SHOULD WE RECONSIDER THE USE OF DEIONIZED WATER AS CONTROL VASE SOLUTIONS?

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Abstract

In *Bouvardia* 'Van Zijverden' flowers held in deionized water leaf wilting started from day 6 of vase life, while flowers placed in tap water did not show any leaf wilting for the 14 days of the experiment. Fresh weight of chrysanthemum 'Cassa' flowers started to decrease after three days in deionized water. In a solution of major tap water components (0.7mM $CaCl_2$+ 1.5mM $NaHCO_3$+ 0.005mM $CuSO_4$) fresh weight of the chrysanthemum flowers did not decrease within the 7 days of the experiment. $CuSO_4$ alone, or a mixture of $CaCl_2$ + $NaHCO_3$ did not have this positive effect on fresh weight. However, none of three rose cultivars (Madelon, First Red, Frisco) showed a difference in fresh weight during vase life between deionized water or the solution of $CaCl_2$ + $NaHCO_3$ + $CuSO_4$.
When copper was replaced by $AgNO_3$ or DICA fresh weight of chrysanthemum did not decrease within the 7 days of the experiment, while it decreased from day 2 when only $CaCl_2$ + $NaHCO_3$ were present in the vase solution. *Bouvardia* flowers in $AgNO_3$ dissolved in deionized water did not show any leaf wilting, while all stems of flowers placed in $NaNO_3$ or deionized water did. The relevance of deionized water as control treatment in vase life experiments was questioned. It is proposed to use a solution of $CaCl_2$ + $NaHCO_3$ + $CuSO_4$ as 'standardized tap water'.

1. Introduction

Because variability in the composition of tap water used as vase water can cause differences in keeping quality of cut flowers (Waters, 1968; Staden and Molenaar, 1975; Staby and Erwin,1978), it was agreed at the Second International Symposium on Postharvest Physiology of Cut Flowers, that deionized or distilled water should be used as a common control vase solution (Reid and Kofranek, 1980). However, the use of deionized or distilled water does neither simulate common practice nor the situation in the intact flower. Zimmermann (1978) showed already that drawing distilled water through stem segments progressively decreased the rate of conductance and that this phenomenon can be eliminated by using tap water or a dilute osmoticum (e.g. 10 mM NaCl). It can be assumed that the same phenomenon happens during vase life. A fast decrease of hydraulic conductance is a phenomenon showed many times in cut flowers. This raises questions about the predictive value of experiments using deionized water.

In chrysanthemum deionized water gave a sharp decrease in fresh weight of the cut flowers after 1-3 days; this decrease was absent in tap water (Van Meeteren *et al.*, 1999). After 4 days in deionized water, hydraulic resistance in the basal part of the stem was about fifty times the value fresh cut flowers had and seven times the value in tap water. Fresh weight as well as hydraulic resistance of flowers during vase life in a solution of calcium chloride, sodium bicarbonate and copper sulfate was similar to that in tap water (Van Meeteren *et al.*, 1999).

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To investigate whether the negative water status caused by using deionized water as vase water is a common phenomenon, we compared the effects of deionized and tap water for chrysanthemum, *Bouvardia* and rose flowers.

The positive effect of tap water on water status should be partly caused by low concentrations of copper ions inhibiting bacterial growth (Van Meeteren *et al*., 1999). The effects of various copper salts on fresh weight during vase life were tested. Also the effects of other chemicals in deionized water, known to inhibit growth of bacteria, were compared to tap water.

2. Materials and methods

2.1. Plant material

Chrysanthemum (*Dendranthema x grandiflorum* Tzvelev cv. Cassa) plants were grown in a greenhouse at Wageningen University in 14 cm diameter plastic pots containing a commercial potting soil. The average temperature was 18ºC and a 16 h photoperiod was maintained until the plants had formed 15-17 leaves longer than 0.5 cm (3-4 weeks). Thereafter, an 8 h photoperiod was maintained until harvest. When necessary, lengthening of the natural photoperiod was achieved by high-pressure sodium lamps (Philips SON/T). Conversely, shortening of the natural photoperiod was by use of black screens.

Bouvardia *(Bouvardia x domestica* cv. Van Zijverden) plants were grown in a greenhouse at Research Station for Floriculture and Glasshouse Vegetables on rockwool slabs and irrigated with a nutrient solution by means of a trickle irrigation system. Temperature set points were 19/18ºC day/night. Plants were about five years old. After the previous flower harvest, they were pruned back and a 14 h photoperiod was maintained using incandescent lamps until the plants had formed about five leaf pairs (about 5 weeks). Thereafter photoperiod was shortened to 11 h until flower harvest (about 8 weeks).

Rose hybrids Madelon®, First Red® and Frisco® were grown in a greenhouse at Wageningen University on rockwool slabs and irrigated with a nutrient solution by means of a trickle irrigation system. They were grown according to the bending system; temperature set points were 19/17ºC day/night. They received supplementary light $^{-1}$. Plants were about five months old.

2.2. Harvest + pretreatment

Flowers were harvested in the greenhouses at commercial maturity. Flowers were brought to the laboratories as soon as possible. Chrysanthemum stems were cut at soil level; length of the stems at harvest was 0.8-0.9 m. Roses were cut near the main stem; length of the stems at harvest was 0.7-0.8 m. Bouvardia stems were harvested near the main stem; their length was about 0.6 m. From all flowers, lower leaves were removed. Thereafter, cut stem ends were trimmed by 1-2 cm in air to get clean stem ends without soil particles. Chrysanthemum and rose flowers were placed for 2-3 h in a bucket with a mixture of ice and tap water (3:2 by volume) in darkness at 4ºC to regain full turgidity (Van Meeteren, 1992). Bouvardia flowers were placed for 24 h in a 'pretreatment solution' (see 2.5) in darkness at 5ºC. Care had been taken that there were no leaves in the water. From chrysanthemum stems 0.30 m was cut off in air after the hydration treatment. Roses were cut at a length of 0.68 m. Thereafter, the fresh weight of the flowers was determined as the initial weight, and flowers were placed in the various vase solutions.

2.3. Vase period conditions and vase solutions

During the vase period, each flower was individually placed in a 'va
deionized water, tap water or the desired solution. The cut flowers were kept in a

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room at 20 $\pm$ 1ºC, 60 $\pm$ 5% RH and a light intensity of 14 µmol· m^{-2}· s^{-1} (Philips, TLD 50W/84HF) with a light period of 12 h. All vase solutions were made with deionized water (0.5-1.0 µS· cm^{-1}); 'tap water' was regular drinking water in our laboratories.

2.4. Fresh weight measurements and leaf wilting

Chrysanthemum and rose flowers were weighed at noon during several days of vase life. For that purpose, flowers were taken out of water for as short a time as possible (20-30 s). Fresh weight of each flower was expressed relative to the initial weight to represent the water status of the flower. Wilting of *Bouvardia* leaves was recorded daily, judged by eye.

2.5. Experimental design

Leaf wilting in Bouvardia in deionized and tap water. During the 24 h 'pretreatment' flowers were placed either in tap water or deionized water. After this period, half of the flowers out of tap water were placed in tap water during the vase experiments, the other half in deionized water. All the flowers out of deionized water were placed in deionized water during vase life. The flowers were randomly placed in a climate room with 15 flowers per treatment. Observed is the survival time till all leaves of a flowering stem were wilted. The experiment was terminated after 14 days.

Fresh weight of chrysanthemum in deionized water or solutions of the main components of tap water: calcium chloride, sodium bicarbonate and copper sulfate. During vase life, flowers were placed in deionized water, or solutions of $CaCl_2.2H_2O$ (0.7 mM), $NaHCO_3$ (1.5 mM) and $CuSO_4.5H_2O$ (0.005 mM) to simulate tap water (Van Meeteren *et al.*, 1999). The design was a randomized complete block with 12 flowers per treatment. The flowers were placed in a climate room with four blocks, and three flowers per treatment in each block. Within a block, the three flowers of one treatment were grouped together to form one sample unit. Daily mean relative fresh weight of sample units was used for data analysis.

Fresh weight of roses in deionized and 'standardized tap water'. After the rehydration in ice-cold tap water, flowers were randomly placed in a climate room in deionized water or a solution of $CaCl_2.2H_2O$ (0.7 mM) + $NaHCO_3$ (1.5 mM) + $CuSO_4.5H_2O$ (0.005 mM). For Madelon® and Frisco® there were 10 flowers per treatment, for First Red® there were 5 flowers per treatment. Daily mean relative fresh weight of each flower was used for data analysis.

Effect of assumed bacteriostatic compounds. With chrysanthemum, the effects on fresh weight changes during vase life of solutions of various copper salts (0.005 mM $CuSO_4$. $5H_2O$, $Cu(NO_3)_2.3H_2O$, $CuCl_2.2H_2O$) as component of 'standardized tap water' were compared. It was argued by Van Meeteren *et al.* (1999) that the positive effect of copper on water status of chrysanthemum was mainly due to the inhibition of bacterial growth. Therefor, the effects of substituting copper sulfate by two bacteriostatic compounds, $AgNO_3$ (0.15 mM) and DICA (dichloroisocyanuric acid) (125mg· l^{-1}), were investigated. To prevent precipitation of AgCl, $CaCl_2$ was replaced by $Ca(NO_3)_2$ (0.7 mM) when $AgNO_3$ was used. The design was a randomized complete block with 12 flowers per treatment. The flowers were placed in four blocks, and three flowers per treatment in each block. Within a block, the three flowers of one treatment were grouped together to form one sample unit. Daily mean relative fresh weight of sample units was used for data analysis.

With *Bouvardia* the effect on leaf wilting of $AgNO_3$ (0.006 mM) in deionized water was compared to tap water and deionized water. As a control a solution of $NaNO_3$ (0.006 mM) was used. Solutions used during pretreatment and vase life had the same composition. There were 10 flowers per treatment, randomly placed in a climate room. Observed is the survival time till all leaves of a flowering stem were wilted. The experiment was terminated after 14 days.

2.6. Statistical analysis

Daily fresh weights were statistically analyzed by analysis of variance using the GENSTAT 5 program (Rothamsted, U.K.), followed by mean separation according to Tukey's HSD-test. Leaf wilting was analyzed by survival analysis (Kleinbaum, 1995) using the Kaplan-Meier procedure of SPSS Advanced Statistics 7.5 (SPSS Inc., Chicago) with right censored data. Of this procedure, the log-rank test of statistical significance was used. P-values for pair wise comparison of treatments are given in the figures.

3. Results

Within the 14 days of the experiment, *Bouvardia* flowers constantly held in tap water did not show any leaf wilting (Fig.1). When flowers were placed in deionized water, leaf wilting started from day 6 of vase life, whether the flowers stood in deionized or in tap water during the 24 h-period in the cold room preceding vase life.

Fresh weight of chrysanthemum flowers started to decrease after three days in deionized water (Fig.2). In a solution of low amounts of $CaCl_2$ + $NaHCO_3$ + $CuSO_4$ fresh weight did not decrease within the 7 days of the experiment. $CuSO_4$ alone, or a mixture of $CaCl_2$ + $NaHCO_3$ did not have this positive effect on fresh weight (Fig.2).

None of the three rose cultivars used showed a significant difference in fresh weight during vase life between deionized water or a solution of $CaCl_2$ + $NaHCO_3$ + $CuSO_4$ as vase water (data not shown).

Whether the solution of $CaCl_2$ + $NaHCO_3$ contained $CuSO_4$, $Cu(NO_3)_2$ or $CuCl_2$ all resulted in fresh weights higher that in the solution without a copper salt (Fig.3). When copper was replaced by $AgNO_3$ or DICA fresh weight did not decrease within the 7 days of the experiment, while it decreased from day 2 when only $CaCl_2$ + $NaHCO_3$ were present in the vase solution (Fig.4).

Bouvardia flowers in tap water or in $AgNO_3$ dissolved in deionized water did not show any leaf wilting, while all stems of flowers placed in $NaNO_3$ or deionized water did (Fig.5). In $NaNO_3$ wilting occurred earlier than in deionized water.

4. Discussion

Compared to tap water, the use of deionized water had a negative effect on the water status of *Bouvardia* flowering stems as expressed in fast wilting of the leaves. This supports the opinion that the results found for chrysanthemum (Van Meeteren *et al.*, 1999) are not specific for the last mentioned crop. However, the use of deionized water had no negative effect on the water status of the rose cultivars used.

The large effect of copper sulfate when added to the combined solution of $CaCl_2$ + $NaHCO_3$ was clearly due to the copper ion (Fig.4). The suggestion by Van Meeteren *et al.* (1999) that low amounts of copper prevented an increase in hydraulic resistance of the stem during vase life, by inhibiting growth of bacteria, was further supported by the strong effect of $AgNO_3$ and DICA. Both compounds are known for their bacteriostatic effect (Van Meeteren, 1978; Van Doorn *et al.*, 1990). It is unclear, however, why rose flowers did not show a negative effect on their water status by the use of deionized water, while rose is very sensitive for bacterial growth, inhibiting water uptake (Van Doorn *et al.*, 1989; Van Doorn, 1995). Cu^{2+}, Ag^{+} and DICA are not specific antibiotics. Besides micro-organisms they will effect various enzymes. It cannot be excluded that copper influences other processes in stem tissue of chrysanthemum and *Bouvardia* involved in impediment of water transport. $CuSO_4$ combined with $NaHCO_3$ could not inhibit the increase in hydraulic resistance during the vase period when measured 3 cm above the cut plane of the stem; calcium-ions could (Van Meeteren *et al*. 1999). Therefor the effect of copper seems to be restricted to the basal stem part, which includes wounded tissue.

Most postharvest research on cut flowers intends to understand, predict or

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improve the vase life behavior of the flowers. However, growers, retailers, or consumers will not use deionized water for their cut flowers. Postharvest experiments using deionized water do not simulate vase life behavior of cut chrysanthemums or *Bouvardia* in practice. Testing of water additives using deionized water could result in overestimating positive effects. For example, addition of $AgNO_3$ had a very positive effect on water status when added to deionized water, while using tap water resulted in the same water status as the $AgNO_3$ solution.

Deionized water in xylem vessels is also not a natural situation of an intact plant. Total solute concentration in xylem sap of *Leucaena leucocephala* and *Zea mays* varied between 10 - 40 and 20 - 100 mOsmol$\cdot$ kg^{-1} respectively (Liang and Zhang, 1997). The effective Ca^{2+} concentrations in our experiments have about the same magnitude as reported for xylem sap from various plants (0.2-13.6 mM) (Atkinson *et al.*, 1992; Ruiz *et al.*, 1993; Schurr and Schulze, 1995).

Tap water may significantly vary in mineral composition and is therefore unsuitable as a standard treatment. Common salts in tap water include $Ca(HCO_3)_2$ and $CaSO_4$, and these salts determine water hardness. Owing to the effects on human health, 60 mg$\cdot$ l^{-1} calcium and 30 mg$\cdot$ l^{-1} HCO_3^- are suggested as minimum concentrations for drinking water (Anonymous, 1980). The concentration of Cu^{2+} in tap water will vary due to the use of pipe material, the period water stays in the pipes, and the copper dissolving ability of the water. However, as long as copper pipe material is used, copper will be present in tap water. In samples of tap water in our laboratory [Cu^{2+}] varied between 0.006 mM (at the beginning of the day) and 0.002 mM (after running the water for 1 h).

We propose to use a 'standard vase solution' in postharvest research of cut flowers. Based on the experimental results as well on their presence in tap water, a simple solution of $CuSO_4$ (0.005 mM) + $CaCl_2$ (0.7 mM) + $NaHCO_3$ (1.5 mM) seems to be a good candidate. When deionized water is used, at least the quality of the water (conductivity) should be mentioned.

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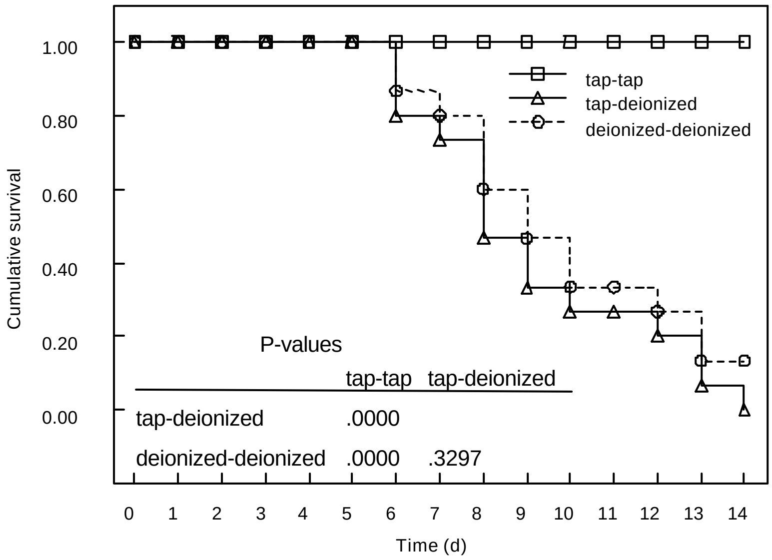
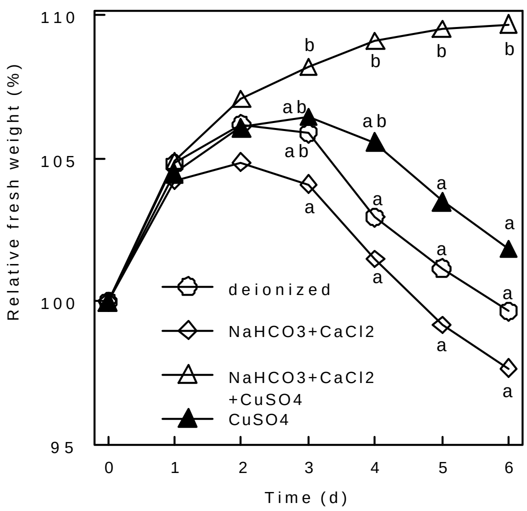
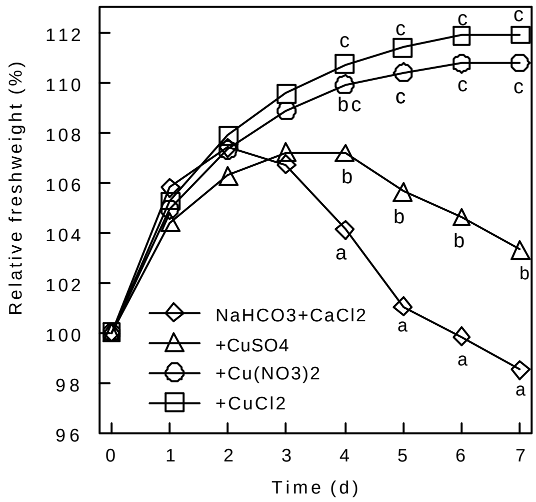
<u>Figures</u>



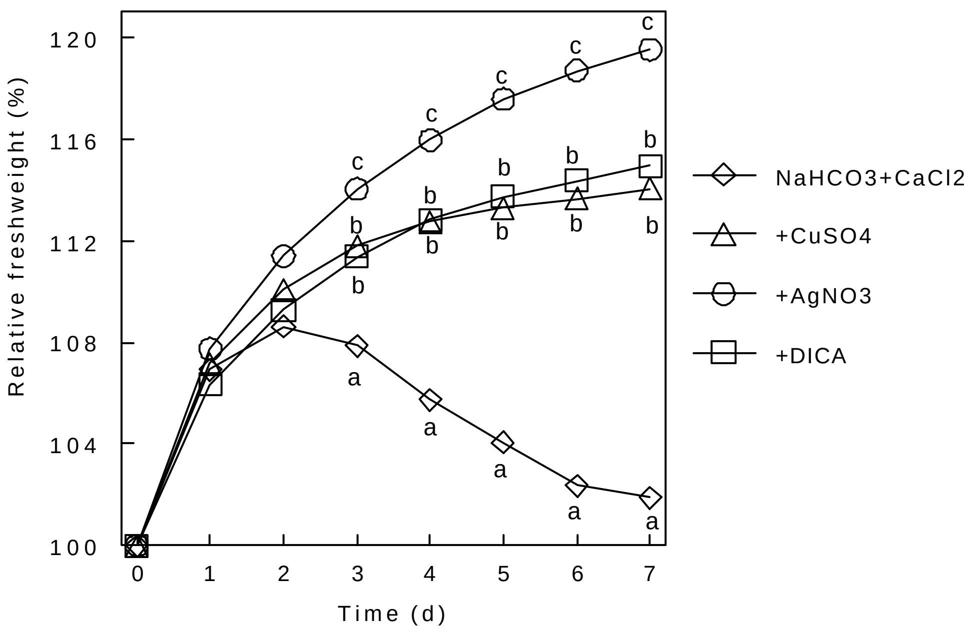
1. The effect of the use of tap or deionized water on survival (no wilted leaves) of *Bouvardia* flowering stems. Cumulative survival as fraction of 15 stems; first day of wilting of all present leaves observed as critical event. Tap-tap = pretreatment and vase life in tap water; tap-deionized = pretreatment in tap water, vase life in deionized water; deionized-deionized = pretreatment and vase life in deionized water.



2. Effect of deionized water and some vase solutions on relative fresh weight of chrysanthemum flowers. Weights at the same day marked by the same letter are not significantly different from each other ($P<0.05$).



3. Effect of various copper salts added to a solution of $NaHCO_3$+ $CaCl_2$ on relative fresh weight of chrysanthemum flowers. Weights at the same day marked by the same letter are not significantly different from each other ($P<0.05$).



4. Effect of copper sulfate, silver nitrate or DICA added to a solution of $NaHCO_3$ + $CaCl_2$ on fresh weight of chrysanthemum flowers. Weights at the same day marked by the same letter are not significantly different from each other ($P<0.05$).

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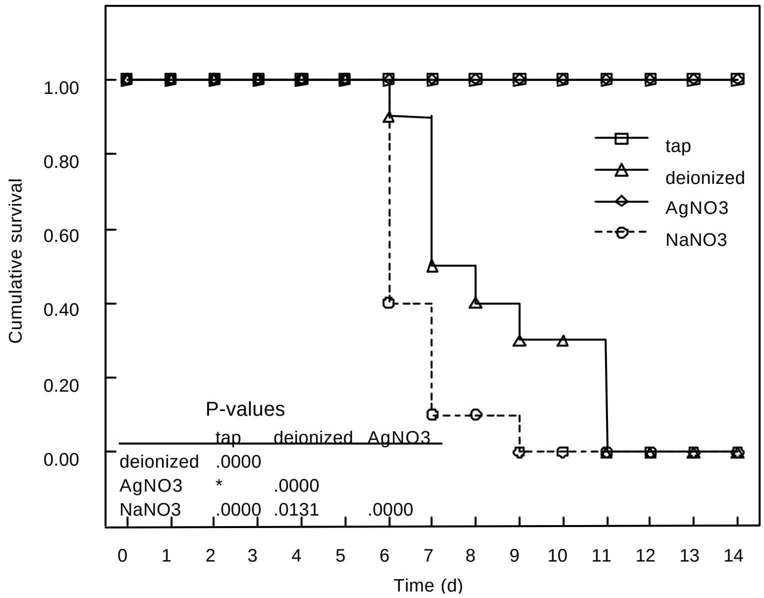

5. The effect of the use of tap water, deionized water, $AgNO_3$ or $NaNO_3$ on survival (no wilted leaves) of *Bouvardia* flowering stems. Cumulative survival as fraction of 10 stems; first day of wilting of all present leaves observed as critical event. *Statistics cannot be computed.