alternative_disinfectants_guidance.pdf>.