Ornamental Section

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CONTROL OF BACTERIA AND STABILITY OF CHLORINE IN CUT FLOWER WATER[1]

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Abstract. **The influence of solution pH, sucrose, and number of carnation** (Dianthus caryophyllus **L.) flower stems on available Cl from solutions of sodium dichloroisocyanurate (DICA) were investigated. DICA solutions held at pH 7.5 retained more chlorine than solutions held at pH 4. Sucrose (SU) decreased the amount of available chlorine from DICA at all pH levels. As the number of stems in DICA solutions increased, the amount of available chlorine decreased. Flowers were evaluated in non-inoculated and inoculated water and floral preservatives (DICA + SU and 8-hydroxyquinoline (8-HQC) + SU). Both non-inoculated preservatives maintained flowers equally but longer than those in water. Inoculated 8-HQC + SU solutions did not sustain flower longevity as well as inoculated DICA + SU. Buffered DICA + SU maintained bacteria-free water for 10 days.**

Untreated cut flower water often becomes contaminated with bacteria. If the microbial population reaches high levels, the results are odoriferous, cloudy water with a concomitant reduction in flower life (1, 7, 8, 9, 10). Slow-release chlorine compounds have been used as sterilizing and cleansing agents for many years (2, 5). However, it is only in recent years that slow-release Cl compounds have been used on cut flowers (4, 7, 8). Under experimental conditions, Cl compounds maintain bacteria-free water for cut flowers (7, 8). In commercial floristry, conditions are much different; usually many stems are placed in vessels holding a relatively small quantity of water and in these situations, bacteria are not controlled by slow-release Cl (9, 10). While much is known about the chemistry and handling of commercial Cl compounds, little is known about handling Cl compounds used in water for cut flowers (2, 5). Commercial Cl compounds are influenced by pH; increased acidity increases the available hypochlorite ion, the reactive moiety responsible for sterilization (2). High temperature and organic matter cause depletion of available Cl (2, 5).

I tested the effects of pH, sucrose, and numbers of flower stems on Cl availability in sodium dichloroisocyanurate solutions.

Materials and Methods

All solutions were freshly made before each experiment using deionized water in sterilized glass jars. Each jar contained 250 ml water or solution, except where noted. All solutions, except where pH was an experimental variable, were buffered with citrate-phosphate to pH 7.5 as outlined by Jensen (3). Five ml of buffer were used per liter of water or solution. Solutions contained 200 mg sodium dichloroisocyanurate (DICA) + 20 g sucrose (SU) per liter. Buffers were added to the water before DICA and SU were added.

Three standard carnations were placed in each jar except where number of stems was an experimental variable. Flower stems were trimmed to 30 cm and leaves were removed from lower one-third of the stem. Flowers were commercially grown and purchased through a local wholesale florist. Flowers were held in a laboratory maintained at 25-27°C under cool-white fluorescent lights at 1.5 klx for 12 hr/day. Relative humidity ranged from 50-70%. Solution pH's and soluble salts were periodically determined during each experiment. Longevity was determined for each flower. Flower life was considered terminated when outer petals lost turgidity and reflexed inward and/or when they discolored. There were 4 jars (replications) per treatment in each experiment.

Available or free Cl was determined daily in solution containing DICA + SU using the O-tolidine method (5). All data were subjected to analysis of variance and only significant effects are discussed.

Buffer effect. Deionized water was buffered to pH 4, 6, or 7.5 with citrate-phosphate according to Jensen (3). Eight ml of buffer per liter of water was used to establish each respective pH. An unbuffered water sample was used as a control. Factorially arranged with each of the 4 buffered waters was a control (no additional chemicals), 200 mg DICA/liter or 200 mg DICA + 20 g SU/liter for a total of 12 treatments. Three flowers were placed in jars and handled as indicated above.

Buffer concentration. Citrate-phosphate buffer was added to water at 0, 5, 10, and 20 g/liter. DICA (200 mg) and 20 g SU/liter were added to the control (no buffer) or each buffered solution.

Number of stems. Each jar contained 250 ml of solution. All solutions contained 200 mg DICA + 20 g SU/liter buffered to pH 7.5. Flowers were prepared as above. Experimental variables were 0, 3, 6, 9, or 12 flowers per jar of solution.

Inoculation and preservatives. Flowers (3/jar) were placed in deionized water, 200 mg 8-HQC + 20 g SU/liter or 200 mg DICA + 20 g SU/liter. The jars holding the flowers and water or solution were left uninoculated or inoculated with 2 ml of a bacterial mixture as previously outlined (9). After 6 days, a 1-ml aliquot of water or solution was collected from each jar. The aliquots were handled aseptically and serially diluted to 10^{-2} to 10^{-6} ml. A 1-ml sample of each diluted water sample was added to sterile petri plates and 20 ml of warm liquid nutrient broth were added. The plates were swirled, allowed to cool, inverted and incubated at 25°C for 48 hr. Colonies on plates were counted and, using appropriate dilution factors, the numbers of bacteria per milliliter were estimated.

Results and Discussion

Buffer effect. DICA solutions without SU buffered to pH 7.5 retained Cl better than those not buffered or those

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buffered to pH 6 and 4 (Fig. 1). DICA solutions buffered to pH 4 with or without SU had less Cl after 1 day than DICA solution with SU at pH 7.5 or 6.0 and those not buffered. DICA solution at pH 4 with or without SU were depleted of Cl after 3 days. At pH 7.5; 6; and 4, DICA solution had more Cl than DICA + SU at each respective pH. Non-buffered DICA solutions with SU were nearly depleted of Cl after 7 days. DICA solution with SU at pH 7.5 retained more Cl than any other buffered DICA solution with SU. Buffers maintained reasonably good pH control; but, pH of non-buffered DICA solution decreased (Table 1).

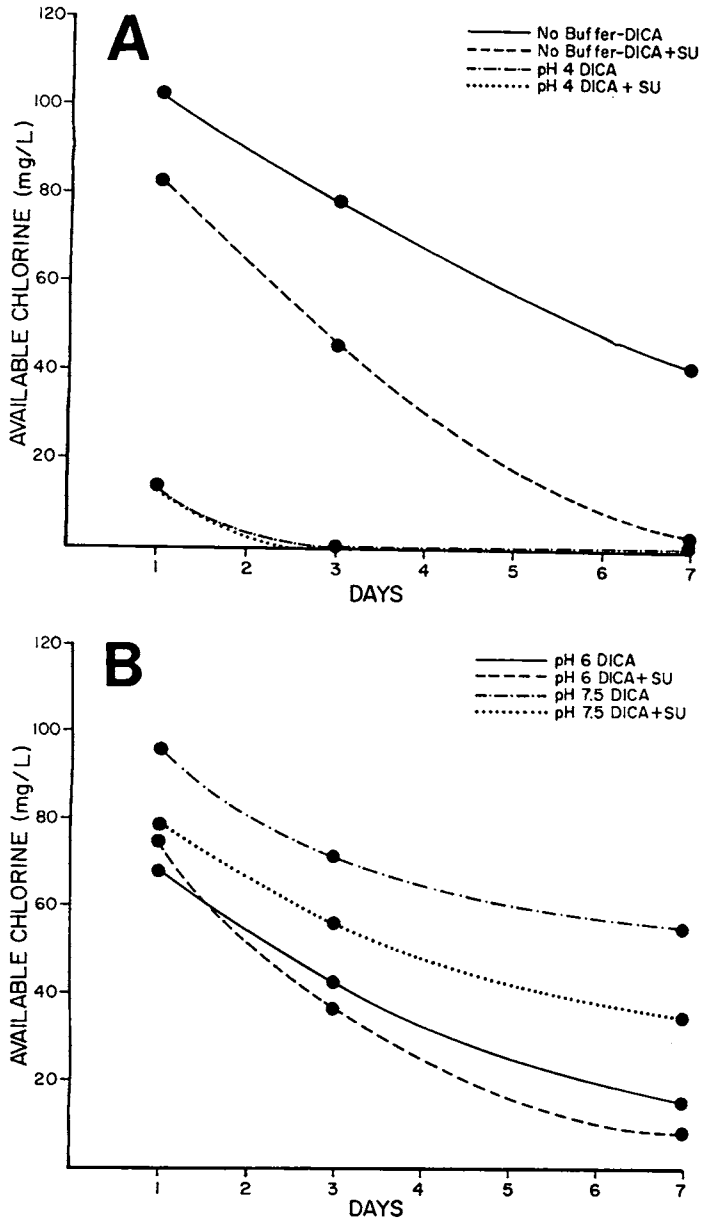

Fig. 1. Influence of buffering and sucrose (SU) on available chlorine in sodium dichloroisocyanurate (DICA) solutions after 7 days. (Curved lines were fitted by eye. Buffer, sucrose main effect and buffer x sucrose interaction significant at the 5% level for day 1, 3 and 7.

Buffer concentration. DICA + SU solutions with 5, 10, or 20 g buffer/liter retained Cl better than non-buffered DICA + SU solution (Fig. 2). Non-buffered DICA + SU solutions rapidly lost Cl and contained no Cl after 7 days. Increased amounts of buffer stabilized solution pH and increased soluble salts but decreased flower life (Table 2).

Table 1. Influence of buffers, sodium dichloroisocyanurate and sucrose on solution pH after 1 and 3 days.

Buffer, Chemicals[z]	Solution pH after 1 day	Solution pH after 3 days
No buffer	5.2[y]	4.6[y]
No buffer, DICA	5.1	4.7
No buffer, DICA + SU	4.3	3.5
pH 4	4.0	4.2
pH 4, DICA	4.6	4.5
pH 4, DICA + SU	4.5	4.3
pH 6	6.1	6.1
pH 6, DICA	6.0	6.0
pH 6, DICA + SU	5.9	5.8
pH 7.5	7.2	7.0
pH 7.5, DICA	7.2	7.0
pH 7.5, DICA + SU	7.1	6.8

[z]Solutions contained citrate-phosphate buffer, 200 mg sodium dichloroisocyanurate (DICA), and/or 20 g sucrose (SU)/liter.
[y]Buffer and chemical main effects and buffer X chemical interaction significant at the 1% level.

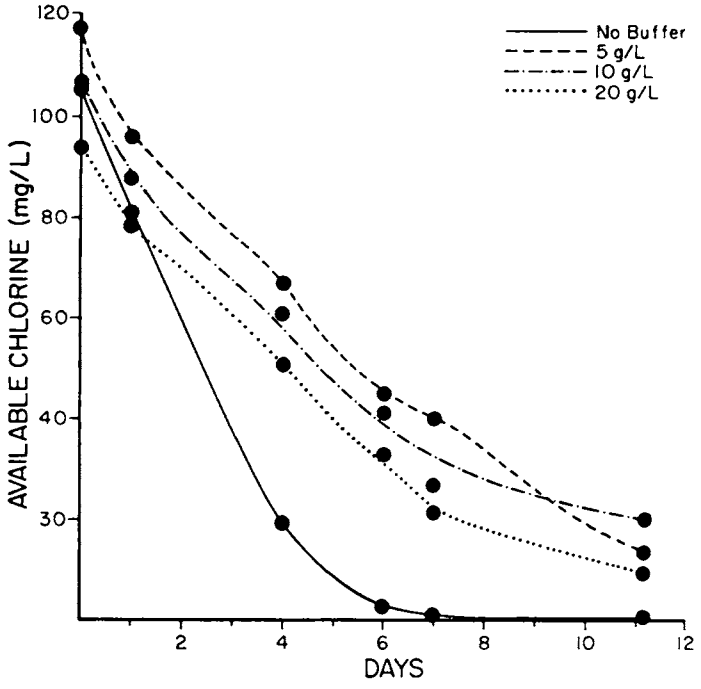

Fig. 2. Influence of citrate-phosphate buffer concentrations on available chlorine in sodium dichloroisocyanurate and sucrose solutions after 11 days. (Curved lines fitted by eye. Means of each buffer concentration are significantly different from control (no buffer); but, not significantly different from each other at day 4, 6, 7, and 11. There are no significant differences at day 1.)

Table 2. Influence of buffer concentration on soluble salts and pH of solutions and longevity of carnation flowers.

Buffer[z] (g/liter)	Soluble salts after Initial	Soluble salts after 7 days	Solution pH after Initial	Solution pH after 7 days	Longevity (days)
none	77	212	4.6 a[y]	3.4 a	13.9 a
5	399	414	7.3 b	7.0 b	10.9 b
10	728	817	7.5 c	7.2 c	8.8 c
20	1309	1398	7.6 c	7.3 d	8.5 c

[z]Citrate-phosphate buffer with 200 mg sodium dichloroisocyanurate + 20 g sucrose/liter.
[y]Mean separation in columns by Duncan's multiple range test, 1% level.

Number of stems. Generally, as the number of stems in DICA + SU solutions increased, the level of Cl and pH decreased (Fig. 3 and 4). After 2 days, Cl in all solution was not significantly different. After 3, 6, and 9 days, DICA + SU solutions holding 0 and 3 stems had similar chlorine levels; but, more than those in DICA + SU solutions holding 6, 9, or 12 stems. After 10 days, sufficient chlorine was present in all solutions to render them bacteria-free.

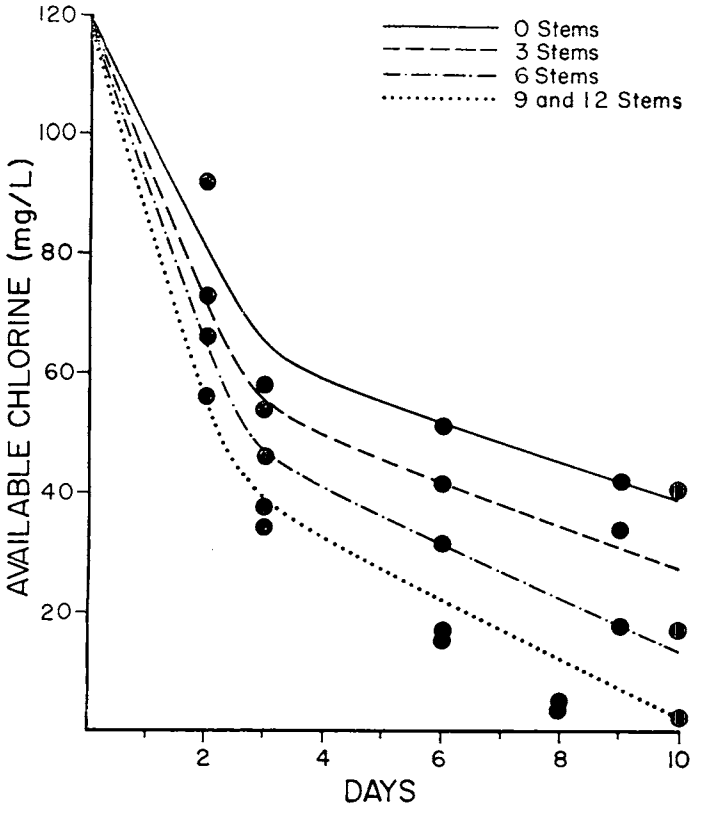

Fig. 3. Influence of the number of carnation stems on available chlorine in sodium dichloroisocyanurate (DICA) and sucrose (SU) after 10 days. (Curved lines fitted by eye.)

After 1; 2; 6; and 9 days, pH's of all DICA + SU solutions holding 0, 3, 6, or 9 stems were significantly different from each other. There were no differences in pH's of DICA + SU solutions holding 9 or 12 stems. Flowers in all solutions had similar longevity, 9 to 9.5 days.

Inoculation. More bacterial colonies were found in inoculated water and 8-HQC solutions than in uninoculated water and 8-HQC solutions (Table 3). The highest number of bacteria were found in 8-HQC + SU inoculated solutions. No bacteria were found in non-inoculated or inoculated DICA + SU solutions. Flowers held in non-inoculated and inoculated water and inoculated 8-HQC + SU solutions had similar longevity but less than flowers held in non-inoculated and inoculated DICA + SU solutions and non-inoculated 8-HQC + SU solution.

In earlier tests, cut flower quality, turgidity, weight, and longevity was maintained only when water or solutions contained sucrose and remained bacteria-free (7, 9). DICA + SU buffered to 7.5 maintained sufficient available Cl for 8 to 10 days to maintain bacteria-free solutions and provided the sucrose necessary to sustain flower quality and longevity. While buffers were necessary to stabilize pH, it was important to use low concentration of buffer to prevent high soluble salts. High soluble salts in cut flower water are a deterrent to long life (3, 11).

The available Cl from DICA + SU in cut flower water followed a pattern of activity similar to those reported for

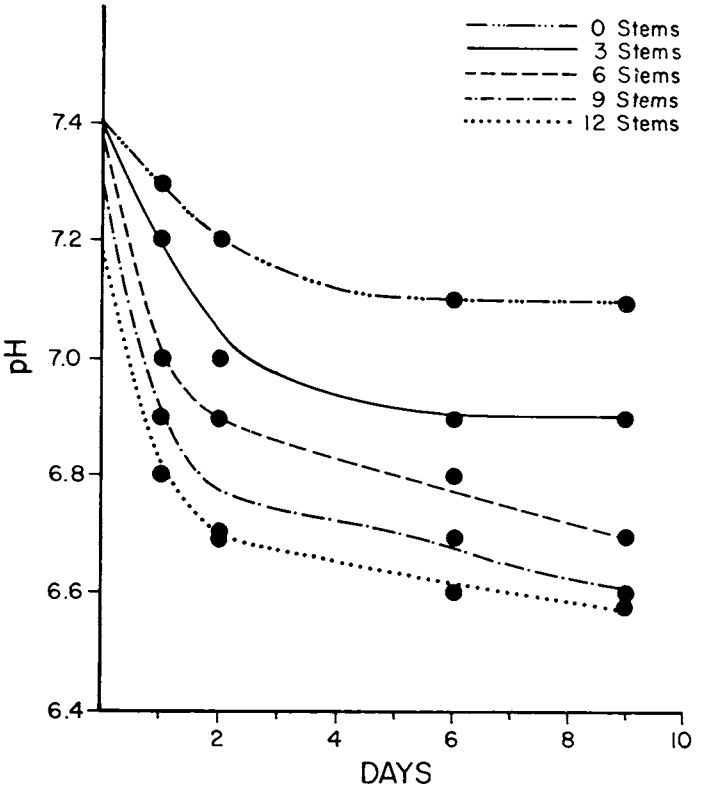

Fig. 4. Influence of the number of carnation stems on pH of sodium dichloroisocynurate and sucrose solutions after 10 days (Curved lines fitted by eye).

Table 3. Influence of 2 floral preservatives and bacteria inoculation on number of bacteria in solutions and longevity of cut carnations.

Preservative[z]	Inoculation	Bacteria (X10⁶/ml)	Flower longevity (days)
Water	Uninoculated	1[x]	7.4[x]
8-HQC + SU	Uninoculated	17	13.3
DICA + SU	Uninoculated	0[y]	13.1
Water	Inoculated	5	6.6
8-HQC + SU	Inoculated	33	7.4
DICA + SU	Inoculated	0[y]	13.0

[z]200 mg 8-hydroxyguinoline (8 HQC) + 20 g sucrose (SU)/liter or 200 mg sodium dichloroisocyanurate (DICA) + 20 g SU/liter.
[y]Lowest dilution 1:100, no colonies. Measured after 6 days.
[x]Preservative, inoculation main effects and preservative x inoculation interaction significant at the 1% level.

other industrial Cl compounds (2, 5). Low solution pH increased the hydrolysis of hypochlorite to hypochorous acid (2). Thus, high pH depressed hydrolysis and hypochlorite was retained. The pH of non-buffered DICA + SU solution decreased rapidly (Fig. 1) and available Cl was depleted.

Previous work showed that 8-HQC + SU inhibited some bacterial species while others grew prolifically (7, 9). This may be the reason for some of the results in the inoculation test (Table 3). After 6 days, inoculated 8-HQC + SU solutions contained about twice as many bacterial cells as uninoculated 8-HQC + SU solutions. Bacterial populations in 8-HQC + SU normally reach a maximum in 2 days (9). Hence, flowers in inoculated 8-HQC solutions would have been exposed to a maximum bacterial population for at least 4 days. Also the species of bacteria in uninoculated solutions probably differed than those in inoculated solutions. This probably accounts for the difference in flower longevity in uninoculated and inoculated

8-HQC + SU solutions. The slow-release Cl, DICA, coupled with sucrose prevented bacterial growth and sustained cut flower life.

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VESICULAR-ARBUSCULAR MYCORRHIZAL INOCULATION AND FERTILIZER LEVEL AFFECT YIELD, MORPHOLOGY, CHLOROPHYLL CONTENT, WATER UPTAKE AND VASE LIFE OF LEATHERLEAF FERN FRONDS[1]

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Abstract. **The vesicular-arbuscular mycorrhizal (VAM) fungus** Glomus intraradices **Schenck & Smith colonized (25% infection) the roots of tissue culture-produced leatherleaf fern**]Rumohra adiantiformis **(G. Forst.) Ching[grown in a sterilized 1:1:1 (sand:peat:perlite) mix in clay pots and fertilized at 3 rates (0, 280, or 560 kg N/ha/yr) with 19-6-12 Osmocote®. Fertilizer level did not affect colonization. Inoculation had no effect on chlorophyll content or plant grade but both increased with increasing fertilization. Frond yield and frond surface areas increased and leaf density thickness decreased with increasing fertilizer level. Inoculation did not affect yield (dry weight basis) but inoculated plants produced fewer and heavier fronds than noninoculated plants. Postharvest longevity of fronds averaged 16.2 days and was not affected by treatments. Average postharvest water uptake was negatively correlated ($r = -0.462$, $P < 0.01$) with vase life. Under the conditions of this experiment, inoculation with** G. intraradices **was of no benefit.**

Leatherleaf fern, besides being a durable cut foliage crop for use in floral arrangements, is a good ground cover for shady locations in central and south Florida (1). Leatherleaf fern, once established as a ground cover, requires little maintenance since its fertilization requirements are relatively low, its pest problems are few (11), and it seldom requires mowing. Potted leatherleaf fern plants are produced commercially for use in the landscape.

Vesicular-arbuscular mycorrhizal (VAM) fungi have been found to associate mutualistically and parasitically with many host plants. Berch and Kendrick (3), Boullard (6), Harley (9), Hepden (12) and Cooper (7) have documented numerous mycorrhizal associations between VA fungi and leptosporangiate ferns. Cooper (7) found 100% of leatherleaf fern samples from 5 locations in New Zealand to be infected, however she did not identify the fungi present. The effects of VAM fungi on leatherleaf fern have not been studied.

The purposes of this experiment were to determine if *G. intraradices* would colonize the roots of leatherleaf fern and if fertilizer level would influence colonization, to see if colonization and/or increasing fertilization levels would improve quality and growth of leatherleaf fern, and to determine the effects of colonization and/or fertilization level on the postharvest longevity of cut leatherleaf fern fronds.

Materials and Methods

A 2 x 3 factorial experiment was initiated May 24, 1983 when individual 10 to 15-cm long terminal leatherleaf fern rhizome pieces were transplanted into 15.2-cm diameter disinfected clay pots. The rhizome source plants had been produced through tissue culture and were found to be free of VAM fungi. The steam-sterilized potting medium contained equal parts of sand, peat and perlite. Half of the plants were inoculated at planting with the mycorrhizal fungus *G. intraradices* by placing a 5-g mixture of chlamydospores, hyphae and infected citrus roots around the rhizomes. The 3 fertilizer levels used were 0, 280 and 560 kg N/ha/yr (0, 0.45 and 0.89 g Osmocote 19-6-12 per pot every 2 months). Treatments were replicated 5 times.

Plants were grown under 73% shade for 1 yr. Production temperatures varied from 4° to 35°C and were not controlled except to maintain the plants above 4°C. Plants were watered as needed. Leaf punches for chlorophyll determinations and root samples for colonization determinations were taken December 21, 1983. Two 0.27-cm² leaf discs were obtained from the most recently matured frond in each pot. Chlorophyll extraction in the dark at −15°C took 48 hr using acidified methanol. Chlorophyll determina-

[1]Florida Agricultural Experiment Stations Journal Series No. 5960.