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Toxicity Responses of Herbaceous and Woody Ornamental Plants to Chlorine and Hydrogen Dioxides

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Abstract

Regular and excessive rates of chlorine dioxide (ClO_2) and hydrogen dioxide (H_2O_2) were sprayed five times at 3-day intervals on eight bedding plant and nine shrub species to determine if plant damage would result. Marketability was judged to be reduced if $\geq$4% surface area of leaves and/or flowers were adversely affected. Rates of 5 and 50 ppm ClO_2 and 900 and 2700 ppm H_2O_2 did not damage most plants tested and will likely control most common pathogen propagules. Rates of 100 ppm ClO_2 and 5400 ppm H_2O_2 did not damage most plants tested if sprayed less than four consecutive times and should control some of the more chemical-tolerant pathogens.

Introduction

Ornamental plant growers routinely use disinfectants to kill pathogen propagules on inert surfaces. Also, bleach products have been used for many years to disinfect surfaces of cuttings from woody plants. The addition of quaternary ammonium compounds (QAC) can extend the vase-life of chrysanthemum flowers (5). QAC products have also been applied directly to plants during production. The use of disinfectants in ornamental plant production is currently limited by the lack of data on efficacy, residual activity, and potential of plant damage.

Disinfectants kill fungal inoculum (spores, mycelia, sporangia, etc.) and bacterial cells by direct contact and provide no systemic nor residual protection. Disinfectants are active for only minutes (depending on chemical reactions with water properties and surfaces [i.e., demand load], volatilization loss of the active chemical, and evaporative drying of the disinfectant from a surface). Pathogen propagules contacted at the time of application are killed but propagules that arrive hours or even minutes after the disinfectant has dried or dissipated are unaffected.

Fungicide and bactericide spray practices have been developed over many years of scientific tests and commercial usage. A very limited research base currently exists to support recommendations for spray intervals for disinfectants or for specific strategies to alternate fungicide, bactericide, and disinfectant applications for the management of diseases on ornamental plants. ZeroTol (27% a.i. hydrogen dioxide [H_2O_2]) is currently labeled and being used as a pre-plant dip or foliar spray on greenhouse and field ornamental plants, cut flowers, and bare-root nursery stock (EPA Registration No. 70299-1). The 2002 ZeroTol label lists rates of 53 to 264 ppm H_2O_2 in mist propagation systems, 530 ppm H_2O_2 in treatment of cut flowers, 1050 ppm H_2O_2 for preventative foliar applications under greenhouse and field conditions, 2640 ppm H_2O_2 for curative foliar applications and in treatment of bare-root nursery stock, and 5270 ppm H_2O_2 in treatment of seed beds and soil.

Chlorine dioxide (ClO_2) is another disinfectant with potential for direct application to plants. It is registered for use in fruit and vegetable processing lines (EPA Reg. No. 5382-43-43553). It is labeled at 3 ppm ClO_2 on cut and peeled fruits and vegetables and with no rate restriction on raw agricultural products with the stipulation that produce be rinsed with water after treatment.

Chastagner and Riley (2) showed that ClO_2 was an effective replacement for formaldehyde in preventing the spread of Fusarium basal rot during the hot water treatment of daffodil bulbs. No adverse growth effects were noted on bulbs that had received a 4-hour-long dip at 10 ppm ClO_2. Carrillo et al. (1) showed that five applications at 0.52 ppm ClO_2 did not harm seedlings of radish (*Raphanus sativus*) cv. Champion and lettuce (*Lactuca sativa*) cv. Great Lakes 118. A single application of ClO_2 at 1.04 ppm also had no negative effect, while five applications had only a small adverse effect from which plants recovered. A single application at 26 ppm ClO_2 water caused a slight reduction in growth, and five applications at 3-day intervals resulted in growth reduction and leaf chlorosis (1).

The use of disinfectants in a foliar disease management program will only be possible if the rate of disinfectant that is required to kill pathogen propagules does not cause injury to the plant. The objective of this research was to evaluate the potential phytotoxicity associated with foliar applications of chlorine and hydrogen dioxides across a range of concentrations that included and exceeded rates of these materials expected to kill pathogen propagules.

Plant Care

Experiments were performed in a greenhouse with temperatures between 70° and 77°F. Plants were potted in Sunshine Mix No. 1/LC1 in 4-inch pots for bedding plants and 1-gal pots for shrubs. Each plant species was watered as needed (once per 1 to 2 days) at the base of each plant. Plants were drench-fertilized weekly with 120 ppm N (2 g Peter's Azalea Fertilizer + 0.02 g calcium nitrate tetrahydrate + 0.01 g potassium nitrate per liter).

Setup, Application, and Rating Methods

For each replication, all plant species were grouped in a single row from smallest to tallest plant across a greenhouse bench. Each rate was tested on four replications of plants that were randomized with in-row spacing of 3/4 to 4 inches, depending on plant size, and between-row spacing of 12 inches. A split-plot design was used in the analysis to test toxicity from disinfectant rate over time (5 applications at 3-day intervals).

All treatments were applied at 3-day intervals. Disinfectant concentrations were mixed in a ½ gal (2 liter) soda bottle immediately before use, quickly capped, and attached to a pressurized CO_2 sprayer that had a TeeJet 8002VS nylon flat fan nozzle. Disinfectants were applied at 44 to 46 psi. The spray bottle was gently inverted before application on each row to maintain a uniform suspension. A tarp-covered PVC frame with an open front and bottom was placed over a plant row prior to treatment. Sprays were made in three passes, one downward and two upward directed sprays, to cover top and bottom surfaces from each side of the plant row. Treatments were applied in order from lowest to highest concentration. The tarp enclosure was shaken to remove drips between replications and rinsed between disinfectants or before being stored.

Phytotoxicity ratings were taken 3 days after an application and earlier in the day before the next application. After the fifth and final spray, plants were rated 6 days later. Symptoms were rated separately for leaves and flowers based on the Barratt-Horsfall scale (0 = 0% area, 1 = 1 to 3%, 2 = 4 to 6%, 3 = 7 to 12%, 4 = 13 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, 7 = 76 to 87%, 8 = 88 to 93%, 9 = 94 to 96%, 10 = 97 to 99%, 11 = 100%) with the aid of diagrammatic representations (6,7). Mid-point values (e.g., mid-point of category 4 = 19%) were used for calculations (e.g., means) for each organ (leaves and flowers) of each plant.

Marketability was considered to be detrimentally affected if $\geq$4% surface area of a plant was adversely affected, although mean toxicity ratings of <4% may have been significantly different than 0. The 4% level was arbitrarily selected based on our opinion that very low levels of damage would not be noticed by most consumers, while damage $\geq$4% seemed to exceed general market standards of acceptability.

Chlorine Dioxide Trials

Six bedding plant and shrub species were utilized in four ClO_2 experiments (Table 1). ClO_2 was generated immediately prior to performing an experiment by mixing IVR-SAN 15 (25% sodium chlorite, 1 to 4.5% sodium chloride) and

Activator-H (14.4% w/w hydrochloric acid) from CH_2O International, Inc. It was kept in a closed, amber bottle that was partially submersed in ice water during the experiment. ClO_2 concentration was calibrated by an iodometric titration (3). Concentrations of ClO_2 tested included 0, 2, 20, 200, and 2000 ppm in Experiment (Exp.) One and 0, 5, 50, 100, and 1000 ppm in Exps. Two, Three, and Four. Rates $\geq$100 ppm ClO_2 were high rates used to verify the risk potential of damage if improper rates are applied.

Table 1. Plants included in chlorine and hydrogen dioxide phytotoxicity experiments.

		Experiment No.					
		ClO_2[a]				H_2O_2[b]	
PLANT	**Organ**	**1**	**2**	**3**	**4**	**1**	**2**
alyssum, sweet (*Lobularia maritima* 'Easter Basket Mix')	Leaf		X	X	X	X	X
	Flower		X	X	X	X	X
candytuft (*Iberis umbellata* 'Purity' (white))	Leaf		X	X	X	X	X
	Flower		X		X		X
coleus (*Coleus X hybridus,* Rainbow mix)	Leaf				X	X	X
evolvulus (*Evolvulus glomeratus* 'Hawaiian Blue Eyes')	Leaf		X	X	X	X	X
	Flower		X	X	X		X
fern (*Drypoteris recurvata* 'Recurved Broad Buckler')	Leaf		X	X			
galium (*Galium odoratum* 'Sweet Woodruff')	Leaf				X		X
oriental poppy (*Papaver orientale* 'Allegro Scarlet Red')	Leaf		X	X			
pansy (*Viola wittrockiana* 'Light Blue')	Leaf		X	X			
	Flower		X	X			
azalea (*Rhododendron* sp. 'Rosy Lights')	Leaf	X	X				
English ivy (*Hedera helix* 'Gold Child')	Leaf		X	X			
Juniper sp.	Leaf					X	X
lilac (*Syringa patula* 'Bridal Memories')	Leaf	X	X				
mountain laurel (*Kalmia latifolia* 'Sarah')	Leaf	X	X		X		
rhododendron (Rhododendron sp.)	Leaf				X	X	X
rhododendron (*Rhododendron* sp. 'Henry's Red' = HR)	Leaf	X	X		X		X
rhododendron (*Rhododendron* sp. 'Yaku Princess' = YP)	Leaf	X	X				
St. Johns-wort (*Hypericum calycinum*)	Leaf	X	X				

a Five applications of ClO_2 were applied at 3-day intervals starting on 29 Oct (Exp. 1), 1999, 8 March (Exp. 2), 19 July (Exp. 3) and 8 Dec (Exp. 4) 2000.
b Five applications of H_2O_2 were applied at 3-day intervals starting on 15 Sep (Exp. 1) and 8 Dec (Exp. 2) 2000.

Phytotoxicity symptoms associated with application of ClO_2 ranged from necrotic tips and margins, necrotic spots and blotches, and death. At 2, 5, and 20 ppm ClO_2, mean toxicity ratings were <4% on leaves and flowers of all plants tested (Table 2). At 50 and 100 ppm ClO_2, mean ratings of $\geq$4% occurred on 5 of the 16 plant selections tested, however ratings did not exceed 4% until after 4 to 5 applications in most plants except alyssum flower, which had ratings $\geq$4% after 1 application. Some plant species had <4% damage even after five applications of 100 ppm ClO_2, such as English ivy (Fig. 1). Most woody plants had <4% damage at rates up to 200 ppm ClO_2, except for mountain laurel after five applications at 200 ppm in Exp. 1 and after four applications at 100 ppm in Exp. 2. Pansy flowers and poppy leaves were the most sensitive organs to ClO_2 with $\geq$4% damage by 50 ppm ClO_2.

Fig. 1. English Ivy with a few small (< 1 to 3 mm), necrotic spots (arrows) from 5 applications of ClO_2 at 100 ppm.

All plant species were damaged by 1000 and 2000 ppm ClO_2 (Table 2). At 1000 and 2000 ppm ClO_2, a mean toxicity rating $\geq$4% occurred after one application on alyssum, azalea, candytuft, coleus, evolvulus, fern, galium, lilac, poppy, and pansy; after two applications on English ivy, mountain laurel, rhododendron ‘HR’, and St. John’s wort; and after three applications on rhododendron ‘YP’. After five applications of 1000 ppm ClO_2, the mean toxicity rating ranged from 9.38% on rhododendron ‘HR’ to 99.6% on alyssum, and most were >50%.

Table 2. Rates and number of applications at 3-day intervals of ClO_2 at which 0%, <4%, and $\geq$4% mean toxicity rating (MTR) resulted. At 0% and <4% MTR, the highest rate(s) and greatest number of applications associated with that MTR are listed. Lower rates, which are not listed, had the same MTR. At $\geq$4% MTR, the lowest rate and fewest number of applications associated with that MTR are listed. The last column lists the number of applications at 1000 or 2000 ppm ClO_2 that first resulted in a MTR $\geq$4%.

			0% MTR		**<4% MTR**		**$\geq$4% MTR**		**$\geq$4% MTR at $\geq$ 1000 ppm**
Plant	**Organ**	**Exp.**	**Rate**	**No.**	**Rate**	**No.**	**Rate**	**No.**	**No.**
alyssum	Leaf	2	100	5					1
		3	100	4	100	5			1
		4	100	5					1
	Flower	2	100	5					1
		3	100	5					1
		4	50	5			100	1	1
candytuft	Leaf	2	100	5					1
		3	100	5					1
		4	100	4	100	5			2
	Flower	2	100	5					1
		4	100	4	100	5			2
coleus	Leaf	4	100	4	100	5			2
evolvulus	Leaf	2	100	4	100	5			1

		3	100	4	100	5			1
		4	100	5					1
	Flower	2	100	5					<4%
		3	100	5					3
		4	100	5					<4%
fern	Leaf	2	50	1	50/100	5/5			1
		3	100	1	100	4	100	5	1
galium	Leaf	4	100	5					2
poppy	Leaf	2	5/50	1/1	5/50 /10000	5/3 /4	50/100	4/5	1
		3	5/50 /100	3/3 /1	5/50 /100	5/3 /3	50/100	4/4	2
pansy	Leaf	2	50/100	2/2	50/100	5/4	100	5	1
		3	100	4			100	5	1
	Flower	2	50	2	50/100	3/1	50/100	4/4	2
		3	50/100	5/1	100	2	100	4	2
azalea	Leaf	1	20/200	5/1	200	3	200	4	1
		2	100	4	100	5			2
English ivy	Leaf	2	100	4	100	5			2
		3	50/100	2/4	50/100	5/5			2
lilac	Leaf	1	200	2	200	4	200	5	1
		2	50/100	5/1	100	5			2
mountain laurel	Leaf	1	200	2			200	3	2
		2	50/100	2/1	50/100	5/2	100	3	3
		4	100	5					5
Rhodo- dendron sp.	Leaf	4	100	5					4
rhodo- dendron (HR)	Leaf	1	20/200	3/4	20/200	5/5			3
		2	100	5					1
		4	100	5					5
rhodo- dendron (YP)	Leaf	1	20/200	3/3	20/200	5/4	200	5	3
		2	100	5					<4%
St. Johns wort	Leaf	1	200	2	200	3	200	4	2
		2	100	5					4

a Five applications of ClO_2 were applied at 3 day intervals starting on 29 Oct (Exp. 1), 1999, 8 March (Exp. 2), 19 July (Exp. 3) and 8 Dec (Exp. 4) 2000.

Predominant symptoms resulting from ClO_2 were necrosis of leaf and flower tissue as spots between and across veins, and marginal necrosis. Early symptoms could start as a yellowing of the margin or tip of leaf or flower. Lesions could have dark brown borders (candytuft leaves, English Ivy leaves, poppy leaves [Fig.2]) or purple borders (coleus leaves, pansy leaves, mountain laurel leaves [Fig. 3]). Lesions could appear desiccated or blanched (azalea, lilac [Fig. 4]). Spots on pansy petals were bleached with no necrotic symptom except at high rates (Fig. 5). Lesions ranged from 1/32 to 1/4 inch diameter but additional applications at high rates resulted in coalescing of lesions and death of the entire leaves and petals.

Fig. 2. Poppy with marginal necrosis from 3 applications of ClO_2 at 1000 ppm.

Fig. 3. Mountain laurel with red and brown spots from 3 applications of ClO_2 at 1000 ppm.

Fig. 4. Lilac with marginal and interveinal necrosis from 2 applications of ClO_2 at 1000 ppm.

Fig. 5. Pansy flower with white bleached spots caused by 4 applications of ClO_2 at 100. Similar damage resulted from 1 application of ClO_2 at 1000 ppm.

ClO_2 is being evaluated for treatment of water to kill pathogens in recirculating systems in greenhouses and in water pumped from catchment ponds to irrigate nursery stock (4). The rates in this study are higher than rates used to treat irrigation water and indicate that ClO_2 should be safe for this use. It would still be prudent to monitor plants under different seasonal extremes to verify safety on all plant selections. Because ClO_2 is volatile at most ambient temperatures, further study is needed to determine if the rates used in this test would be effective for application on production and plant surfaces.

Hydrogen Dioxide Trials

Five bedding plant and three shrub species were used in two H_2O_2 experiments at 0, 900, 2700, 5400, and 10200 ppm (0.4, 1.3, 2.6, and 4.8 oz Zerotol per gal, respectively) (Table 1). Rates of $\geq$5400 ppm H_2O_2 were high rates used to verify the risk potential of damage if improper rates are applied.

Phytotoxicity symptoms were observed following applications of H_2O_2 (Table 3). The flowers of candytuft and evolvulus had damage on $\geq$4% of the flower's surface area from 900 to 10200 ppm H_2O_2 after 5 to 2 applications, respectively, while leaves had <4% damage at 10200 ppm. Rhododendron leaves were sensitive to H_2O_2 (Figs. 6 and 7); however, the mean toxicity rating $\geq$4% developed after three applications at rates $\geq$2700 ppm in Exp. 1, but only at 10200 ppm in Exp. 2. Coleus was very sensitive to H_2O_2 and had a mean toxicity rating $\geq$4% at rates $\geq$900 ppm in Exp. 2 and $\geq$2700 ppm in Exp. 1 (Fig. 8).

Table 3. Rates and number of applications at 3-day intervals of H_2O_2 at which 0% or <4% and ≥4% mean toxicity rating (MTR) resulted. At 0 and <4% MTR, the highest rate(s) and greatest number of applications associated with that MTR are listed. Lower rates, which are not listed, had the same MTR. At ≥4% MTR, the lowest rate and fewest number of applications associated with that MTR are listed.

			0% MTR		<4% MTR		≥4% MTR	
Plant	**Organ**	**Exp.**	**Rate**	**No.**	**Rate**	**No.**	**Rate**	**No.**
alyssum	Leaf	1	10200	5				
		2	10200	5				
	Flower	1	5400/ 10200	5/ 3	10200	5		
		2	5400/ 10200	3/ 1	5400/ 10200	5/ 5		
candytuft	Leaf	1	10200	5				
		2	10200	5				
	Flower	2	900/ 2700/ 5400/ 10200	4/ 4/ 4/ 1	2700	5	900/ 5400/ 10200	5/ 5/ 2
coleus	Leaf	1	900/ 2700/ 5400/ 10200	3/ 2/ 1/ 1	900/ 2700/ 5400/ 10200	5/ 3/ 2/ 2	2700/ 5400/ 10200	4/ 3/ 3
		2			900/ 2700	3/ 1	900/ 2700/ 5400/ 10200	4/ 2/ 1/ 1
evolvulus	Leaf	1	10200	5				
		2	5400/ 10200	3/ 1	5400/ 10200	5/ 5		
	Flower	2	5400/ 10200	4/ 1	5400/ 10200	5/ 2	10200	3
galium	Leaf	2	900/ 2700/ 5400/ 10200	2/ 1/ 1/ 1	900/ 2700/ 5400/ 10200	5/ 5/ 5/ 5		
juniper	Leaf	1	10200	5				
		2	10200	5				
Rhododendron sp.	Leaf	1	900/ 2700/ 5400/ 10200	3/ 2/ 2/ 2	900/ 2700	5/ 3	2700 5400/ 10200	4 3/ 3
		2	5400/ 10200	5/ 2	10200	3	10200	4

a Five applications of H_2O_2 were applied at 3-day intervals starting on 15 Sep (Exp. 1) and 8 Dec (Exp. 2) 2000.

Fig. 6. *Rhododendron* sp. with <4% injury on upper leaf surface from water (blue tag, check) and from 5 applications of ZeroTol at 10200 ppm (orange tag).

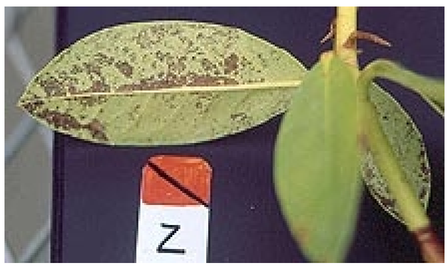

Fig. 7. *Rhododendron* sp. with necrotic spots on the lower leaf surface from 4 applications of ZeroTol at 10200 ppm (orange tag). Damage also resulted from 4 applications of ZeroTol at 2700 and 5400 ppm. No injury resulted from water.

Fig. 8. Coleus with loss of red pigment from 3 applications of ZeroTol at 5400 ppm. Similar damage resulted from 4 and 2 applications of ZeroTol at 2700 and 10200 ppm, respectively.

Predominant symptoms resulting from H_2O_2 were necrosis and desiccation of leaf and flower tissue as spots between veins and blotches at the margin. Tissue of desiccated lesions appeared as if tissue was dehydrated with faded color tone that might have a gray tint. Coleus leaves had a blotchy loss of color, particularly red color, with retention of light green color (Fig. 8). Spots on evolvulus petals were bleached with no necrotic symptom except at high rates. Necrotic lesions on rhododendron leaves were angular in outline and restricted to the lower surface. In general, lesions ranged from 1/32 to 5/8 in. in diameter but repeated applications at high rates resulted in coalescing of lesions.

Concepts Common to the Use of ClO_2 and H_2O_2

Damage from both disinfectants was not always consistent within or between experiments. This is an indication that variable responses are possible under different environmental or cultural conditions. Variability was highest on flowers. Interestingly, flowers were generally less sensitive than leaves to ClO_2 (with the exception of pansy) and generally more sensitive than leaves to H_2O_2. Flowers were short-lived (3 to 6 days) and no attempt was made to record flower longevity or number of applications received on a flower before natural senescence. Maturity of plant organs may have been another source of experimental variability. Some variability was judged to be from drops that originated from the protective trap-covered PVC pipe frame that was used to protect neighboring plants from spray.

Coleus was more sensitive to H_2O_2 and pansy and poppy were more sensitive to ClO_2 than other plants. This is an indication that sensitivities to disinfectants can vary by plant species. Plant producers need to test a wider range of plant species before broad scale use of disinfectants. Regional climatic differences and cultivar differences may be important factors in determining safety of disinfectant applications to crops.

The damage reported in this study resulted from applications made every 3 days with no flushing of residual salts from the leaves because plants were watered at the base of the plant. At the rates that caused damage, often damage was not observed until after 3 to 5 applications. If applications were made at 7-day intervals and plants were watered from overhead, then the potential for damage might be lower because of less frequent exposure and reduced salt accumulation on leaves.

Disinfectants will likely be used against the propagules of pathogens, such as gray mold (*Botrytis cinerea*), powdery mildew (*Podosphaera* spp., *Erysiphe* spp.), and downy mildew (*Peronospora* spp.), which are common aerially disseminated pathogens present on exposed plant surfaces (flowers, leaves, stems). While disinfectants such as ZeroTol are currently registered for use on plants, limited research has been published to indicate appropriate intervals, rates, and alternation strategies with bactericides and fungicides needed to control foliar pathogens. Since disinfectants provide no residual protection, the level of disease pressure (amount of inoculum in or around the production site) may affect the selection of a spray strategy and the need to switch to traditional pesticides. Rate may be further affected by the pathogen species and propagule type (thin- versus thick-walled spore, etc.) being targeted (4). Concern has also been expressed about low levels of injury from a disinfectant providing an infection court for opportunistic pathogens such as *Botrytis cinerea*. Future research will determine if this concern is justified or if regular applications of a disinfectant maintain a low enough level of inoculum to prevent disease progress.

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