SUCROSE ENHANCES THE POSTHARVEST QUALITY OF CUT FLOWERS OF *EUSTOMA GRANDIFLORUM* (RAF.) SHINN.

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Abstract

The postharvest life of cut *Eustoma grandiflorum* flowers is limited by poor bud opening and bent neck in open flowers. Vase solutions containing up to 6% sucrose or glucose improved the quality and vase life of the flowers. The additional carbohydrate improved petal color, increased bud opening, strengthened pedicels, and extended overall inflorescence longevity by up to 8 days. The principal sugar in the petals of open flowers was glucose, with lower concentrations of sucrose and fructose. Presence of sugar in the vase solution greatly increased the concentration of sugars in the perianth of buds and open flowers. *Eustoma* flowers are affected by exposure to ethylene and pre-treatment with 1-MCP or STS delays final senescence of flowers that have been held in solutions containing sugar.

1. Introduction

Lisianthus, or the Texas gentian, *Eustoma grandiflorum* (Raff.) Shinn., native to the prairie states of North America, is now an important commercial cut flower (Halevy and Kofranek, 1984). Hybrids developed in Japan provide a wide range of colors, color patterns, and both single and double forms. Lisianthus has become very popular as a cut flower, because of the range of colors available, and the fact that each inflorescence comprises a long, straight stem bearing as many as 10 individual flowers. In addition, the plants (which may be annual or biennial depending on the growing environment) bear as many as 10 inflorescences over the production season (Tjia and Sheehan, 1986).

Although there have been many studies of the environmental conditions required for lisianthus production, there has been little examination of the postharvest characteristics of the flowers. Lisianthus is usually harvested commercially when the first one or two flowers on the stem have opened. A good quality inflorescence will usually have ten or more buds and flowers. If the flowers are placed in water, few if any of these buds open, and the longevity of the inflorescence is therefore determined by the life of the open flowers. Frequently, too, the life of the flowers is shortened by premature wilting and bending of the pedicels of the open flowers.

In other bud-type cut flowers, it has long been known that bud opening (and consequently display life of the inflorescence) can be enhanced by the addition of carbohydrate. This can be achieved either by using a postharvest pulse with a high concentration of sugar, as in gladiolus (Mayak *et al*., 1973) or by using a vase solution combining sugar with an appropriate biocide, as in hybrid *Limonium* (Doi and Reid,

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1995) and *Liatris spicata* (Han, 1992). Halevy and Kofranek (1984) recommended a pulse with 5-10% sucrose for 24 hours to improve postharvest life of Lisianthus flowers, and Farina *et al*. compared a number of such pulses. They found that pulsing with Chrysal or TOG (with 4% sugar) was ineffective, but that TOG with 10% sugar or 8-HQS with 10% sugar were both effective pulses. Song *et al*. (1994) tested the effects of STS pre-treatment, and found a modest improvement in bud opening, but also showed that a standard holding solution containing biocides and 2% sugar increased flower life three-fold. Ichimura and Korenaga (1998) suggested that it was the biocide (8-HQS) rather than sucrose that was important in improving the postharvest life of lisianthus.

Recently, Ichimura *et al*. (1998) suggested that ethylene was an important postharvest factor for lisianthus flowers. They showed that the life of aging flowers was significantly reduced if they were exposed to low concentrations of ethylene, and that there was substantial production of ethylene (primarily from the pistil) during flower senescence. We report here an examination of the hypothesis that provision of carbohydrate as a pulse, or in the vase solution, would improve the postharvest quality and longevity of lisianthus flowers. We also examined the role of ethylene in the life of these flowers by determining the effect of STS and 1-MCP pre-treatment and of ethylene exposure on the life of the flowers.

2. Materials and Methods

2.1. Plant Material

Lisianthus flowers (var. Heidi Pink) were obtained from a commercial grower in Salinas, California, harvested at commercial maturity and immediately transported to Davis in an insulated container over melting ice. The flowers were stored at 2°C, for no more than 24 hr, prior to use in experiments.

2.2. Effects of carbohydrates and biocides

The stems were recut to ca. 45 cm., and leaves on the basal 5-10 cm of the stem were removed. Flowers were then randomized, and placed individually (six replicate stems per treatment) in sterile plastic containers containing a range of solutions, including commercial flower preservatives, common floral vase biocides, and combinations of sucrose at different concentrations with a range of biocides. Control flowers were placed in DI water; vase solutions tested included DI water containing 50 $mg.L^{-1}$ NaOCl, 200 $mg.L^{-1}$ Physan (a proprietary mixture of quaternary ammonium compounds), 200 $mg \cdot L^{-1}$ 8-hydroxyquinoline citrate (8-HQC) and 200 $mg \cdot L^{-1}$ aluminum sulfate alone, or with sucrose, or sucrose alone.

Commercial cut flower preservatives tested included 20 $mL \cdot L^{-1}$ Crystal Clear (Floralife Inc. Burr Ridge, IL), 9 $g \cdot L^{-1}$ Oasis Floral Preservative (Smithers-Oasis, Kent, OH), 20 $mL \cdot L^{-1}$ Chrysal Professional 3 (Pokon & Chrysal, Naarden, Holland) and 10 $mL \cdot L^{-1}$ Rogard (Gard Products, IL.). In experiments to test the effect of pulse treatments with sucrose, flowers were placed in solutions containing higher concentrations of sucrose with or without a biocide (50 ppm NaOCl or 200 $mg \cdot L^{-1}$ aluminum sulfate) for 12 to 48 h. After the pulse treatment, flower quality and longevity was tested in DI or in vase solutions containing different sucrose or glucose concentrations.

2.3. Treatment with ethylene and ethylene action inhibitors

Freshly-harvested lisianthus flowers were treated with 4mM STS for 1 hour and then placed in water, or for 6 hours with 500 $nL.L^{-1}$ 1-MCP, or held in air. Replicate flowers from these different treatments (6 per treatment) were placed in DI in the evaluation room. To test the effects of ethylene, replicate flowers were exposed for 2

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days to 1 $\mu L.L^{-1}$ ethylene prior to vase life evaluation.

2.4. Evaluation of flower quality and longevity

2.4.1. Vase life

The effects of the different treatments on flower longevity and quality were examined by holding the flowers in a controlled environment room (vase life room) maintained at 20±1°C under 12 $h.d^{-1}$ cool-white fluorescent illumination (15$\mu mol.m^{-2}.sec^{-1}$ PAR), and an RH of 40% to 60% (Reid and Kofranek, 1980). The most recently opened flower was tagged at the beginning of the experiment to allow us to determine the number of buds that opened during the postharvest period. Flowers were examined daily for stem discoloration or pedicel bending, and the number of open and wilted flowers was noted. The vase life of the inflorescence was considered terminated when 50% of the open flowers had wilted.

2.4.2. Color

To determine the effect of various preservatives on color development during bud opening, we measured the color (L*, a*, b*, CIE) of the center of the outer surface of a petal on the oldest flower in each inflorescence, and that of a petal on the second opened bud, using a Minolta CR-300 ChromaMeter (Minolta, Ramsey, N.J.).

2.4.3. *Pedicel rigidity*

After 21 days in the vase, the effects of different treatments on rigidity of the pedicels was measured using a locally-constructed electronic force gauge based on a commercial force transducer (Interface, Inc. Scottsdale, AZ.). Pedicels of similar diameter were laid across a 5 cm gap, and the force required to cause a 5 mm deflection in the pedicel was determined.

2.5. Changes in Sugar Content

The changing sugar content of lisianthus flowers during vase life was determined for flowers held in DI and in a commercial preservative. At the start of the experiment we tagged buds at five distinct stages (Fig.1). The tagged buds and flowers were harvested at intervals, the petals were dissected, and weighed, and then frozen pending carbohydrate analysis. Petals were extracted in 2 or 5 mL 80% ethanol depending on the weight of the sample, by shaking for three hours. 1 mL of the extract was then evaporated to dryness in a water bath, redissolved in 1 mL DI, and used directly for HPLC analysis of sugars. Sugars were separated on two 10 cm long Aminex Fast Carbohydrate Columns connected in series, and concentrations determined by refractive index of the eluent peaks and comparison with peak area of an inositol internal standard.

3. Results

3.1. Effects of biocides and commercial preservatives

Lisianthus flowers placed in DI water lasted for 6 days (Table 1), and only two to three buds developed into flowers during that time. Of all the preservatives and biocides tested, only the solution of 1.5% sucrose in DI increased the vase life of the flowers, and that increase was only by 30%. The biocides 8-HQC and Physan[20] were phytotoxic, as evidenced by browning of the stem tissues, and similar symptoms were observed with all of the commercial preservatives. The end of vase life was associated with petal wilting

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and bending of the pedicels of buds and flowers. Solutions containing 1.5% sucrose alone, or in combination with aluminum sulfate or hypochlorite did not show the pedicel bending found in most treatments.

3.2. Effect of higher sucrose concentrations

When lisianthus flowers were pulsed with 6% sucrose for 24 or 48 hours and then placed in DI water, flower opening was considerably increased (Table 2). The pulse pre-treatments had no effect on longevity of the first opening bud (Table 2), but greatly increased the life of the next two (as much as 3-fold). Flowers that were held continuously in 6% sucrose performed as well as those pulsed for 24 or 48 hours with that concentration. Pulsing with 20% sucrose was ineffective and caused premature drying or yellowing of the leaves.

As the sucrose content of the vase solution increased, the postharvest performance and quality of the flowers changed dramatically (Fig. 1). The number of flowers opening per inflorescence and the rigidity of the pedicels increased significantly between 0 and 1.5% sucrose and did not increase further with increasing sugar concentrations. In contrast, the life of the first opened bud continued to increase with the sugar content of the vase solution, and was more than double that of control flowers in solutions containing 6% sugar. This increase was not reflected in life of the inflorescence which plateaued (at about 50% greater than that of controls) at a concentration of 3% sucrose. 3% sucrose. Pulse pre-treatments with high sugar concentrations also had a benefical effect on color (data not shown).

3.3. Effect of a biocide

When stems of lisianthus were placed in $Al_2(SO_4)_3$, a standard 'biocide' used in commercial preservatives, their life and opening was not significantly different from that of control flowers in DI (Table 3). The addition of sucrose greatly improved opening and vase life of the later opened flowers but $Al_2(SO_4)_3$ provided no significant additional benefit.

3.4. Fresh weight changes during opening and effects of sugar on opening.

In harvested inflorescences, the fresh weight of flower buds at different stages of opening t increased more than six-fold from the immature stage to fully open. The fresh weight of buds that were tagged at those same stages then allowed to develop in water or preservative for 18 days shows the dramatic effect of sugar on opening of lisianthus flowers. By 18 days, the flowers that had been open at harvest were senescent (as indicated by a low fresh weight) whether the inflorescences had been in DI or preservative.

Although there was some enlargement of buds on inflorescences held in DI, none of them achieved the weight of a fully open flower. The fresh weight of buds at all stages had increased in the inflorescences held in preservative, and even the buds that had been immature at harvest were starting to open after 18 days in the vase.

3.5. Sugar content of developing lisianthus flowers

Analysis of the sugar content of replicate buds and flowers from the treatments in Figure 4 demonstrated the presence of low concentrations of sucrose at all developmental stages, and a marked increase in hexose (particularly glucose) in the open flowers (Table 3). This pattern was repeated in the flowers analyzed after 18 days in the vase, by which time small buds had developed into open flowers which also contained high levels of glucose. Sugar content was considerably higher in the flowers that had been provided

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with a commercial preservative during their vase life, but the ratio of the three detectable sugars was almost identical to that of the open flowers on freshly harvested inflorescences.

3.6. Evaluation of possible sugar treatments

Any of the continuous or pulse treatments with sugars improved the number of flowers that opened on lisianthus inflorescences (Fig. 4). However, after 18 days in the vase there were significant numbers of open flowers remaining only for those treatments that included at least 3% sugar in the vase solution. Although pulse treatments improved flower opening and diameter of the first opened flower, the optimal postharvest performance was obtained from continuous treatment with vase solutions containing 3 or 6% sugar. Many flowers opened in the 1% sucrose vase solution, but the flowers were shorter lived that those opened in higher sucrose concentrations or any of the glucose concentrations.

3.7. Effects of ethylene and ethylene action inhibitors

Although there was no significant effect of pretreatment with the ethylene action inhibitors, STS or 1-MCP, on the life of lisianthus flowers placed in air in the vase-life room, these materials did significantly extend the life of the flowers that had been exposed to 1 μL.L-1 ethylene for 2 days at the start of their vase-life evaluation. The inhibitors improved the life of flowers exposed to ethylene by more than 50% (to ca. 13 days from 8 days).

4. Discussion

Our data support the suggestions of a number of previous workers that the postharvest life of lisianthus is greatly improved by providing sugars in the vase solution. Although we obtained some benefit from the pulsing treatment suggested by Halevy and Kofranek (1984), we found that the longevity of the flowers was further improved if sucrose was present continuously in the vase.

Unlike many other flowers, where 1.5% sugar gives maximal benefit, we found continued improvement to 3% or even 6% sugar. Both sucrose and glucose were effective, although our data suggest slightly superior performance with glucose. Unfortunately, we found that two of the biocides commonly used in some of the most popular commercial floral preservatives caused symptoms of phytotoxicity – browning of the stem and premature bent neck. $Al_2(SO4)_3$ and NaOCl did not cause these problems. Ichimura and Korenaga (1998) recommended 8-HQS as a biocide for lisianthus; presumably this salt did not cause the phytotoxicity that we saw with 8-HQC.

The opening of lisianthus flowers is associated with substantially increased concentrations of glucose in the corolla. The glucose probably serves to provide osmotic potential for the expansion of the petal cells, and the availability of soluble carbohydrate for that purpose is probably partly responsible for the improved opening of the flowers in preservative solution. Similarly, the availability of carbohydrate may be important for the synthesis of the lignin that is the likely reason that pedicels of sugar-treated flowers are stronger, and for the anthocyanins that are the basis for color in these flowers. Kawabata *et al.* (1996) found that flowers of lisianthus grown under reduced light intensity had paler color, and also showed that the inclusion of 0.5M sucrose in the vase solution improved color.

Although we did find a significant negative effect of ethylene exposure on the vase life of the flowers, inhibition of ethylene action by application of inhibitors did not appear to extend the life of flowers held in ethylene-free air. Ichimura *et al.* (1998) demonstrated a climacteric-like pattern of ethylene production by pistils, and petals

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during the senescence of lisianthus flowers. The modest response of the flowers to pretreatment with STS and 1-MCP suggests that inhibition of ethylene action is not warranted in commercial handling of lisianthus. Clearly, though, providing a vase solution with at least 3% sugar has a very significant effect on flower life and flower quality.

Acknowledgments

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Tables

1. Effects of various vase solutions (prepared in DI) on the postharvest performance of cut flowers of *Eustoma grandiflorum.*

Preservatives	*Days to damage*		*Number of opened flowers*	*Longevity (days)*[z]
	Stem browning	*Pedicel bending*		
DI[x]	15.0 a[y]	7.3 cd	2.7 bcd	6.0 b
Crystal Clear	8.5 c	13.0 b	2.3 bcd	7.5 ab
Oasis	9.6 b	8.8 c	3.3 abc	6.0 b
Chrysal	7.8 c	6.3 d	2.0 dc	6.3 ab
Rogard	8.0 c	9.0 c	1.7 d	6.0 b
50ppm bleach+1.5% S[a]	14.5 a	14.5 ab	3.2 abc	6.5 ab
20ppm Physan+1.5% S	9.5 b	7.0 d	2.3 bcd	6.1 ab
250 $mg \cdot L^{-1}$ 8-HQC+1.5% S	9.5 b	6.6 d	2.8 abcd	6.3 ab
200 $mg \cdot L^{-1}$ $Al_2(SO_4)_3$+1.5% S	15.0 a	15.0 a	4.0 a	7.5 ab
1.5% sucrose (S)	15.0 a	15.0 a	3.7 ab	8.3 a

[z]Days to wilting of 50% of the open flowers
[y]Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$
[x]DI : Deionized water
[a]S: Sucrose

2. Effects of pretreatments with different concentrations of sucrose on postharvest performance of inflorescences and individual flowers of *Eustoma grandiflorum.* The % of open flowers was determined after 18 days in the vase. All the treatments contained $Al_2(SO_4)_3$ at 200 $mg \cdot L^{-1}$

Treatments	*% open flowers*	*Longevity(days)*		
		1st flower	*2nd flower*	*3rd flower*
DI[x]	18.81 b[y]	4.67 ab	3.83 c	2.67 b
6% S for 24h then DI	36.27 ab	3.33 ab	9.33 ab	6.67 a
6% S for 48h then DI	37.82 a	4.50 ab	9.50 ab	7.17 a
6% Sucrose (S)	46.75 a	7.00 a	10.83 a	7.33 a
20% S for 24h then DI	18.89 b	1.00 b	5.00 bc	3.33 ab
20% S for 48h then DI	17.68 b	1.00 b	5.67 abc	2.00 b

[y]Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$
[x]DI : Deionized water.

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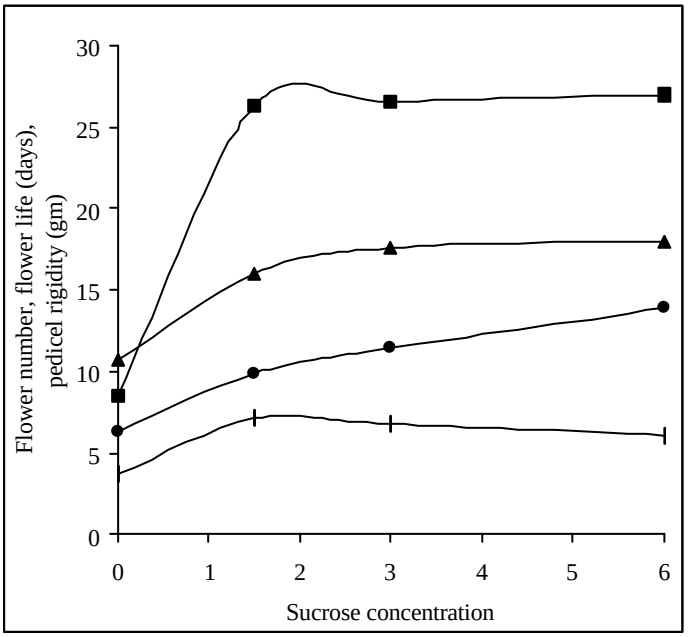
3. Sugar content of lisianthus flowers during development. Flowers at different developmental stages were analyzed after harvest, or after they had been in DI or a commercial preservative (CC) for 18 days.

Stage at harvest		*Petal sugar content (% fresh weight)*		
		Glucose	Fructose	Sucrose
At harvest				
Open flower		0.44±0.22[x]	0.07±0.00	0.21±0.12
Opening bud		<0.02	0.03±0.00	0.08±0.03
Large bud		<0.02	0.03±0.03	0.10±0.04
Small bud		<0.02	<0.02	0.09±0.03
Immature bud		<0.02	<0.02	0.17±0.06
After 18 days in DI	*Stage at Day 18*			
Open flower	Dead	<0.02	0.16±0.10	0.07±0.00
Opening bud	Dead	<0.02	0.04±0.02	0.02±0.01
Large bud	Dead	<0.02	0.03±0.02	0.01±0.00
Small bud	Opening	0.19±0.10	0.10±0.03	0.12±0.08
Immature bud	Wilting	<0.02	0.04±0.04	<0.02
After 18 days in CC				
Open flower	Dead	<0.02	0.67±0.22	0.14±0.08
Opening bud	Wilting	0.06±0.06	0.01±0.01	0.04±0.02
Large bud	Open	0.13±0.07	0.02±0.02	0.10±0.06
Small bud	Open	0.30±0.04	0.04±0.00	0.14±0.05
Immature bud	Opening	0.40±0.22	0.07±0.00	0.19±0.14

[x] Data are the means of three replicate flowers or buds

Figures



1.Effect of increasing sucrose concentrations in the vase solution on the postharvest performance of Lisianthus. Flowers were held in solutions containing 200 $mg \cdot L^{-1}$ $Al_2(SO_4)_3$ and different concentrations of sucrose and we determined the number of flowers that opened ( ), life of the first opening bud ( ), life of the inflorescence determined as days to senescence of half of the opened flowers ( ), and rigidity of the pedicels on each flower after 21 days in the vase ( ).

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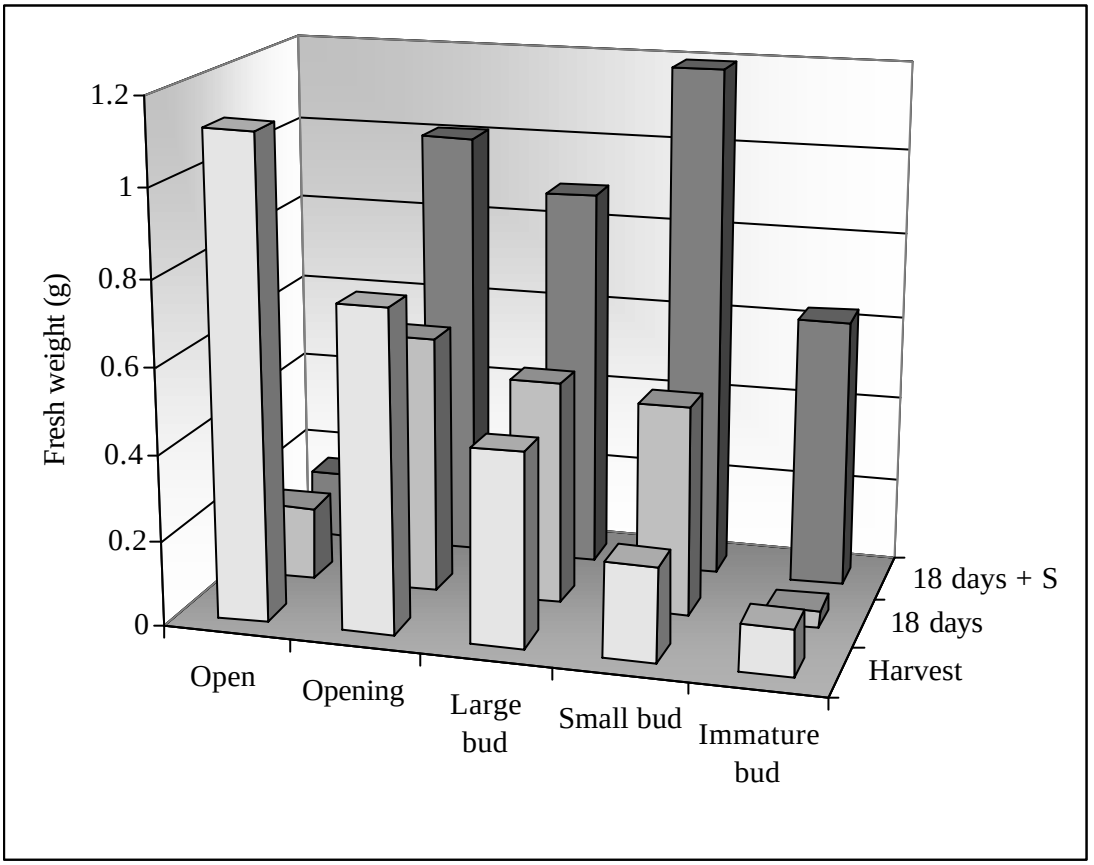

2. Fresh weight of different stages of lisianthus flowers at harvest and of tagged replicates of the different stages after 18 days in DI or in a commercial preservative.

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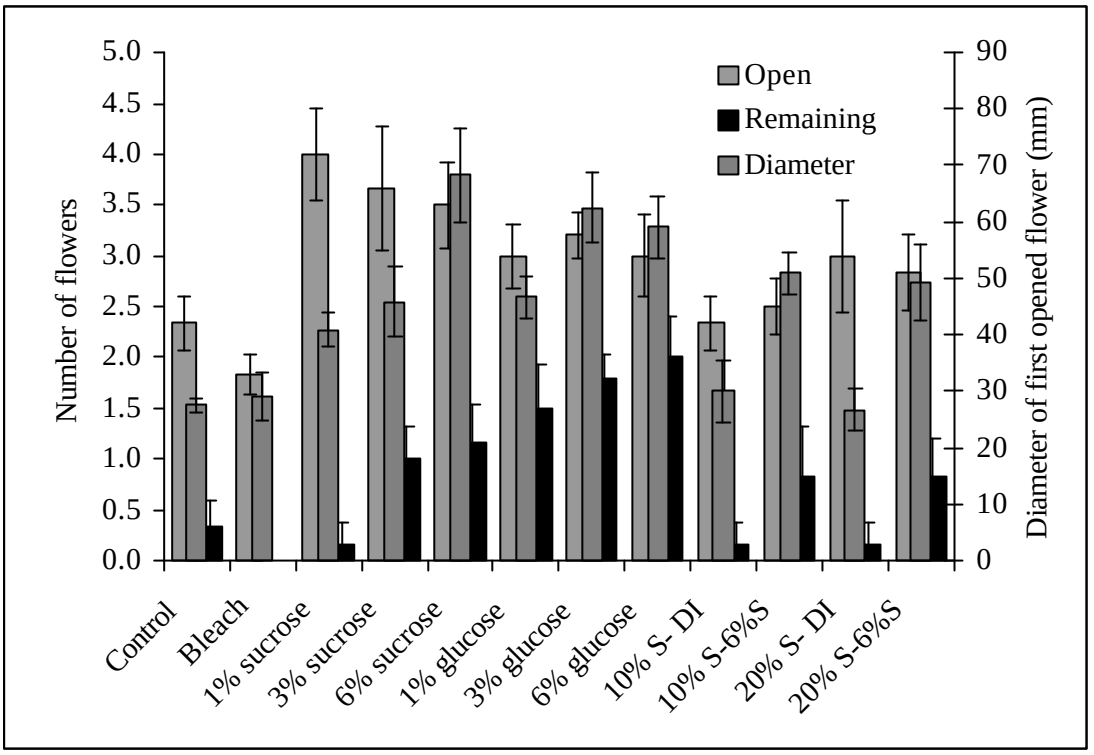
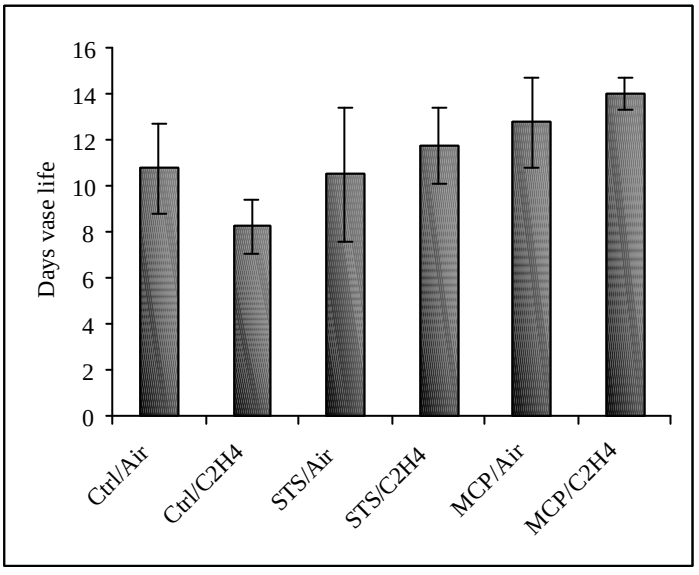


3. Effect of different pulsing and vase solutions on the postharvest performance of lisianthus flowers. Flowers were either placed directly in vase solutions, or pulsed first for 24 hours with different concentrations of sucrose. Postharvest quality parameters measured included diameter of the first bud after it opened, number of flowers remaining at day 18, and number of flowers opened on each inflorescence. Means of 6 flowers per treatment; error bars show the SD.



4. Effect of pretreatment with STS or 1-MCP and/or exposure to ethylene on the vase life of lisianthus flowers.

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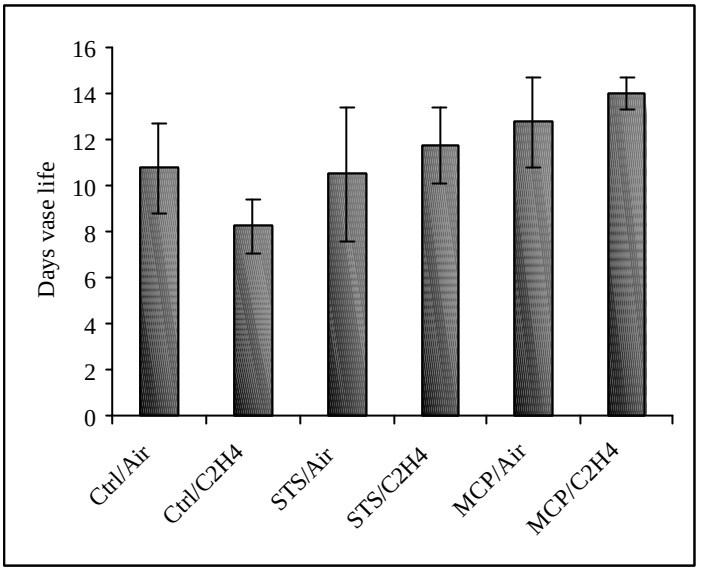

5. Effect of pretreatment with STS or 1-MCP and/or exposure to ethylene on the vase life of lisianthus flowers.

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