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Response of Container-grown Nursery Plants to Chlorine Used to Disinfest Irrigation Water

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Abstract. **The recycling of irrigation water may cause the dispersal of plant pathogens. Irrigation water disinfected with 2.4 mg·L^{-1} of free chlorine for 5 min was overhead-applied to 17 container-grown nursery plants for 11 weeks in a commercial nursery to evaluate the response of container-grown nursery plants to chlorine. No visual symptoms of injury or growth reduction were observed on the evergreen shrubs (*Juniperus horizontalis*, *Thuja occidentalis*, *Buxus microphylla*, *Picea glauca*, *Rhododendron catawbiense*, *Taxus media*, and *Chamaecyparis pisifera*), but there were visual injuries and/or growth reduction on some of the deciduous shrubs (*Salix integra*, *Hydrangea paniculata*, *Prunus ×cistena*, *Weigela florida*, *Physocarpus opulifolius*). Symptoms of anthracnose were reduced on *Cornus alba* plants treated with chlorinated water. The chlorine treatment did not affect leaf chlorophyll content. The chlorine treatment killed all fungi and oomycetes in the irrigation water (DNA multiscan). Although there were visible leaf injuries and growth reduction on some of the deciduous plants, chlorine injury did not render them unsalable. Results suggest that irrigation water treated with 2.4 mg·L^{-1} free chlorine for 5 min will effectively control the dispersal of common plant pathogens without reducing the market value of container-grown plants.**

Some nurseries and greenhouses have implemented the recovery and reuse of irrigation water to reduce water consumption and mitigate the potential release of fertilizers and pesticides into the environment (Bush et al., 2003; Hong and Moorman, 2005; Hong et al., 2003). This practice generally involves collecting excess irrigation water and leachate in a reservoir such as a pond for subsequent irrigation. However, recycling of the water may disperse plant pathogens into crops through the irrigation system (Bush et al., 2003; Hong and Moorman, 2005; Newman, 2004).

Evidence demonstrates that contaminated irrigation water is one of the principal sources of inoculum of *Phytophthora* and *Pythium* pathogens in numerous crops (Hong and Moorman, 2005). In the United States alone, crop losses attributed to phytophthora diseases can exceed several billion dollars annually and the worldwide cost for chemical management of phytophthora diseases can represent over 25% of the annual fungicide market (Kuhajek et al., 2003). In several studies, recycled irrigation water was found to harbor substantial densities of pathogen propagules (Bush et al., 2003; Wangsomboondee and Ristaino, 2002), and disease was more severe when plants were irrigated with contaminated water (Grech and Rijkenberg, 1992; Klotz et al., 1959; McIntosh, 1966; Whiteside and Oswalt, 1973). Hong and Moorman (2005) reported 17 *Phytophthora* sp., 26 *Pythium* sp., 27 genera of fungi, eight species of bacteria, 10 viruses, and 13 species of plant parasitic nematodes in ponds, rivers, canals, streams, lakes, runoff water, watersheds, reservoirs, wells, holding tanks, effluents, ebb and flow, recirculating and hydroponic systems. Seven *Phytophthora* sp. and several *Pythium* sp. have been recovered from the recycling irrigation system at a perennial container nursery in southwestern Virginia (Bush, 2002; Bush et al., 2003; Hong et al., 2003).

Our previous research found that 2.0 mg·L^{-1} of free chlorine can kill *Pythium aphanidermatum* and *Phytophthora cactorum* zoospores and *Phytophthora infestans* sporangia (Cayanan et al., 2008a). Cayanan et al. (2008b) found that a free chlorine concentration less than 2.5 mg·L^{-1} did not adversely affect the growth or appearance of several species of 1-year-old nursery liners during the late growing season (fall) in a small research setting. Frink and Bugbee (1987) and Brown (1991) reported on the phytotoxic effects of chlorine on several greenhouse crops and their free chlorine thresholds, which ranged from 2 mg·L^{-1} to 77 mg·L^{-1}. However, there is no known published information regarding the use of chlorinated irrigation water under typical nursery practices.

The objective of the present study was to determine whether a free chlorine concentration of 2.4 mg·L^{-1} would harm common container-grown nursery plants when applied under commercial nursery practices during the period of shoot emergence and growth and whether such a treatment would minimize the dispersal of common plant pathogens in recycled irrigation water.

Materials and Methods

Plant material and treatments. The experiment was conducted at Canadale Nurseries Ltd. (St. Thomas, Ontario, Canada) from 7 June 2007 to 22 Aug. 2007. Seventeen 1-year-old container-grown nursery plant species (Table 1) were tested. All plants were obtained from Canadale Nurseries Ltd. except for the *Rhododendron*, which were obtained from Blue Sky Nursery Ltd. (Beamsville, Ontario, Canada). Before the experiment, all the plants were grown in hoop houses over winter and in the early spring. Plants were grown in 9.1-L containers except *Syringa* (4.5 L) and the *Juniperus*, *Thuja*, *Picea*, *Taxus*, *Chamaecyparis*, *Rhododendron*, and *Euonymus* (13.6 L). The container substrate (Gro-Bark Co., Georgetown, Ontario, Canada) contained composted pine bark, sphagnum peatmoss, coarse perlite, and composted leaf, yard, vegetable, and wood waste amended with surfactant, micronutrient mix, and dolomitic limestone. Container substrate was amended by incorporating 15N–3.9P–10K resin-coated, controlled-release fertilizer at the rate of 1 kg·m^{-3} (Osmocote Plus 15-9-12, 5–6 month 21 °C; Scotts Co., Marysville, OH). Plants of each species were uniform in height at the beginning of the experiment.

There were two irrigation treatments: (1) nonchlorinated water and (2) chlorinated water. Plants of all species were laid out in a completely randomized split block design with four blocks and two treatments. Five plants of each species in each block were grouped together as one experimental unit

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Table 1. Plant species used to test the effects of chlorinated irrigation water on spray-irrigated, container-grown shrubs.

Plant type	Plant species	Common name	Cultivar name
Deciduous	*Cornus alba*	Ivory halo dwarf silver dogwood	Bailho
	Euonymus fortunei	Emerald gaiety euonymus	Emerald Gaiety
	Hydrangea paniculata	Peegee hydrangea	Grandiflora
	Physocarpus opulifolius	Coppertina ninebark	Coppertina
	Prunus ×cistena	Purpleleaf sandcherry	Not available
	Salix integra	Japanese variegated willow	Alba Maculata
	Spiraea japonica	Goldmound spirea	Goldmound
	Syringa meyeri	Dwarf Korean lilac	Palibin
	Viburnum ×carlcephalum	Fragrant viburnum	Not available
	Weigela florida	Fine wine weigela	Not available
Evergreen	*Buxus microphylla*	Green velvet boxwood	Green Velvet
	Chamaecyparis pisifera	Sungold threadleaf cypress	Filifera
	Juniperus horizontalis	Limeglow™ golden juniper	Not available
	Picea glauca	Dwarf Alberta spruce	Conica
	Rhododendron catawbiense	Rhododendron	Album
	Taxus media	Hick's yew	Hicksi
	Thuja occidentalis	Emerald cedar	Smaragd

with each plant as a subsample. Plants were overhead-irrigated with impact spray emitters (Rittenhouse, Niagara, Ontario, Canada). The spray application rate was 46.2 $L \cdot min^{-1}$ over an area of 63.6 m^2. Plants were watered for 30 min three times a day (at 0500 HR, 0700 HR, and 0900 HR) for 11 weeks. Each day, the 4.5-, 9.1-, and 13.6-L containers received a total volume of 227, 300, and 391 mL of water, respectively.

All water used for irrigation was taken directly from the collection pond. The free chlorine concentration of the nonchlorinated water was measured weekly and had an average free chlorine concentration of 0.16 with a SD of ± 0.03 $mg \cdot L^{-1}$. Pond water was disinfected using a chlorination system from ClearTech (ClearTech, Mississauga, Ontario, Canada), which pumped pond water from the collection pond into a 1386-L mixing tank. A chlorine stock solution of 12,500 $mg \cdot L^{-1}$ was injected into the tank and mixed until the free chlorine concentration of the water was 2.4 $mg \cdot L^{-1}$ and was held at this concentration for 5 min before plants were overhead-irrigated. The chlorine stock solution was prepared with 5.25% sodium hypochlorite (Javex Bleach; Colgate-Palmolive Canada Inc., Toronto, Ontario, Canada) and well water. The free chlorine concentration of the well water averaged 0.03 ± 0.01 $mg \cdot L^{-1}$. The pH of the chlorinated water was adjusted to 6.5 to 7.0 with 5% acetic acid (Pure White Vinegar; Loblaw Inc., Toronto, Ontario, Canada) because chlorine is reported to be the most effective at this pH range (Frink and Bugbee, 1987). A 9184sc Amperometric Free Chlorine Sensor (Hach Co., Loveland, CO) was used to measure the free chlorine concentration inside the mixing tank and a C201 Oakton Colorimeter (Oakton Instruments, Vermont Hills, IL) was used to measure the free chlorine concentration of the irrigation water collected at the impact spray emitter. As a result of the short distance between the mixing tank and the spray emitter, no chlorine concentration difference was detected during the experiment.

A free chlorine concentration of 2.4 $mg \cdot L^{-1}$ and a contact time of 5 min was chosen based on our previous research, which indicated that a free chlorine concentration less than 2.5 $mg \cdot L^{-1}$ should not have any adverse effect on the growth or appearance of most plants (Cayanan et al., 2008b), but 2.0 $mg \cdot L^{-1}$ or greater of free chlorine with a minimum contact time of 3.0 min should kill the common plant pathogens *P. aphanidermatum*, *P. cactorum*, and *P. infestans* (Cayanan et al., 2008a).

Samples and measurements. Visual injury of plant leaves, detected by the unaided eye, was expressed as the percentage of leaves or flowers on the plant exhibiting the visual injury (i.e., flower necrosis, marginal necrosis, chlorosis, curling, and loss of leaves) on the whole plant. Plants were rated once a week for 11 weeks.

On 23 Aug. 2007, after 11 weeks of growth, three plants from each experimental unit were randomly selected for growth and physiological measurements. Plant height was measured from the substrate surface and canopy spread was the average of two perpendicular measurements. Leaf chlorophyll content index (CCI) of the youngest fully expanded leaf without visible injury was determined using a CCM-200 (Opti-Sciences, Tyngsboro, MA). Plant shoots were divided into stems and leaves. Leaf area (LA) was measured using a leaf area meter (LI-3100; LI-COR, Lincoln, NE). Plant tissues were dried at 60 °C. Specific leaf area (SLA) was calculated by dividing LA by leaf dry weight. Leaf area, leaf and stem dry weight, SLA and CCI were not determined for *Chamaecyparis*, *Juniperus*, *Thuja*, *Taxus*, and *Picea*.

Rhododendron, *Hydrangea*, and *Syringa* were further analyzed to represent the three sizes of containers (13.6, 9.1, and 4.5 L, respectively) used to grow the plants. At harvest, leachate was collected from the containers of harvested plants using the pourthrough method as described by Wright (1986) and analyzed for chloride (Cl^-) using high-performance liquid chromatography (HPLC), DX500 (DIONEX, Oakville, Ontario, Canada) as described by Zheng et al. (2004). In addition, the amount of free chlorine input (mg) per kilogram of growing substrate was calculated in this study as follows:

$$\text{Free chlorine input} = (V_w/t/Cl)/m$$

where V_w is the average volume of irrigation water applied daily ($L \cdot d^{-1}$), t is the length of the experiment (day), Cl is the average free chlorine concentration of the irrigation water ($mg \cdot L^{-1}$), and m is the mass of the substrate (kg). The mass of the substrate was measured by drying the growing substrate under 105 °C until the weight was constant.

Samples of nonchlorinated and chlorinated irrigation water were collected on Day 6 and Day 71 for detection of plant pathogens using DNA multiscan analysis (University of Guelph Laboratory Services Pest Diagnostic Clinic). Water samples (three samples for both chlorinated and nonchlorinated water at each time) were stored in a cooler with ice and submitted for testing 2 h after sample collection. In the tests, membrane-type "fungi and oomycetes" was used to analyze irrigation water samples collected on Day 6 and membrane type "G" on Day 71. Both membrane types detect common species of *Pythium* and *Phytophthora,* including the pathogens *P. aphanidermatum*, *P. cactorum*, and *P. infestans*.

Statistical analysis. All data were tested for normality and homogeneity before analysis of variance with SAS software Version 9.1 (SAS Institute, Cary, NC). Contrast comparisons using a Type I error rate of 0.05 were used to determine the chlorine effects on each plant species.

Results

Visual injury. None of the evergreen shrubs (*Juniperus*, *Thuja*, *Buxus*, *Picea*, *Rhododendron*, *Taxus*, and *Chamaecyparis*) exhibited visual injury caused by chlorine; visual injuries were observed on five of the 10 deciduous shrubs tested.

No visual injury was observed on deciduous shrubs from Day 1 to 56. On Day 57, 5% of the juvenile leaves on *Salix* displayed marginal necrosis as a dark brown color. Five percent of the mature leaves on *Hydrangea* displayed marginal necrosis as a dark brown color. Five percent of flowers on *Hydrangea* also displayed necrosis as an orange–brown color.

On Day 76, *Salix* and *Hydrangea* displayed the same extent of visual injuries as observed on Day 57, but new visual injuries were observed. Ten percent of the juvenile leaves of *Salix* displayed curling and *Hydrangea* displayed a 10% leaf drop compared with the plants treated with nonchlorinated water. Five percent of the leaves of *Prunus* displayed marginal necrosis as a grayish brown color. Fifteen percent of *Weigela* leaves displayed chlorosis as a light green color and 10% of the juvenile leaves of *Weigela* also exhibited curling. Fifteen percent of the leaves of *Physocarpus* displayed chlorosis as a light yellow color. All *Cornus* exposed to nonchlorinated water during this experiment

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displayed anthracnose (*Discula destructiva*), a leaf spot disease. However, there was a 65% reduction of anthracnose on *Cornus* treated with chlorinated water.

Growth. The growth of plant species responded differently to the chlorine treatment (Tables 2 and 3). LA, leaf dry weight, stem dry weight, total aboveground dry weight, and height of *Salix* were reduced by chlorine, but there was no effect on spread and SLA. For *Hydrangea*, *Prunus*, *Physocarpus*, and *Euonymus*, only LA was reduced, but no treatment effect was observed for the leaf dry weight, stem dry weight, total aboveground dry weight, height, spread, and SLA. The SLA of *Cornus* treated with the chlorinated water was greater than that of *Cornus* plants treated with the nonchlorinated water, but no treatment effect was observed for the LA, leaf dry weight, stem dry weight, total aboveground dry weight, height, and spread. None of the growth parameters of the remaining deciduous shrubs (*Syringa*, *Weigela*, *Spiraea*, and *Viburnum*) and all of the evergreen shrubs (*Rhododendron*, *Buxus*, *Chamaecyparis*, *Juniperus*, *Thuja*, *Taxus*, and *Picea*) were affected by chlorine.

Leaf chlorophyll content. Chlorine treatment had no effect on the leaf CCI for any of the 17 plant species (Table 3).

Free chlorine input and growing substrate. Total free chlorine input during the 11-week experiment was 9, 6, and 5 $mg \cdot kg^{-1}$ of substrate in the 4.5-, 9.1-, and 13.6-L containers. HPLC analysis showed no difference in the Cl^- content of the nonchlorinated and chlorinated growing substrates. For *Rhododendron*, *Hydrangea*, and *Syringa*, the leachate Cl^- concentration measured by the pourthrough method was 321.4 ± 26.71, 1724.4 ± 26.71, and 330.7 ± 26.71 $mg \cdot L^{-1}$, respectively.

DNA multiscan. On Day 6, nonchlorinated water had low levels of *Fusarium* sp., moderate levels of *Phytophthora* sp., high levels of *Pythium* sp., and low levels of *Verticillium dahliae*. On Day 71, the nonchlorinated water had low levels of *Pythium* sp. and there was no detection of any fungi or oomycetes in the chlorine treatment on both Days 6 and 71.

Discussion

In this study, chlorine had no phytotoxic or growth effects on any of the evergreen (broad-leaf or needle-type) shrubs. Visual injuries were observed on several of the deciduous shrubs. Surface wax on the leaves may limit or prevent the retention of irrigation water on the foliage of evergreen shrubs. In contrast, deciduous shrubs have relatively soft, nonwaxy, and hairy leaves, which may allow retention of irrigation water on the plant surface, prolonging exposure of the leaves to any chlorine in the water and potentially resulting in leaf injury. Nonetheless, many of the observed injuries were mild and difficult to detect.

Our previous research (Cayanan et al., 2008b) indicated that a free chlorine concentration less than 2.5 $mg \cdot L^{-1}$ did not adversely affect *Physocarpus opulifolius*, *Salix integra*, *Hydrangea paniculata*, *Weigela florida*, or *Spiraea japonica*. In our present study, the 2.4 $mg \cdot L^{-1}$ of free chlorine caused visual injury on *Weigela florida*; caused both visual injury and growth reduction on *Physocarpus*, *Salix integra*, and *Hydrangea*; but *Spiraea japonica* was unaffected.

Differences in results may be the result of the fact the plants in the previous study were irrigated with chlorinated water long after the new growth had hardened off, whereas in the present study, plants were irrigated between June and August when they were more actively growing. It is worth noting also that injuries appeared after 8 weeks (56 d) of exposure to chlorinated water in the present study, whereas our previous study was only 6 weeks (42 d) in duration.

Although results of this study show that chlorinated water could be applied for up to 8 weeks, in actual practice, the chlorinated irrigation water need not be applied continuously once plant pathogens are under control. Intermittent exposure to chlorine may reduce the phytotoxic effects that were observed in some species as a result of 8 weeks of continuous chlorine exposure.

Lack of any difference in the Cl^- content of the substrate of all three container sizes also suggests that short-term use of 2.4 $mg \cdot L^{-1}$ of free chlorine may not result in excess Cl^- being released into the environment from leachate. Concurrent laboratory studies found that it required 30,000 mg of free chlorine applied to 1 kg of growing substrate (same substrate used in the current study) to establish a detectable chlorine residual (chlorine not consumed by organic matter; data not shown). After 11 weeks of irrigation with chlorinated water in our study, the total free chlorine input to the growing substrate was 9, 6, and 5 mg per kilogram of growing substrate in the 4.5-, 9.1-, and 13.6-L containers, respectively. These values are considerably less than 30,000 mg of free chlorine per kilogram of growing substrate and would not be enough to establish free chlorine residual. In addition, free chlorine applied to the growing substrate would be oxidized by the organic matter of the growing substrate, which would prevent the establishment of a free chlorine residual.

In agreement with our previous work, Hong et al. (2003) reported that zoospores of *Phytophthora* sp. may be killed with free chlorine concentrations ranging from 0.25 to 2 $mg \cdot L^{-1}$. Our previous work also indicated that zoospores of *P. infestans* were killed with 1.0 $mg \cdot L^{-1}$ of free chlorine with a contact time of 3.0 min; zoospores of *P. cactorum* with 0.3 $mg \cdot L^{-1}$ and contact time of 6.0 min; and sporangia of *P. aphanidermatum* with 2.0 $mg \cdot L^{-1}$ and contact time of 3.0 min. These results are in agreement with our DNA multiscan results because all fungi and oomycetes detected in the nonchlorinated water were killed in the chlorinated water in our present study.

Table 2. The leaf area (LA), leaf dry weight, stem dry weight, total aboveground dry weight, height, spread, specific leaf area (SLA), and leaf chlorophyll content of 17 container-grown nursery plant species overhead irrigated with chlorinated water (2.4 $mg \cdot L^{-1}$) and nonchlorinated water (0 $mg \cdot L^{-1}$).

Plant species	LA (cm^2)	Leaf dry wt (g)	Stem dry wt (g)	Total aboveground dry wt (g)	Ht (cm)	Spread (cm)	SLA ($cm^{-2} \cdot g$)	Chlorophyll (CCI)
Prunus ×cistena	1,048*	8.7	18.0	26.7	49	38	88	37
Salix integra	8,416***	62.6*	116.9*	179.5*	114*	142	146	15
Hydrangea paniculata	4,541*	22.9	18.9	41.8	57	66	164	32
Physocarpus opulifolius	5,032*	42.0	39.4	81.4	79	67	111	53
Syringa meyeri	81	1.5	1.4	2.9	19	13	54	16
Weigela florida	1,449	11.5	5.5	17.0	41	389	126	30
Spiraea japonica	3,388	17.0	8.7	25.7	27	41	199	7
Viburnum ×carlcephalum	2,347	29.2	36.4	65.6	47	54	80	45
Cornus alba	2,968	16.8	12.4	29.2	412	50	170*	27
Euonymus fortunei	1,331	24.2	11.7	35.9	31	35	55	73
Rhododendron catawbiense	6,494*	61.1	71.4	132.5	44	51	104	103
Buxus microphylla	6,133	106.6	72.7	179.3	36	43	58	107
Chamaecyparis pisifera	ND	ND	ND	125.7	33	42	ND	ND
Juniperus horizontalis	ND	ND	ND	60.3	28	31	ND	ND
Thuja occidentalis	ND	ND	ND	282.7	57	38	ND	ND
Taxus media	ND	ND	ND	35.3	31	20	ND	ND
Picea glauca	ND	ND	ND	231.7	67	29	ND	ND

ND, *, ***Not determined, chlorine treatment effect significant at $P < 0.05$ and $P < 0.0001$, respectively.
Values with * and *** are the mean of the nonchlorinated treatment. All of the others are the mean of the nonchlorinated and chlorinated treatments.

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Table 3. Percent reduction of leaf area (LA), leaf dry weight, stem dry weight, total aboveground dry weight, height, and specific leaf area (SLA) caused by chlorinated irrigation water (2.4 mg·L^{-1}) compared with the nonchlorinated plants on container-grown nursery plant species.

	Percent reduction (%)					
Plant species	LA	Leaf dry wt	Stem dry wt	Total aboveground dry wt	Ht	SLA
Prunus ×cistena	50					
Salix integra	44	51	49	50	16	
Hydrangea paniculata	38					
Physocarpus opulifolius	15					
Cornus alba						–24
Euonymus fortunei	8					

Percentage reductions were presented only when there was a significant treatment effect within the tested species at $P < 0.05$ level. Positive numbers represent reduction and negative numbers represent an increase in growth.

We also observed the suppression of a foliar disease, dogwood anthracnose, caused by *Discula destructiva* (Carr and Banas, 2000; Daughtrey and Hibben, 1994; Dudt and Shure, 1993; Redlin, 1991). It is a serious disease known to cause necrosis (Carr and Banas, 2000; Daughtrey and Hibben, 1994; Redlin, 1991), leaf spot, leaf blotch, blight, dieback (Daughtrey and Hibben, 1994; Redlin, 1991), twig dieback (Redlin, 1991), and defoliation (Daughtrey and Hibben, 1994) in *Cornus* sp. *Cornus* in this experiment displayed anthracnose on both the nonchlorinated and chlorinated plants. However, foliar symptoms of anthracnose on *Cornus* treated with chlorinated water was reduced by 65% compared with the nonchlorinated *Cornus*. Also, free chlorine concentration of 2.4 mg·L^{-1} did not cause any visual injuries or negative growth effects on the *Cornus*. To manage dogwood anthracnose, growers are recommended to use best management practices such as reducing leaf wetness periods (Daughtrey and Hibben, 1994). Growers may also use fungicides to manage the disease; however, these methods do not kill the disease inoculum. Moorman and Lease (1999) reported that pruning of *Cornus* branches infected with anthracnose improved the appearance of the plants but did not eliminate the disease. Pruning is also time-consuming and other labor-intensive efforts managing the disease. The reduction of anthracnose severity with chlorinated water may reduce the number of applications of fungicides.

In conclusion, our research found that 2.4 mg·L^{-1} of free chlorine with a contact time of 5.0 min in the nursery irrigation water killed all fungi and oomycetes and was safe for all seven evergreen shrubs tested. Visual injuries on the deciduous shrubs appeared on only some of the plants and were not considered sufficient to render them unsalable. We also determined that the amount of chlorine used to chlorinate 1000 L of irrigation water with a free chlorine concentration of 2.4 mg·L^{-1} is 494.4 mL of 5% bleach, which currently costs $0.23 U.S. This research provides new information for developing economic water treatment systems to reduce the dispersal of common plant pathogens without affecting the market value of plants.

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