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Effects of Postharvest Treatments with Nanosilver on Senescence of Cut Lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) Flowers

Ewa Skutnik *, Aleksandra Łukaszewska and Julita Rabiza-Świder

Section of Ornamental Plants, Institute of Horticultural Sciences, Warsaw University of Life Sciences, Nowoursynowska 166, 02−787 Warszawa, Poland; aleksandra_lukaszewska@sggw.edu.pl (A.Ł.); julita_rabiza_swider@sggw.edu.pl (J.R.-Ś.)

* Correspondence: ewa_skutnik@sggw.edu.pl; Tel.: +48-225932271

Abstract: Lisianthus is among the most popular cut flowers. Regarding the postharvest losses, these experiments were designed to compare the effects of a nanosilver (NS) based preservative to the standard preservative containing 8-hydroxyquinoline citrate (8-HQC) and sucrose (S). Additionally, the effect of 24 h conditioning in the NS solution on the postharvest longevity and the general condition of lisianthus (*Eustoma grandiflorum* 'Mariachi Blue') was tested. The vase life of flowers on conditioned and non-conditioned stems was extended by the preservatives, more so by NS + S than by 8-HQC + S (44–54% *versus* 13–23%). Conditioning had no detectable effect on longevity. Daily water uptake showed alternative peaks and drops, with a general tendency of the uptake rate to decrease over time. The highest uptake intensity and the highest transpiration rate were in stems in the NS + S solution while the lowest was in 8-HQC + S. Conditioning negatively affected the average fresh weight of the flowering stems in all holding solutions with stems in preservatives being heavier than those in water. Preservatives did not induce accumulation of the total soluble or reducing sugars in petals; such accumulation was promoted by conditioning, but only in the upper flowers. The free proline content increased in senescing lower flowers on non-conditioned stems; conditioning limited this increase in flowers in preservatives. In the upper flowers, free proline increased in both water controls while the preservatives and conditioning generally reduced the proline contents below the initial level. Conditioning lowered the hydrogen peroxide contents in senescing lower flowers, relative to the initial level and the non-conditioned stems. The catalase activity kept dropping during the vase life in both the lower and upper flowers, in conditioned and non-conditioned stems, with the exception of flowers from water where the activity remained the highest from all three treatments. It appears that the NS preservative with sucrose improves the overall condition of lisianthus flowers and extends their vase life.

Keywords: longevity; conditioning; flower preservatives; water balance; soluble carbohydrates; free proline; oxidative stress

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1. Introduction

Lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) has become one of the most popular cut flowers. With its multi-flower stems, single, semi-double, and double rose-like flowers in a variety of colors, it is attractive for consumers worldwide. Its postharvest longevity varies between cultivars, ranging from five days [1], to eight or nine days [2,3] and up to 28 days [4]. However, as each stem bears both fully open flowers and closed buds, it is not always clear how the reported vase life lengths were evaluated. As the structure of the lisianthus "cut flower" is complex, it is difficult to develop a postharvest treatment that would simultaneously delay senescence of the open flowers and promote bud opening. Studies on senescence and postharvest treatments of lisianthus have been carried out for

over two decades to improve its keeping qualities: the vase life, bud opening, and petal coloration [5–7].

The longevity of cut flowers is shortened by several factors: disruption of the water uptake and transport, due mainly to microbial vessel blockage; depletion of the respiratory substrate, which limits the energy available to sustain life processes; and harmful effects of the reactive oxygen species (ROS) emerging during the oxidative stress after the flowering stems are detached from mother plants as well as the senescence-accelerating ethylene action in the ethylene-sensitive species [8]. All these factors have been found to play a role in lisianthus. Reports on biocides capable of prolonging the lisianthus vase life by assuring uninterrupted water transport in cut stems include 8-hydroxyquinolite citrate [3,4,9–11], aluminum sulfate [2], sodium hypochlorite [7,9], silver nitrate [9], nanosilver [12,13], nanoparticles of silicon and silver [14], and peracetic acid [15]. All of these efficiently limit the microbial population in vase water and prolong the vase life, especially when applied with sucrose.

Exogenous sucrose is indispensable for most cut flowers as a respiratory substrate. Cut flowers are able to absorb and metabolize sugars from vase water to provide energy required to support life and to open flower buds [8,16]. Sucrose is mostly used as a flower food and for lisianthus, it is effective in concentration between 2% and 6% [10,14], but glucose and fructose can also be used [10]. Sugar accumulation in the vacuoles reduces the osmotic potential, thus enhancing water influx into petals and promoting their expansion during bud opening [10].

Ethylene is involved in the flower senescence of lisianthus [17]. Treatments with inhibitors of the ethylene action such as silver thiosulphate (STS) [17], 1-methylcyclopropene (1-MCP), or salicylic acid (SA) [18] extended flower longevity. A positive effect of acetaldehyde on decreasing ethylene synthesis and extending lisianthus longevity was reported by Seighalani et al. [19], and that of silver nanoparticles by Kiamohammadi [14]. According to Asil and Karimi, [20] cytokinin benzyladenine (BA) also improved lisianthus longevity by delaying ethylene production. The enhanced resistance to ethylene may also be related to the action of exogenous sucrose [5].

Numerous results suggest that chemicals such as polyamines [21] or SA and sucrose [3,22] can increase the vase life of lisianthus cut flowers by improving the antioxidant system and reducing the damage caused by the oxidative stress during senescence.

The aim of this work was to evaluate the effect of conditioning lisianthus flowering stems with nanosilver and to assess the efficiency of the NS-based preservative *versus* the standard preservative containing 8-HQC in improving longevity, water balance, and senescence-related processes in petals of *Eustoma*. Silver is a strong antibacterial agent, which nowadays is applied in a form of nanoparticles. According to Damunpola and Joyce [23], nanosilver (NS) interacts with bacteria membranes and destroys them. As it has relatively little toxicity to the environment, it has become a common substance used for postharvest treatments of cut flowers [24].

2. Material and Methods

Bifurcated flowering stems of *Eustoma grandiflorum* (Raf.) Shinn. 'Mariachi Blue', each bearing three flowers/buds on each of the two side shoots (Figure 1), were harvested from mother plants and trimmed to 60 cm in length. The stems were stripped off excessive leaves, leaving only the upper pairs of leaves. One half of the stems were placed directly into calibrated cylinders (volume 0.5 dm^3) with the following holding aqueous solutions: 200 mg dm^{-3} 8-hydroxyquinoline citrate with 2% sucrose (8-HQC + S), 1 mg dm^{-3} nanosilver with 2% sucrose (NS + S), and distilled water serving as a control. NS was obtained as a commercial preparation (Altermedica Laboratories, Żywiec, Poland). The other half of the stems were conditioned for 24 h in 5 mg dm^{-3} nanosilver solution and then placed into the same holding solutions as above. Each treatment contained 10 stems, individually tagged, in individual cylinders, and treated as single replicates. The preservatives and

water were not exchanged during the experiment, but they were replenished when the holding solution level dropped to 5 cm.

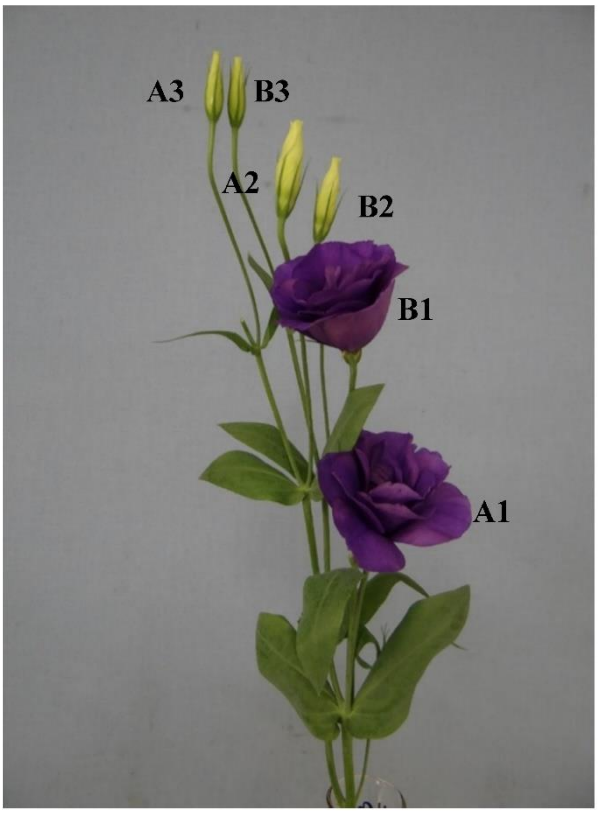

Figure 1. Flowering stem of lisianthus 'Mariachi Blue'.

The experiment was carried out in a room with controlled temperature of 20 ± 1 °C, a relative humidity of 60%, and quantum irradiance of 35 µmol m^{-2} s^{-1}, under the 12 h day/12 h night regime; the 24 h conditioning with NS was performed under the same conditions.

The vase life was expressed as a number of days elapsed between placing flowers into solutions (conditioning or holding) and the loss of decorative value of each flower in an inflorescence such as petal withering or peduncle drying and bending. Longevity of the youngest flower should be regarded as a total vase life of the whole flowering stem.

The water balance parameters were determined in a separate experiment where flowering stems were placed individually into 250 mL cylinders, 10 stems per each treatment.

Water uptake (a drop in solution volume) was measured on each day of the experiment and expressed in mL. The fresh weight of flowers was taken daily after solutions had been replenished, and expressed in grams (g). The transpiration rate was expressed as the difference between water uptake and weight change, and expressed in gram per stem per day.

Samples for all biochemical analyses were collected from a separate batch of flowers immediately after harvest (Day 0), and Days 9 and 20 after harvest, separately from the lower (open at harvest) and upper (buds at harvest) flowers (Figure 1). For each analysis, four flowers were sampled from each treatment. The petals were finely cut, mixed, and three samples of 0.5 g each were weighed. Three extracts were prepared and three aliquots were taken from each extract, giving a total of nine readings for each data point. Additionally, three samples were taken for the dry weight (DW) measurements: plant material was dried at 105 °C until constant weight, as in Strzelecka et al. [25].

Total soluble sugars were measured as described by Dubois et al. [26] and expressed in mg glucose per g of DW. The material was homogenized in 80% ethanol (Chempur, Piekary

Śląskie, Poland). The extracts were incubated for 20 min in a boiling water bath with 5% phenol (Chempur, Piekary Śląskie, Poland) and 96% H_2SO_4 (Stanlab, Lublin, Poland), and the extinction was measured spectrophotometrically (Shimadzu UV-1800, Japan) at 490 nm. The total sugar content was calculated from a previously plotted standard curve, prepared for glucose.

The reducing sugars content was measured by the Somogyi method as modified by Nelson [27] and expressed in mg glucose g^{-1} DW. The material was homogenized in 80% ethanol (Chempur, Piekary Śląskie, Poland). The extracts were incubated for 20 min in a boiling water bath with the copper reagent; the molybdenum arsenic reagent (Merck, Darmstad, Germany) was added and the extinction was measured at 520 nm. The amounts of reducing sugars were calculated from a previously plotted standard curve, prepared for glucose.

The free proline content in petals was tested according to Bates et al. [28] by measuring the quantity of a colored reaction product of proline with ninhydric acid (Sigma, Saint Louis, USA). The absorbance was read at 520 nm. The amount of proline was calculated from a previously plotted standard curve and expressed in µmol g^{-1} of DW.

The petal hydrogen peroxide (H_2O_2) content was measured spectrophotometrically after the reaction with potassium iodide (KI) (Sigma, Saint Louis, USA) as described by Jędrzejuk et al. [29] and expressed at 390 nm as µg of hydrogen peroxide per gram on a DW basis.

The catalase (CAT) activity (EC 1.11.1.6) was determined spectrophotometrically as the rate of H_2O_2 disappearance at 405 nm according to Goth [30] and expressed as mkatals per gram DW.

Results were statistically evaluated by ANOVA 1 or ANOVA 2 using IBM SPSS Statistics program (SPSS, Poland). Duncan's test at $\alpha = 0.05$ was applied to assess the significant differences between the means.

3. Results

3.1. Vase Life

The vase life was determined for each of six flowers situated parallelly on two side shoots (A and B) of the bifurcated flowering stem (Table 1). Both factors, the position of a flower on the stem and the treatment, significantly affected flower longevity (Table 1).

Table 1. The effect of the postharvest treatments on the vase life of cut lisianthus 'Mariachi Blue' flowers.

Treatment	Vase Life (Days)						Mean for Treatment
	A1	A2	A3	B1	B2	B3	
water	12.1 a [1]	19.0 fg	20.0 fghi	10.9 a	18.2 ef	21.9 jkl	17.0 a [2]
8-HQC + S	17.6 de	22.0 jk	24.8 mn	15.2 cd	22.2 jk	23.3 klm	20.9 b
NS + S	15.4 cd	30.2 p	33.1 r	16.9 de	27.3 o	34.0 s	26.2 c
NS/water	12.8 ab	19.1 fg	20.7 ghij	12.1 a	19.1 fg	21.8 jk	17.6 a
NS/8-HQC + S	15.9 cd	21.8 ijk	19.8 fgh	16.0 cd	21.1 hij	24.2 lm	19.8 b
NS/NS + S	14.4 bc	26.3 no	34.9 s	17.0 de	26.7 no	32.6 pr	25.3 c
Mean for Flower	14.7 a [3]	23.0 b	25.6 c	14.7 a	22.4 b	26.3 c	

[1] All means within the table followed by the same letter do not differ significantly at $\alpha = 0.05$. [2] Means in the column followed by the same letter do not differ significantly at $\alpha = 0.05$. [3] Means in the line followed by the same letter do not differ significantly at $\alpha = 0.05$.

The lower flowers (numbered 1, Figure 1) on both side shoots A and B, open at harvest, lasted in water for 11–12 days while the upper buds (2 and 3) lasted significantly longer, 18–22 days; the latter included the time to open the buds. Generally, both preservatives prolonged the vase life of the first flowers on the side shoots of the non-conditioned and conditioned stems. Additionally, the longevity of the second and third flowers on both conditioned and non-conditioned stems were increased by the preservatives, with NS + S being more effective than 8-HQC + S. Conditioning did not increase the longevity of the second and third flowers from flowering stems held in water or in the standard

preservative, and even reduced it in the A3 flower in the latter treatment by 25% relative to non-conditioned stems. In flowers on stems held in the NS + S solution, conditioning reduced longevity (A2, B3), increased it (A3) or had no effect (B2).

3.2. Water Balance

The water uptake rate during vase life is shown on Figure 2A. The daily volume of absorbed solution started to differ after only one day, and ranged between 10.4 (water) to 16.6 g (NS + S) per stem. On Day 1, the stems after conditioning absorbed less holding solution than the non-conditioned stems, and this pattern continued for the entire duration of the experiment for stems held in the NS solution. On the other hand, conditioned stems placed into water or the preservative solution absorbed more or equal amounts of the vase solutions than the non-conditioned stems. The intensity of daily water uptake showed alternative peaks and falls, observable every 2–3 days with a general tendency of the uptake rate to drop. The maximum water uptake was 21.5 g per stem (Day 3) and it dropped to 2.0–4.9 g on Day 27. Generally, the highest uptake intensity was noted for stems placed into the NS + S solution (with or without conditioning), while the lowest was for the stems in the standard preservative.

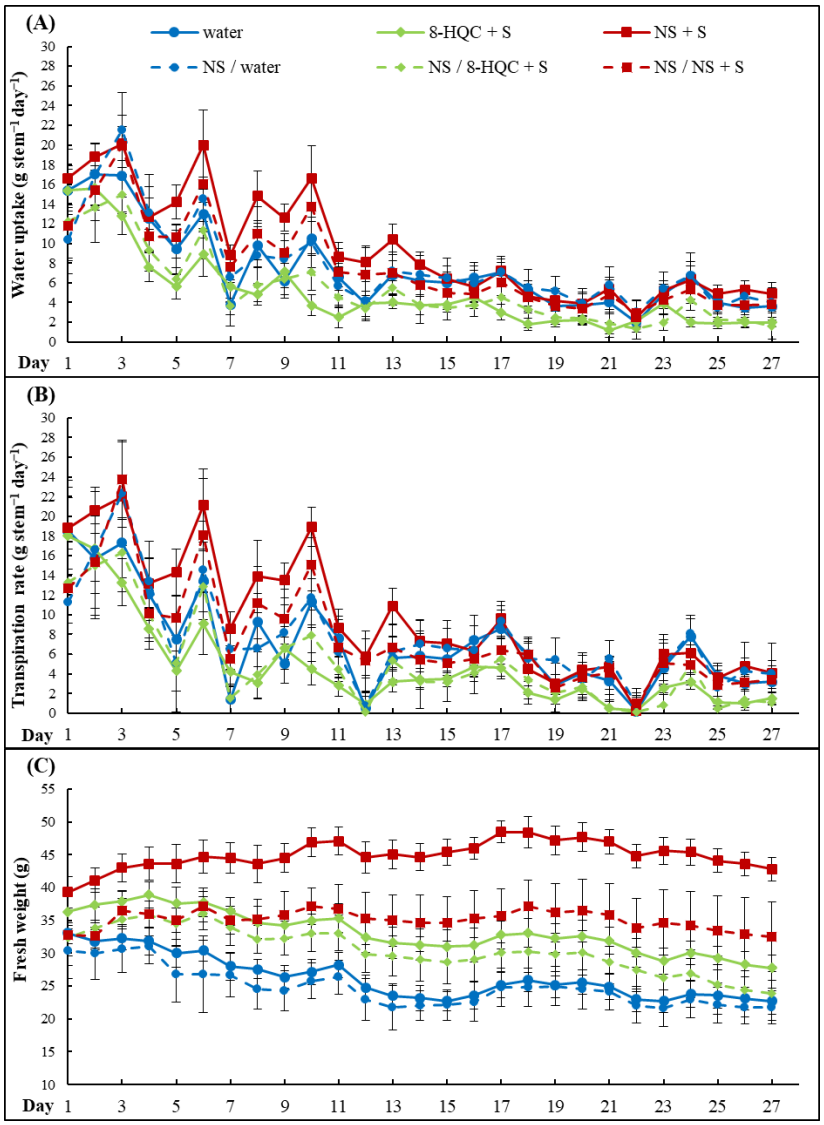

Figure 2. The effect of preservatives on the water balance in cut lisianthus flowers. Water uptake (**A**), transpiration rate (**B**), and fresh weight (**C**) of cut lisianthus flowers.

The transpiration rates are shown in Figure 2B. On Day 1, the transpiration of non-conditioned stems was higher than that in the conditioned stems, ca. 18–19 g *versus* 11–13 g per stem. The intensity peaked on Day 3, being the highest in conditioned stems in water and in NS + S (22–24 g per stem). The general tendency of the transpiration rate was comparable to that of the water uptake, with peaks and drops every few days, and a gradual reduction in all treatments over time. The volumes of water loss were the lowest in conditioned and non-conditioned stems into the standard preservative. The amount of transpired water on the last day ranged between 1 g (8-HQC + S) and 4 g (NS + S) per stem.

Changes in the fresh weight of stems during the vase life are shown in Figure 2C. They were gradual and devoid of dramatic day-to-day changes. Stems kept in water started losing weight after 4–5 days. Their weight dropped by 25–30% during 27 days, to ca 23 g at the end of experiment, less than that in stems in preservative solutions. Stems in the 8-HQC + S solution had lower fresh weight after 27 days relative to the initial value, but started losing weight later than those in water, after 11–12 days. Stems in the NS + S solution maintained their fresh weight at higher levels than at the beginning of the experiment, which was apparent, especially in the non-conditioned stems, where fresh weight increased by 30%. Generally, all conditioned stems had lower fresh weights than the non-conditioned stems held in the same solutions.

The average values of the water balance parameters calculated from 27 daily measurements are shown in Table 2.

Table 2. The effect of preservatives on the water balance parameters in cut lisianthus 'Mariachi Blue' flowers.

Treatments	Water Uptake (g stem^{-1} day^{-1})	Transpiration Rate (g stem^{-1} day^{-1})	Fresh Weight (g)
water	7.37 c [1]	7.09 b	26.46 b
8-HQC + S	4.95 a	4.73 a	33.22 d
NS + S	9.50 e	9.71 d	44.63 f
NS/water	7.80 d	7.52 bc	24.98 a
NS/8-HQC + S	5.30 b	5.03 a	30.48 c
NS/NS + S	7.71 d	7.73 c	35.00 e

[1] Means in each column followed by the same letter do not differ significantly at p = 95%. Values are expressed as the means of 27 days after harvesting.

The average daily water uptake was the lowest in stems into the standard preservative, both in conditioned and non-conditioned stems, and a similar tendency was observed in the average transpiration values. However, the lowest average fresh weight was in stems held in water, while stems from the standard preservative had intermediate weight between that from water and the NS + S solution. The highest weight was in conditioned and non-conditioned stems in NS + S. There was a clear negative effect of conditioning on the flower stem weight in all three holding solutions. The same was true for the uptake and transpiration in stems in NS + S, where conditioning reduced both parameters by nearly 20%.

3.3. Soluble Carbohydrates

Changes in the total soluble sugars in the lower flowers are shown in Figure 3A. Both the date and the treatment significantly affected the sugar contents. It increased during 20 days of the vase life in flowers from non-conditioned stems by over 20% over the initial value; after conditioning, some sugar losses were observed at the end of the vase life by 10% in flowers held in water and by 20% in those from the standard preservative. A small (6%) but significant increase in the sugar level was observed in conditioned flowers placed into the NS + S solution. The highest sugar accumulation occurred on Day 9 in flowers on stems that held the NS + S: 41% and 33% relative to Day 0 in the conditioned and non-conditioned flowers, respectively.

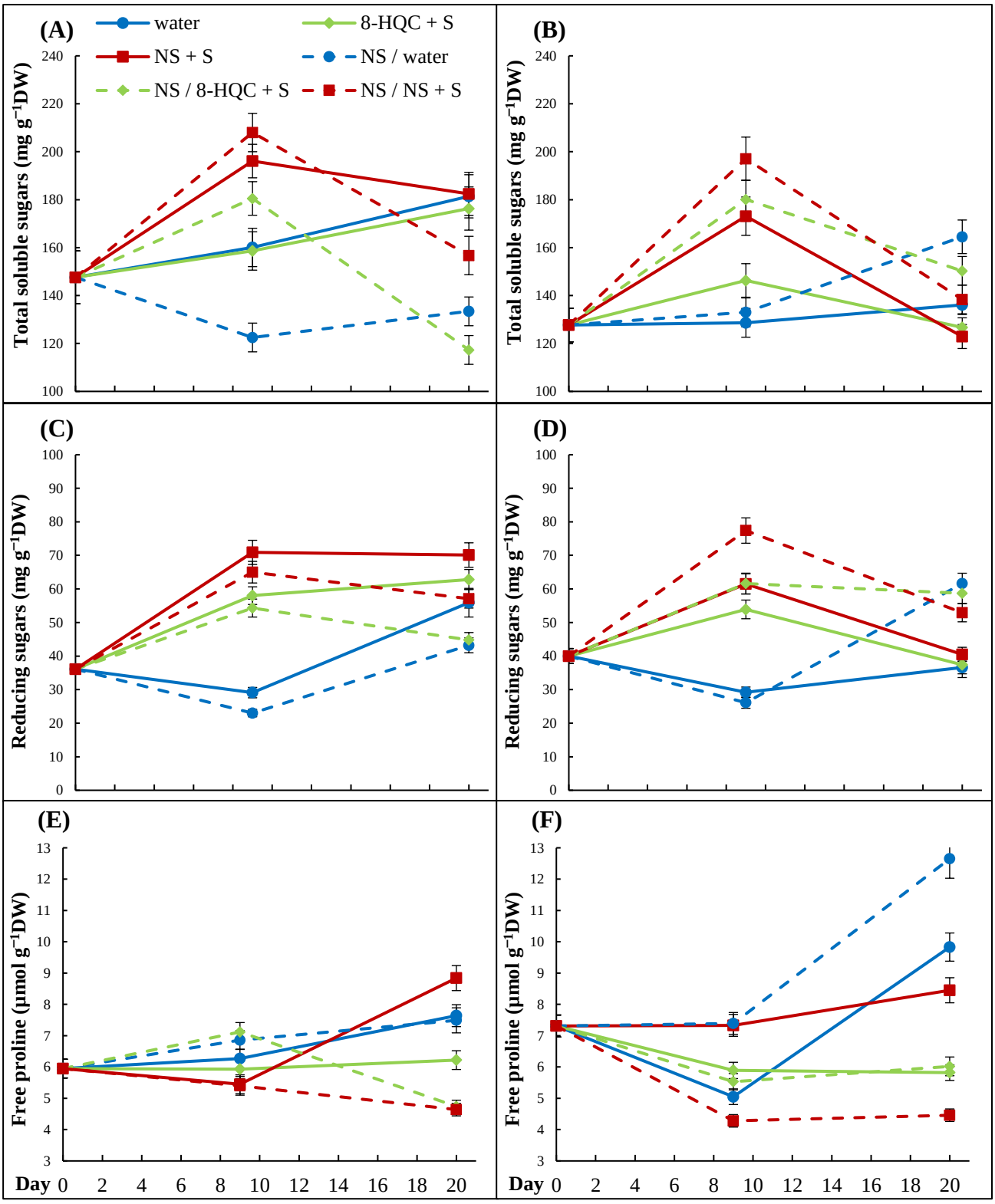

Figure 3. Total sugar contents (**A**,**B**), reducing sugars contents (**C**,**D**), and free proline contents (**E**,**F**) in the lower (**A**,**C**,**E**) and the upper (**B**,**D**,**F**) flowers of cut lisianthus. Values are expressed as the mean ± SD. Vertical bars represent standard deviations of the means.

Changes in the total soluble sugars in the upper flowers are shown in Figure 3B. Both the date and the treatment significantly affected the sugar contents. The initial sugar level in the upper flowers was 13% lower than that in the lower ones. On Day 9, the sugar

contents increased relative to the initial level in non-conditioned and conditioned flowers held in both preservatives, and this increase was more pronounced in flowers from NS + S than from 8-HQC + S. The total soluble sugar contents on Day 20 did not differ significantly from that on Day 0 in flowers from non-conditioned stems, but it increased in all treatments after conditioning, with the highest in flowers held in water by 38% compared to the initial value. The highest sugar accumulation was observed on Day 9 in the conditioned flowers placed into the NS + S solution where it reached 154% of the initial level.

Changes in the contents of reducing sugars in the lower flowers are shown in Figure 3C. Both the date and the treatment significantly affected the parameter. In all cases, the levels were higher on Day 20 than on Day 0. In flowers from the non-conditioned stems held in water, the amount of reducing sugars doubled in 20 days of the vase life while in those kept in both preservatives, it increased 2.3 and 2.6 times for 8-HQC + S and NS + S, respectively. Conditioning lowered the reducing sugar levels in flowers from all three vase solutions even though they were still higher than on Day 0. Here, only the flowers held in NS + S solution had significantly more sugars than those held in water.

Changes in the contents of reducing sugars in the upper flowers are shown in Figure 3D. Again, both the date and the treatment significantly affected the parameter. The initial level was 46% higher than in the lower flowers. On Day 9, a drop in reducing sugars was noted in both flower batches (conditioned and non-conditioned) standing in water while their levels increased in flowers held in the preservatives, more so in flowers in NS + S—over 90% and 50% relative to the initial level in the conditioned and non-conditioned stems, respectively. On Day 20, the reducing sugar levels in flowers on the non-conditioned stems were similar as on Day 0, while in conditioned flowers from all three vase solutions, the sugar accumulation was observed, producing values 32 to 54% higher than on Day 0.

3.4. Free Proline

Both the date and the treatment significantly affected the free proline content in the lower flowers (Figure 3E). On Day 9, the amino acid content did not differ from the initial level in non-conditioned flowers from all three solutions, while on Day 20, the levels increased in flowers from water and NS + S. The conditioned flowers from water and the standard preservative had more free proline on Day 9 than those held in NS + S, and significantly more than on Day 0. On Day 20, the free proline contents further increased in flowers on stems held in water while in those kept in the preservative solutions, it dropped to below the initial level.

Changes in the contents of free proline (μmol per g DW) in the upper flowers on flowering stems are shown in Figure 3F. At the start of the vase life, the upper flowers contained over 20% more free proline than the lower ones. Compared to the initial level, in flowers on stems held in water—both from conditioned and non-conditioned stems—the increase in free proline occurred on Day 20 by 73% and 34%, respectively. In flowers from stems placed into the preservative solutions, the proline contents either remained unchanged or dropped by nearly 40% in flowers from the conditioned stems held in NS + S.

3.5. Hydrogen Peroxide

Changes in the hydrogen peroxide content as affected by the postharvest treatments are shown in Figure 4A. On Day 9, it increased from 1414 μg g^{-1} DW to ca. 1700–1800 μg g^{-1} DW in flowers on non-conditioned flowering stems kept in the preservative solutions, while a small reduction was observed in the control. A more pronounced reduction—by 20%—was observed in control flowers on conditioned stems. In turn, in flowers from conditioned stems in the preservative solutions, a rise in the H_2O_2 level was observed, up to 145% of the initial value in NS + S. On Day 20, non-conditioned flowers had more hydrogen peroxide than immediately after harvest. Its highest level, 125% of the initial level, was observed in flowers from NS + S. On that date, the H_2O_2 levels in flowers

on conditioned stems from all three treatments were lower than the initial values, with the lowest, 73% of the initial level, in water-held flowers.

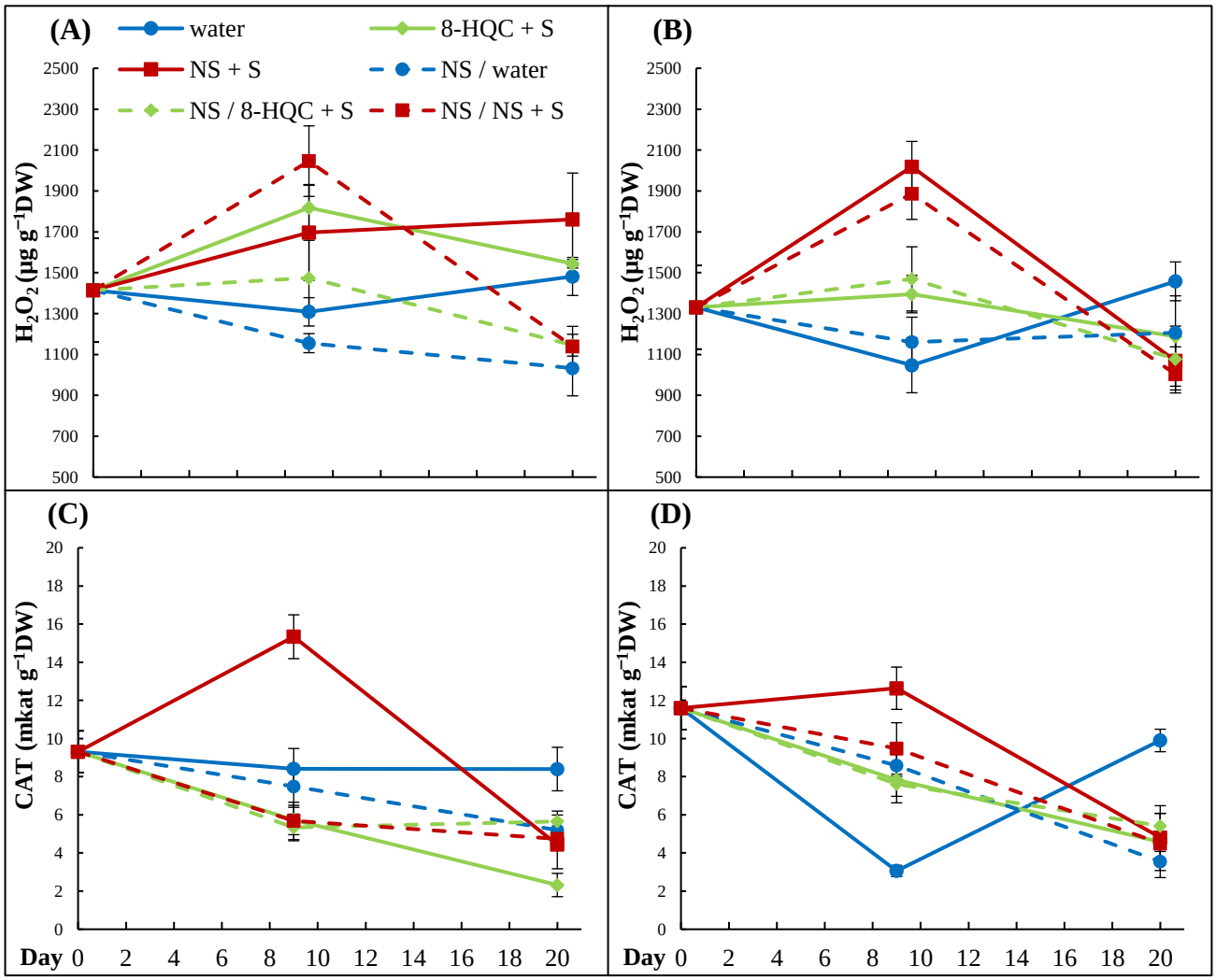

Figure 4. Hydrogen peroxide (H_2O_2) content (**A**,**B**) and catalase (CAT) activity (**C**,**D**) in the lower (**A**,**C**) and the upper (**B**,**D**) flowers of cut lisianthus. Values are expressed as the mean ± SD. Vertical bars represent standard deviations of the means.

Figure 4B shows changes in the H_2O_2 contents in the upper flowers. The initial content was comparable to that in lower flowers, and the general pattern of changes was similar. On Day 9, after a drop in flowers from conditioned and non-conditioned stems held in water, the hydrogen peroxide levels increased in the non-conditioned flowers and remained almost unchanged in the conditioned ones. In non-conditioned flowers in the standard preservative, a small reduction was observed on Day 9, and the levels dropped further on Day 20. In flowers on conditioned stems in the standard preservative, after a small increase on Day 9, the H_2O_2 level dropped on Day 20 to the value below that of non-conditioned flowers. In flowers on stems from conditioned and non-conditioned batches kept in NS + S, the hydrogen peroxide levels increased by 40–50% on Day 9, followed by a dramatic drop on Day 20 to the levels below the initial value, and the values for flowers from the other two treatments.

3.6. Catalase

Changes in the CAT activity in lower flowers are illustrated in Figure 4C. Generally, it was lower at the end of the vase life than immediately after harvest. In flowers on the water-held stems, the activity dropped during the vase life in both conditioned and non-

conditioned stems by 44% and 10%, respectively. This drop was even more pronounced in non-conditioned flowers placed into the standard preservative where on Day 20, it was only 25% of the initial value. In flowers from conditioned stems in 8-HQC + S, the CAT activity was more than double that of non-conditioned stems. In flowers from both batches held in NS + S, this reduction in the CAT activity was similar, 52–41% relative to the initial value. CAT showed an unexpectedly high activity on Day 9 in flowers on non-conditioned stems in NS + S, 165% of the initial activity.

Changes in the CAT activity in the upper flowers are presented in Figure 4D. The initial value was 25% higher than in the lower flowers, but the pattern of changes was similar: on Day 20, the activity was lower than at the beginning of the vase life, followed by a drop to 85–30% of the initial activity. There was little difference in the effects of preservatives on the CAT activity, but in conditioned flowers from water, the levels dropped by 70% compared to 15% in non-conditioned flowers. Additionally, in upper flowers, a stimulation of CAT activity by NS + S was evident on Day 9 in non-conditioned flowers, but this increase was not as dramatic as that in the lower flowers.

4. Discussion

Multi-flowered lisianthus (*Eustoma grandiflorum*) stems are very showy so there is a growing demand worldwide for cut flowers, with cultivars from the Mariachi group considered particularly desirable. As the structure of the lisianthus "cut flower" is complex, it is difficult to design a postharvest treatment that on one hand would delay senescence of the flowers already opened, while on the other, would promote bud opening. Moreover, as each cut lisianthus stem bears fully open flowers and closed buds, it is not always clear how to evaluate the vase life length with considerable confusion in the literature. Bahrami et al. [3] did not list the number of open flowers at harvest and considered the vase life ended when 50% of all flowers on a stem had wilted. Islam et al. [4] used stems with three open flowers and several buds (without providing exact numbers), and also adopted the criterion of 50% wilted flowers. In the "half open stage', the flowering stems were cut for the experiment of Kazemi et al. [31] terminated when 50% flowers had wilted. Similarly, Ichimura [5], Huang and Chen [16] "recorded the vase life as a number of days from the end of pulse treatment until the when the last flower senesced". However, again, no number of flowers per stem has been specified. For this study, we harvested flowering stems with one flower *per* shoot open, reduced the bud number to two on each of the two side shoots, and evaluated the longevity of individual flowers.

Despite some confusion as to the exact state of the experimental material, all cited authors showed that a sugar combined with biocides, 8-HQS or 8-HQC, was an important factor in prolonging the vase life of lisianthus while the biocides alone were ineffective. This suggests that the microbial presence in vase water is a minor problem for cut lisianthus, and vessel blockages do not heavily obstruct water uptake and reduce the vase life. Such a conclusion was indeed drawn by Islam et al. [4]. However, there are numerous reports on the positive effects of different biocides on the lisianthus vase life. Recently, a positive effect of silver nanoparticles in a concentration of 40 mg dm^{-3} was reported by Kamiab et al. [1], especially when combined with silicon nanoparticles and 4% sucrose. That mixture eliminated the microbial population and prolonged the vase life from five to 17 days. However, we consider such a concentration of silver nanoparticles in the holding solution as unnecessarily high, even though Jowkar et al. [32] used NS at a similar range of concentrations (10–50 mg dm^{-3}) as a biocide for 'Cherry Brandy' roses. Very high NS concentrations—75 and 125 mg dm^{-3}, with 2% sucrose—were tested as optimal for roses 'High and Magic' [33]. Lü et al. [12] used even higher concentrations of NS—50–250 mg dm^{-3}—for 1 h pulsing of 'Movie Star' roses, but the high end concentration turned out to be phytotoxic. We set out to compare the effectiveness of nanosilver against 8-HQC, which is a component of the long-known standard preservative [8] and to test its effectiveness as a conditioner. However, just like Liu et al. [34], who used 5 mg dm^{-3} NS for conditioning gerberas, the concentrations we applied were much lower than those high

ones listed above: 5 mg dm^{-3} nanosilver for conditioning and 1 mg dm^{-3} as a component of a vase solution. We were inspired by the fact that even lower NS concentrations—0.5 mg dm^{-3}—extended the vase life of 'Movie Star' roses [13]. This is in line with now common tendency to use chemicals in the lowest concentrations possible, both for economic and environmental reasons.

Depending on the treatment, the longevity of the oldest flowers on our test stems ranged from 12 to 16 days while that of the youngest flower from 18 to 35 days. The latter included time to open the bud. Conditioning as such did not affect the longevity of individual flowers, but longevity was significantly increased by both preservatives containing 2% sucrose with nanosilver being more effective than 8-HQC + S. The overall mean for the vase life in NS + S for all six flowers/buds was 54% and 44% higher than for water controls in non-conditioned and conditioned stems, respectively, while such values for 8-HQC + S were 23% and 13%.

Numerous reports showed that exogenous sugar is indispensable for sustaining lisianthus flower life, both as a respiration substrate [16] and as an osmolyte that promotes water influx into expanding petals, thus enhancing bud opening [35]. The number of open buds on stems held in sucrose solutions was always higher than those in solutions without sugar [15,36]. With sucrose present in a holding solution, the reported increase in vase life was from 15% [15] to 340% [1]. Even a short 18 h pulse of a solution with 5% sucrose resulted in a 4.5 day longer vase life [3].

As sucrose is a transportable form of carbohydrates within lisianthus stems [35], it has mainly been used to extend the vase life, and generally it is more effective than glucose or fructose [10]. The reported sucrose concentrations in holding solutions for lisianthus range between 2% [4,10,17] and 6% [14], while 5% has been used for 18 h conditioning [3].

When sugar is transported from a vase solution to flower, it accumulates in leaves, causing severe damage, so a general rule is to use sucrose concentrations lower than 2% in leafy cut flowers such as roses, lilies, or alstremerias [8]. To avoid potential leaf toxicity, we used 2% sucrose in both preservatives under study and did not observe any foliage problems. No information on leaf damage was given by Kiamohammadi [14], who found 6% sucrose—in combination with an unusually high silver concentration (120 mg dm^{-3} of $AgNO_3$)—as the best for lisianthus even though 4% sucrose was already harmful for leaves in the trials carried out Shimizu-Yumoto and Ichimura [6] and Shimizu-Yumoto [36].

As ethylene is involved in the senescence of lisianthus flowers [6,17,36], pulse treatments with chemicals inhibiting its activity and limiting its biosynthesis should be beneficial in prolonging the postharvest longevity of cut flowers. Conditioning with silver thiosulfate (STS) has been applied to the ethylene-sensitive flowers for four decades, and its positive effect on lisianthus has also been reported [6,17,36]. However, conditioning was more effective when STS was combined with sucrose. The role of sucrose in improving the postharvest physiology of lisianthus by delaying the ethylene production or reducing the sensitivity to C_2H_4 cannot be underestimated. To protect lisianthus flowers against the ethylene action, treatments with BA and sucrose resulted in the lowest ethylene production [16].

Conditioning flowering stems with 5 mg dm^{-3} NS did not prolong the vase life in our experiment. Perhaps the concentration of silver transported from the vase solution to flowers was too low to affect the ethylene synthesis and to increase flower longevity. According to Lü et al. [12], silver concentration in pulsed rose stems was the highest in their basal ends and much lower in leaves and flowers. The authors used nanosilver in concentrations 10–50 times higher than ours, and successfully improved keeping qualities of roses with concentrations of 50 and 100 mg dm^{-3}. Even higher NS concentrations were used by Kasir et al. [33]: 75 and 125 mg dm^{-3} for 2 h pulse of 'High and Magic' roses. The concentration ranging between our 5 and 125 mg dm^{-3} of the above cited authors leaves room for further trials to find the lowest but optimal NS concentration for conditioning lisianthus. As for NS as a biocide, the concentration of 1 mg dm^{-3} appeared to be very low yet its efficiency—when applied with sucrose—was higher than that of the standard preservative. In our experiments with cut peonies, 1 mg dm^{-3} NS affected

keeping qualities in a manner comparable to 8-HQC, and has been recommended as its alternative treatment for peonies [37].

Water balance is a crucial factor in controlling the longevity of cut flowers. When the water balance becomes negative due to dominance of transpiration over water uptake, flowers begin to wilt. Water deficiency affects not only the visual appearance of cut flowers but also accelerates the processes of senescence. The rate of water uptake is known to decline as lisinathus flowers senesce, regardless of the holding solution [9] and this tendency was observed here. Treatments improving water balance (i.e., minimizing transpirational losses and stimulating uptake by eliminating bacterial vessel blockages) are commonly used in the cut flower industry. 8-HQC has long been known as a potent biocide as well as an antitranspirant [8], so it is no surprise that when used in the standard preservative, it positively affected water balance in our experiments. However, it is noteworthy that higher average stem weights from the standard preservative—compared to water controls—appeared to be due to a reduced transpiration rate and not to stimulation of the uptake. Contrary to Lü et al. [12], where NS inhibited the stomatal conductance and reduced the leaf water loss in roses, in our experiment, the transpiration rate was the highest in the NS + S-treated flowers. However, as nanosilver also stimulated the water uptake, it is the combination of the two processes that produced the highest average weights of flowering stems. It was also unexpected that conditioning with NS negatively affected the fresh weight, expressed both by its average daily values and the course of changes during 27 days of the vase life. Perhaps less sugar was delivered to the stems, as during the first day of the vase life, with a high uptake intensity, only pure nanosilver was absorbed. Sugar is the "driving force" for the solution uptake.

The fresh weight of flowering lisianthus stems depended not only on the volume of absorbed holding solutions, but on sucrose present in both of them. A crucial role of sucrose in enhancing the vase solution uptake was shown by Bahrami et al. [3]. Stems from our sucrose containing solutions had their fresh weight significantly higher than those from water controls. Similar observations were made by Huang and Chen [16], who kept cut lisianthus flowers in 2% sucrose or glucose, and observed increases in their fresh weight while that in the water-held stems decreased during vase life. They showed that exogenous sugars were absorbed from holding solutions where their concentrations dropped after several days, to be translocated to buds and flowers and promoted their opening and maintained water balance. Glucose, fructose, and sucrose were identified as endogenous carbohydrates in lisianthus petals and they accumulated in petals of flowers standing in the sugar-containing solution while decreased in flowers kept in water. Such a reduction in total soluble sugars in lisianthus flowers—regardless of the postharvest treatments and temperatures—were reported by Cavasini et al. [18]. Apart from the above listed sugars, *myo*-inositol and D-bornesitol were found in petals by Norikoshi et al. [35], but glucose and fructose were the major carbohydrates in petals. Their accumulation in the vacuoles of flowers supplemented with exogenous carbohydrates contributed to the reduction of the osmotic potential, which in turn promoted water influx associated with flower opening. We did not identify individual sugars in petals; however, differences between the amounts of the total soluble and reducing sugars suggest that the latter constituted an important proportion of soluble carbohydrates in lisianthus. At harvest, open flowers were well provided with carbohydrates by the mother plant so the concentration of total soluble sugars was higher than that in the buds. At the same time, the initial reducing sugar concentrations were comparable in both flower types. Generally, flowers held in water had less endogenous carbohydrates during the vase life than those placed into the preservatives, and the highest sugar content in flowers from NS + S may be due to more intensive uptake of the vase solution in this treatment. While conditioning lowered the water uptake rate and thus affected the amounts of sugars delivered to petals, the upper, younger buds on conditioned stems had more total soluble and reducing sugars than those on non-conditioned stems. Carbohydrates needed for bud opening might have come from leaves, which are an intermediate station in the exogenous sugar translocation from the

vase solution to flowers. This is only a speculation as unfortunately, we did not analyze the sugar levels in leaves. This might have elucidated certain ambiguities in carbohydrate changes in lisianthus supplemented with sucrose.

The rise in free proline is taken as an indicator of the progress of senescence and such a rise was observed during the vase life of cut lisianthus by Kazemi et al. [31] and Bahrami et al. [3]. Treatments extending the vase life limited proline accumulation and both authors found SA as a chemical effective for both purposes. Additionally, in cut peonies, an accumulation of free proline occurred at the end of the vase life and sugar-containing preservatives—either with 8-HQC or NS—reduced this process several-fold relative to water controls. In our experiments, free proline increased in senescing lower and upper flowers standing in water, while the preservatives generally reduced the free proline contents relative to their controls. Unexpectedly, relative to non-conditioned flowers, conditioning enhanced the accumulation of free proline in upper flowers on stems held in water. In contrast, conditioning limited the rise in free proline in lower and upper flowers on stems held in NS + S. Here, an extra dose of nanosilver absorbed during the first 24 h of the vase life might have supplemented the amount of silver taken up by the stems from the vase solution and tipped the balance, making the NS concentration sufficient for the antisenescence action. However, as both sugar-containing solutions reduced free proline level in flowers, it might be sucrose that acted here as the antioxidant defense factor [38] and this remains to be elucidated.

During senescence, cell damage appears, caused by oxidative stress. Reactive oxygen species (ROS) such as hydrogen peroxide are generated, damaging cells and hastening their death. Living organisms have developed antioxidant defense mechanisms against ROS to reduce the damage caused by the oxidative stress during senescence. Scavenging enzymes such as superoxide dismutase (SOD), CAT, or ascorbate peroxidase (APX) neutralize ROS to maintain the redox balance.

In our experiments, changes in the hydrogen peroxide contents were relatively small and the senescing lower flowers on conditioned stems contained less H_2O_2 than non-conditioned ones. In the upper flowers, striking differences in the H_2O_2 contents appeared on Day 9 between control flowers and those held in NS + S, the latter having the highest hydrogen peroxide concentration. In this case, the CAT activity peaked in synchrony with a rise in H_2O_2 which, however, did not reduce its content. Generally, the CAT activity was reduced with time in lower and upper flowers of all treatments, reaching the values at the end of the vase life than the values below the initial one. This does not confirm earlier reports that treatments limiting the rise in hydrogen peroxide and/or increasing the activity of antioxidant enzymes extends the vase life of cut lisianthus. Ataii et al. [21] found such a positive effect of polyamine putrescine. Furthermore, SA extended longevity in lisianthus, reducing the H_2O_2 accumulation and enhancing the CAT activity in petals [22]. A delay in senescence in lisianthus by endogenous hydrogen was also associated with increased activity of CAT [39]. In cut peonies, the CAT activity increased during the vase life and was higher in flowers held in preservatives, especially in NS + S [40].

5. Conclusions

This manuscript presents new treatments for lisianthus with NS and sucrose, based on various physiological and biochemical parameters (water uptake, transpiration, carbohydrates, free proline content, hydrogen peroxide levels, and catalase activity), which have not been previously reported. In conclusion, NS in concentrations as low as 1 mg dm^{-3} in combination with 2% sucrose acted comparably to the standard preservative by extending vase life and controlling certain senescence-related processes in petals. Using the nanosilver particles for cut flowers including lisianthus is still a novelty, therefore there is no ready-to-use protocol on how to handle the material during a postharvest life. The presented results show that NS may be recommended as a biocide in vase solutions for lisianthus, and at low concentrations, it is safe for the environment. The ability to use silver

nanoparticles offers new possibilities in the choice of ways to extend cut flowers including the postharvest vase life of cut lisianthus flowers.

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