The Effect of Germicides on the Longevity of Cut Flowers

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Abstract. The effect of DICA (50 mg-liter⁻¹), BCDMH (12 mg available chlorine/liter), and HQC (250 mg-liter⁻¹) on the longevity of 14 popular cut flower species was assessed. Longevity was significantly extended in: *Rosa hybrida* L. 'Gabrielle' and *Scilla campanulata* L. Squill. by all germicides; *Lilium parkmannii* L. 'Nepal', *Gerbera jamesonii* L. 'Mercy', and *Narcissus tazetta* L. 'Fortune' by DICA and BCDMH; *Gypsophila paniculata* L. 'R22' by DICA and HQC; and *Freesia hybrida* Eckl. ex Klatt 'White Bergunden' by BCDMH. No effect on longevity was found in *Dendranthema grandiflora* (Ramat) Kitamura. 'Horim', *Dianthus caryophyllus* L. 'Medea', *Dianthus barbatus* L., *Iris hollandica* L. 'Pearl', and *Gerbera jamesonii* L. 'Double Delight'. Longevity was significantly reduced by DICA in *Alstroemeria aurantiaca* L. 'Mona Lisa' and *Tulipa hybrida* L. 'Apeldoorn'. Analysis of microbial concentrations showed that proliferation was effectively controlled by DICA and BCDMH, but not by HQC. Levels of up to 10⁶ cfu·ml⁻¹ were detected in water, indicating that species not affected by germicides can tolerate these microbial quantities. Fresh weight and solution uptake data indicated that germicides acted primarily by improving solution uptake. Longevity was significantly reduced in *R. hybrida* 'Gabrielle' and *D. caryophyllus* 'Medea' flowers placed in solutions containing high counts of microorganisms (>10⁸ cfu·ml⁻¹) isolated from *D. caryophyllus* or *R. hybrida*. Chemical names used: 1-bromo-3-chloro-5,5-dimethylhydantoin (BCDMH); sodium dichloroisocyanuric acid (DICA); 8-hydroxyquinoline citrate (HQC).

The most common cause of the termination of vase life in cut flowers is water stress (Halevy, 1976). The rapid proliferation of microorganisms in vase water is thought to result in xylem blockage, water stress, and a subsequent reduction in cut flower longevity (van Doorn and Perik, 1990); a variety of germicides have been suggested to prevent this problem (Aarts, 1957; Larsen and Cromarty, 1966; Larsen and Cromarty, 1967; Larsen and Frolich, 1969; Marousky, 1968, 1969, 1971; Kofranek et al., 1974; Halevy and Mayak, 1981). Many studies have correlated an increase in bacterial counts in the vase water with decreased longevity (Larsen and Frolich, 1969; Clerkx et al., 1989; van Doorn and Perik, 1990). While Zagory and Reid (1986) isolated three microorganisms from the holding solutions of cut carnation flowers that specifically reduced longevity of cut carnations, roses, and chrysanthemums, it has not been shown conclusively how the presence of microorganisms adversely affects longevity. It has been suggested, however, that the presence of microorganisms in vase water can: 1) cause physical plugging of the cut stem (Burdett, 1970; Lineburger and Steponkus, 1976; van Doorn and Perik, 1990), 2) release toxic metabolites and/or enzymes (Mayak and Accati, 1979; Accati et al., 1980; Put and Rambouts, 1989), or 3) evolve damaging levels of ethylene (Fujino et al., 1983).

Despite these results, the response of many cut flowers to germicides is highly variable among species and variety. Longevity was consistently improved in cut roses, for example, with the addition of germicides (Marousky, 1969; Clerkx et al., 1989; van Doorn and Perik, 1990). Vase life in cut carnations, however, has

been improved with germicides in some studies (Aarts, 1957; Larsen and Frolich, 1969; Mayak et al., 1977), while in others there was no effect (Marousky, 1976; van Doorn et al., 1991). In fact, van Doorn et al. (1991) found that vase life of cut carnation flowers was unaffected when high levels of bacteria (10⁸ cfu·ml⁻¹) or 250 mg-liter⁻¹ DICA were added to vase water. Similar conflicting results were found with HQC on *Freesia* (Woodson, 1987; Accati-Garibaldi and Deambrogio, 1990). Further, many studies tested the effect of commercial preservatives where it is difficult to determine whether any beneficial effect on the flowers resulted from the sugar, the germicide, or some other compound contained in the formulation (Staby et al., 1978; Healy and Lang, 1989; Dan and Griffith, 1990).

Results from most studies are, therefore, variable and difficult to compare due to a lack of consistency in establishing criteria for termination of vase life and in experimental conditions. This study attempts to clarify the effect of DICA, BCDMH, and HQC on the vase life and microbial counts found in the vase water of 14 popular species of cut flower. Additionally, assessment of the impact of microbes on flower longevity was made. Little information is available on the efficacy of BCDMH, which is being used in increasing amounts by flower growers and wholesalers as it has the advantages of being more effective than chlorine in lower concentrations and maintains efficacy over a longer period (H. Tayama, personal communication).

Materials and Methods

Effect of germicide solutions. Flowers were collected from commercial flower growers and transported to the laboratory in water within 4 h of harvest. Stems of each species were trimmed to a uniform length and placed in test tubes containing the following treatments: 1) tapwater (control), 2) 50 mg (a.i.) sodium dichloroisocyanurate/liter (DICA) 60% a.i. Bioguard pool sanitiser,

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Abbreviations: cfu, colony-forming units; FW, fresh weight.

Table 1. Criteria used for determining end of vase life.

Genus	Termination of vase life
Alstroemeria	First sign of leaf yellowing
Dendranthema	First sign of petal wilting or drop, leaf yellowing
Dianthus	First sign of petal inrolling/wilting
Freesia	Half the open flowers wilted
Iris	First sign of petal wilting
Gerbera	More than one-third of the petals brown/wilted
Gypsophila	50% florets wilted
Lilium	First sign of petal browning or drop
Narcissus	First sign of petal wilting
Rosa	More than one-third petals wilted or bent neck
Scilla	First sign of petal wilting
Tulipa	First sign of petal wilting or drop

Biolab, Melbourne, Australia), 3) BCDMH 12 mg available chlorine/liter (Nylate, Wobelea Pty, Melbourne); and 4) 250 mg HQC (Ajax Chemicals Pty, Melbourne). The pH of each solution was determined as: distilled water, 6.9; DICA, 5.9; BCDMH, 6.5; HQC, 3.6.

Test tubes and solutions, with and without the flower, were weighed daily to determine fresh weight (FW) and solution uptake. Solutions were replaced every 3 days to ensure germicides remained active. Ten replications of each treatment were arranged in a randomized-block design, and each trial was conducted twice. Flowers were placed at $20\text{C} \pm 1\text{C}$, $65\% \pm 3\%$ relative humidity (RH), under 24 h light of $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Vase life was assessed daily and was considered terminated according to the criteria listed in Table 1.

Microbial suspensions. Samples of vase water were taken from *R. hybrida* 'Gabrielle' and *D. caryophyllus* 'Medea' at the start of the experiment (0 days), and 2, 6, and 9 days after placement in 50 mg·liter⁻¹ DICA, BCDMH (12 mg·liter⁻¹ available chlorine), and 250 mg·liter⁻¹ HQC. After decimal dilutions, samples were plated out on Kings B media, incubated at 20C for 5 days, and counted.

Concentrated microbial suspensions were made by placing 10 stems each of *R. hybrida* 'Gabrielle' and *D. caryophyllus* 'Medea' in tapwater at 20C for 10 days. Samples of the suspensions were plated on Kings B medium to determine counts. Freshly cut stems of *R. hybrida* 'Gabrielle' and *D. caryophyllus* 'Medea' were placed in the vase water containing $\approx 10^8$ cfu·m⁻¹; control stems were placed in distilled water. Vase life was assessed at 20C.

Results and Discussion

Effect of germicide solutions. The most effective germicides were DICA and BCDMH; both improved longevity in six species, but DICA had no effect on six other species, and BCDMH had no effect on eight others (Table 2). HQC increased vase life in three species and had no significant effect on 11 species. Vase life was significantly increased in *R. hybrida* 'Gabrielle' and *S. campanulata* by DICA, BCDMH, and HQC; *L. parkmannii* 'Nepal', *G. jamesonii* 'Mercy' and *G. paniculata* by DICA and BCDMH; *N. tazetta* 'Fortune' by DICA and HQC; and *F. hybrida* 'White Bergunden' by BCDMH (Table 2). The pH of the germicide solutions did not appear to affect FW (Fig. 1) or vase life (Table 2) in species treated with HQC solutions (pH 3.6) compared with DICA and BCDMH (pH 5.9 and 6.5, respectively).

Previous studies have shown that the longevity of roses, gerberas, and *Gypsophila* has been consistently improved with germicide solutions. Longevity of cut 'Sonia', 'Ilona', 'Polka', and 'Frisco' roses, for example, was increased with HQC and DICA in similar concentrations to those used in this study (Marousky, 1969; van Doorn, 1988; van Doorn and Perik, 1990). Alternatively, increasing the number of microorganisms in the vase water resulted in poor vase life in cut 'Sonia' roses and 'Fleur' gerberas (Clerkx et al., 1989). Longevity has been improved in *G. jamesonii* 'Wageningen Rood' with sodium hypochlorite (van Meeteren, 1978) and in *G. paniculata* with quaternary ammonium compounds (Tandler et al., 1986).

Longevity was unaffected by any germicide in several species, namely *D. grandiflora* 'Horim', *D. caryophyllus* 'Medea', *D.*

Table 2. Effect of DICA, BCDMH, and HQC on vase life (days) of selected cut flower species. Flowers were placed in DICA (50 mg·liter⁻¹), BCDMH (12 mg·liter⁻¹ available chlorine), or HQC (250 mg·liter⁻¹), with tap water as a control, and vase life was assessed under the criteria in Table 1. Each species was tested twice, and values are the means of both trials. Data was subjected to ANOVA using the Genstat 5 package. LSD was determined at $P = 0.05$.

Species	Vase life (days)				LSD
	Control	DICA	BCDMH	8HQC	
<i>A. aurantica</i>	10.4	9.4	10.2	10.7	0.8
<i>D. grandiflora</i>	13.9	16.4	13.6	13.4	3.2
<i>D. caryophyllus</i>	6.0	6.3	6.6	6.6	0.7
<i>D. barbatus</i>	11.3	11.8	11.6	11.5	2.0
<i>F. hybrida</i>	6.5	6.5	7.3	7.0	0.8
<i>I. hollandica</i>	5.0	5.3	5.4	5.7	1.7
<i>Gerbera jamesonii</i>					
'Double Delight'	10.6	11.9	13.1	12.2	3.1
'Mercy'	12.1	15.8	14.8	11.6	1.9
<i>Gypsophila</i>					
<i>paniculata</i>	9.4	10.7	10.2	10.9	1.0
<i>L. parkmannii</i>	6.5	8.1	8.0	7.6	1.4
<i>N. tazetta</i>	3.2	4.0	3.7	3.9	0.7
<i>R. hybrida</i>	10.8	14.5	15.3	12.8	1.6
<i>S. campanulata</i>	11.7	16.6	13.6	13.7	1.7
<i>T. hybrida</i>	5.7	5.2	5.5	5.3	0.5

A

A

B

B

C

C

Fig. 1. Effect of tapwater (▲), 50 mg DICA/liter (□), BCDMH (12 mg available chlorine/liter) (■), and 250 mg HQC/liter (○) on FW of cut *R. hybrida* 'Gabrielle' (A), *D. caryophyllus* 'Medea' (B), and *T. hybrida* 'Apeldoorn' (C) flowers. FW is expressed as percentage of initial fresh weight (IW). LSD was calculated at $P = 0.05$.

Fig. 2. Effect of tapwater (▲), 50 mg DICA/liter (□), BCDMH (12 mg available chlorine/liter) (■), and 250 mg HQC/liter (○) on solution uptake of cut *R. hybrida* 'Gabrielle' (A), *D. caryophyllus* 'Medea' (B), and *T. hybrida* 'Apeldoorn' (C) flowers. Uptake is expressed as milliliters/gram FW per day. LSD was calculated at $P = 0.05$.

Table 3. Microbial counts in vase water solutions containing individual stems of *R. hybrida* 'Gabrielle' and *D. caryophyllus* 'Medea'. Solutions consisted of: tapwater, DICA (50 mg·liter⁻¹), BCDMH (12 mg·liter⁻¹ available chlorine), or HQC (250 mg·liter⁻¹), and were sampled 0, 2, 6, and 9 days after harvest. Values are the mean of six samples each from a tube containing an individual stem.

Species and compound	Microbial counts (cfu·ml ⁻¹)			
	Days			
	0	2	6	9
<i>R. hybrida</i>				
Water	2 × 10 ⁴	10 ⁶	10 ⁵	4 × 10 ⁵
DICA	0	0	0	0
BCMH	0	0	0	0
HQC	0	10 ²	2 × 10 ⁵	10 ⁶
<i>D. caryophyllus</i>				
Water	5 × 10 ⁴	10 ⁶	4 × 10 ⁶	10 ⁶
DICA	0	0	0	0
BCDMH	0	10 ⁶	6 × 10 ⁵	3 × 10 ⁶
HQC	0	7 × 10 ³	10 ⁶	10 ⁷

barbatus, *I. hollandica* 'Pearl', and *G. jamesonii* 'Double Delight' (Table 2). Responses to germicides can, therefore, vary widely among species, as well as between cultivars of the same species as longevity was improved by DICA and BCDMH in *G. jamesonii* 'Mercy', but not in 'Double Delight'. Similarly, HQC improved longevity in *F. hybrida* 'Aurora' (Woodson, 1987), but had no effect on *F. hybrida* 'Royal Crown' (Accati-Garibaldi and Deambrogio, 1990).

Other studies have also found a variable effect of germicides on carnation and chrysanthemum longevity. The application of DICA or silver nitrate had no effect on longevity in *D. caryophyllus* (van Doorn et al., 1991), and HQC did not affect longevity in *D. caryophyllus* 'Red Sim' (Marousky, 1976). Mayak et al. (1977), however, found that silver nitrate improved longevity in 'White Sim' carnations. In contrast to our results, longevity in *D. grandiflora* improved when quaternary ammonium compounds were used as germicides (D'hont and van der Sprong, 1989).

Longevity was significantly reduced in *A. aurantiaca* 'Mona Lisa' and *T. hybrida* 'Appeldoorn' by 50 mg DICA/liter. Vase life has been extended in *T. hybrida* and *A. aurantiaca* by commercial preservatives (Staby, 1978; Healy and Lang, 1989), but it is not known whether this resulted from the germicide contained in the formulation. Certain cut flower species are known to be sensitive to specific germicides. Vase life of 'Sonia' roses, for example, declined with the application of DICA at 50 mg·liter⁻¹ (D. Joyce, personal communication), while the Australian native flowers *Thryptomene calycina* (Lindl.) Stapf and *Telopea speciosissima* R.Br. are both sensitive to Physan (200 mg·g⁻¹) and benzalkonium chloride at 50 mg·g⁻¹ (J. Faragher, unpublished data).

Rosa hybrida 'Gabrielle', *D. caryophyllus* 'Medea', and *T. hybrida* 'Apeldoorn' were chosen as examples of flowers where longevity was improved, unchanged, or decreased, respectively, by the application of germicides (Table 2). Fresh weight was maintained at a significantly higher level in stems treated with DICA, BCDMH, or HQC in *R. hybrida* 'Gabrielle' (Fig. 1A) than in stems treated with water. Fresh weight of stems treated with DICA, BCDMH, and HQC continued to increase up to 8 days after harvest, then declined (Fig. 1A). Solution uptake improved with all treatments for 5 days (Fig. 2A), then declined rapidly in roses kept in water. Uptake continued to improve with DICA and remained relatively constant with HQC, while uptake in BCDMH declined, but at a lower rate than in water.

There was no significant difference in FW and solution uptake between *D. caryophyllus* stems treated with germicides or water (Figs. 1B and 2B). Although FW was maintained in stems treated with HQC for 7 days, this had no significant effect on vase life (Table 2). Possibly, vase life was at least partially determined by ethylene production by the flowers, as the carnations were not treated with silver thiosulphate after harvest.

Fresh weight of tulips (Fig. 1C) was similar for flowers placed in water or germicides for 5 days after harvest, but was significantly lower in stems treated with DICA after 6 days (Fig. 1C). Solution uptake was not affected by germicide solutions for the life of the cut tulips (Fig. 2C), indicating that the detrimental effect of DICA was not due to a decrease in solution uptake and FW, but possible tissue toxicity.

Microbial suspensions. DICA and BCDMH effectively controlled microbial growth during the vase life of *R. hybrida* 'Gabrielle' (Table 3), but up to 10⁶ cfu·ml⁻¹ were consistently detected in water and in solutions containing HQC. Microbial growth in solutions containing cut 'Medea' carnations was controlled by DICA only, as up to 10⁶ cfu·ml⁻¹ were recorded in tapwater, BCDMH, and HQC solutions after 9 days (Table 3). The uniform appearance of bacterial colonies on plates inoculated with BCDMH solutions indicated that one microbial strain may have been resistant.

The addition of two mixed microbial populations grown from *D. caryophyllus* 'Medea' and *R. hybrida* 'Gabrielle' flower solutions at the higher concentration of 10⁸ cfu·ml⁻¹ significantly reduced vase life in 'Gabrielle' roses and 'Medea' carnations

Table 4. Vase life of individual stems of *R. hybrida* 'Gabrielle' and *D. caryophyllus* 'Medea' placed in: sterilized water, or with mixed microbial populations (adjusted to 10⁸ cfu·ml⁻¹) grown in vase water containing 10 stems each of *R. hybrida* 'Gabrielle' or *D. caryophyllus* 'Medea'. LSD was determined at *P* = 0.05

Source of microbes	Vase life (days)	
	<i>R. hybrida</i> 'Gabrielle'	<i>D. caryophyllus</i> 'Medea'
Water (none)	11.5	11.0
<i>R. hybrida</i>	5.6	5.5
<i>D. caryophyllus</i>	1.5	6.7
LSD	1.8	1.8

(Table 4). Microbes grown from *D. caryophyllus* 'Medea' were particularly deleterious to 'Gabrielle' roses (Table 4), indicating the possibility that microbial populations from carnation flowers are more toxic to other cut flower species. Other soluble or suspended materials not measured in these trials may have been responsible for the observed decline in longevity. Preliminary trials indicated that a mixed microbial population (10^8 cfu·ml⁻¹) isolated from *D. caryophyllus* was significantly more toxic to 'Gabrielle' roses than microbes isolated from several other flower species, including *Protea neriifolia* and *T. hybrida*. Similarly, Zagory and Reid (1986) found that longevity in 'Cara Mia' roses was significantly reduced by pure strains of *Pseudomonas* and yeast isolated from carnation flowers.

Van Doorn et al. (1991) reported that a mixed microbial population (10^7 cfu·ml⁻¹) had no effect on the vase life of *D. caryophyllus* 'Scania' and 'White Sim', but higher levels ($>10^8$ cfu·ml⁻¹) significantly reduced vase life (van Doorn et al., 1992). Our results support these data as microbial levels of 10^6 cfu·ml⁻¹ (Table 3) did not affect longevity in *D. caryophyllus* 'Medea', whereas longevity was significantly reduced by 10^8 cfu·ml⁻¹ (Table 4).

Apparently, sensitive species, exemplified by roses in this study, are adversely affected by relatively low microbial levels ($<10^6$ cfu·ml⁻¹) and benefit from the application of germicides (Table 2). Microbial concentrations of 10^6 cfu·ml⁻¹ were routinely recorded in the untreated tapwater 3 days after flower stems were added (Table 3). Based on our results (Table 2), lilies, gerberas, gypsophila, daffodils, freesia, and bluebell may behave in a similar manner. However, carnations appeared to be tolerant of microbial counts up to $\approx 10^7$ cfu·ml⁻¹. Other species that may be included in this class are *Dendranthema* (chrysanthemums), iris, alstroemeria, and tulip, i.e., those species unaffected by germicides (Table 2). Higher microbial levels ($>10^8$ cfu·ml⁻¹), however, may lead to accelerated wilting (Table 4). Microbial concentrations of this magnitude can occur under commercial conditions, as counts as high as 10^8 cfu·ml⁻¹ have been recorded in vase water in a survey of European and American florists and supermarkets (W.G. van Doorn, 1992, personal communication).

In tolerant species, such as *D. caryophyllus*, placed in water with microbial counts $<10^8$ cfu·ml⁻¹, bacterial blockage of xylem vessels may not be the primary cause of wilting (Table 2). Probably, cut flower senescence is controlled by a combination of factors, some physical, such as xylem vessel blockage caused by bacteria or air embolism, and others genetically determined, such as a loss of membrane integrity (Woodson, 1991). When water uptake is not hindered by physical xylem blockage, genetically determined factors may result in the decline in the ability of flowers to take up water and retain water in cells resulting in wilting.

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