Postharvest Treatments Improve Quality of Cut Peony Flowers

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Abstract: Peony is one of the most important ornamental plants in the international flower market, but has a relatively short vase life in water. This study tested the effects of 8-hydroxyquinoline citrate (8-HQC) and nanosilver (NS) in combination with sucrose, as well as two commercial preservatives, on the longevity and some physiological and biochemical aspects of senescence of cut flowers of 14 cultivars. Responses varied both by cultivar and treatment. The preservatives extended the vase life in only five cultivars; however, in nine cultivars, preservatives increased the flower diameter and improved the general flower appearance. Blockages in xylem vessels started to appear soon after harvest. Both NS and 8-HQC with sucrose prevented tylose formation, while bacterial blockages were reduced only by the NS solution. Reduction in stem blockages did not translate into better water balance or flower longevity. The highest carbohydrate accumulation in petals was observed in the NS solution. Preservatives mitigated the rise in free amino acids, including free proline. They did not prevent an increase in H₂O₂ content but flowers in preservatives generally had higher catalase activity than in the control. As solutions with NS produced comparable or even better results than 8-HQC, we recommend the latter as a component of a preservative for cut peony flowers. However, cultivar-specific responses indicate that postharvest treatments must be individually tailored to each cultivar.

Keywords: blockages; carbohydrates; catalase; nanosilver; free proline; preservatives; senescence; sucrose; vase life; water balance

1. Introduction

Large flowers, bright attractive colors, elegant shapes, and rich fragrance make peony (Paeonia lactiflora Pall.) a highly decorative plant. As such, it has recently become one of the most important ornamental plants on the flower market [1]. Peonies are one of the most beautiful flowers in the world, known in Europe as the “Queen of the Garden”. They are considered a symbol of good luck, fortune, health, wealth, and prosperity and are said to possess medicinal qualities. However, cut peony flowers have a very limited vase life; they may not even fully open or show early petal drop [2]. The natural peony vase life is around 7 days [3], but this depends on the cultivar [4], the stage of maturity at which flowers are harvested, and the environment in which the plants are grown [5]. To sustain the cut peony industry development, mechanisms/treatments need to be developed capable of prolonging the vase life while maintaining high flower quality during the vase period. Due to research on low-temperature requirement for flowering [6], peonies can now be grown for cut flowers almost everywhere, from Chile and Australia to Alaska, providing a nearly year-round supply for the global market [7].
Flower opening and senescence of cut flowers consist of a series of physiological, biochemical, and molecular changes resulting from separation of stems from a mother plant. Postharvest water relations, the carbohydrate status, the hormonal balance, and environmental conditions are the main factors affecting the postharvest longevity of cut flowers. Generally, continued water uptake and an energy supply are essential for flower opening during this period. Carbohydrates, especially sugars, are provided as a nutrition source in preservative solutions; they serve as a respiration substrate and for osmotic regulation [8,9], prevent damage to proteins, and improve water balance by closing stomata [10,11]. Lack of sugar is one of the main causes of petal senescence [12]. Supplementing vase solutions with sucrose or glucose has a positive effect on flower opening, increases flower size, improves petal color, and prolongs the vase life in a wide range of species [12,13].

Dehydration and wilting, caused by either air embolisms in the stem xylem or by bacteria entering the stem from the vase water and blocking water uptake [9], can affect both the ability of flower buds to open and their vase life. Stem end blockage is a major factor in the imbalance between water uptake and water loss in cut flowers [9,14]. The most common method of extending the vase life is by using various preservative solutions. These usually contain a biocide to control the bacterial growth; surfactants, which help to quickly rehydrate flowers because a reduction of the surface tension of water improves water uptake [15,16]; and a sugar as an energy source [17]. The most commonly used and efficient solution is the so-called “standard preservative”, composed of sucrose and 8-hydroxyquinoline citrate (8-HQC) [18]. Recently, after silver nitrate has been banned for its toxicity, nanosilver (NS) has emerged as a new biocide for cut flowers [19]. NS has small-sized particles, a strong antibacterial activity [19–21], a wide activity spectrum, and is said to be safe for the environment [22,23]. Because of these attributes, preservative solutions with NS have been tested in a range of different species and reported as effective in prolonging the postharvest life of cut flowers [3,24–35].

Considering the economic importance of cut peony flowers and their relatively short shelf life, it appears advisable to develop effective treatments capable of delaying the rate of quality deterioration, and to extend the postharvest life. The experiments described herein were designed to assess the effects of various preservative solutions on the flower quality in a range of peony cultivars, on some parameters of the water balance, including the formation of blockages in stem vessels, as well as on some aspects of senescence occurring in flowers, such as changes in the reducing sugars, soluble proteins, free amino acids and free proline, \( \text{H}_2\text{O}_2 \) levels, and the catalase activity.

2. Material and Methods

2.1. Plant Material

Flowering stems of 14 cultivars of herbaceous peony (\textit{Paeonia lactiflora} Pall.: ‘Albert Crousse’, ‘Charles Binder’, ‘Duchesse de Nemours’, ‘Festiva Maxima’, ‘Gayborder June’, ‘Graziella’, ‘Hania’, ‘Jadwiga’, ‘Kabata’, ‘Königin Wilhelmina’, ‘Laura Dessert’, ‘Ursynów’, ‘Vogue’, and ‘Wiesbaden’) were harvested from the collection of perennials of the Section of Ornamental Plants at the Warsaw University of Life Sciences, Warsaw, Poland. All harvested stems had flowers in the marshmallow bud stage, with no visible symptoms of disease, pests, or mechanical defects, as recommended by Eason et al. [36] and Yu et al. [37]. Cut stems were stripped off excessive leaves, trimmed to 55 cm, and placed into vases with one of the following solutions: distilled water (control), 8-hydroxyquinoline citrate (8-HQC) at 200 mg L\(^{-1}\) and 20 g L\(^{-1}\) sucrose (S), distilled water with 1 mg L\(^{-1}\) nanosilver (NS) and 20 g L\(^{-1}\) sucrose, and two commercial preservatives: Chrysal Clear sachet and Florafile 300. Exact formulations of these commercial preservatives are proprietary. Commercial preservatives were prepared according to the producers’ instructions. In the experiments testing the parameters of senescence, we omitted commercial preservatives. During the experiment, solutions were not exchanged, but only replenished as needed. The experiments were carried out in a room with a controlled temperature of 20 ± 1 °C,
relative humidity of 60%, and quantum irradiance of 35 $\mu$mol m$^{-2}$ s$^{-1}$ (Fluorescent lamp LF80 36W/850, Natural Daylight, Philips Factory, Pila, Poland) under a 12 h day/12 h night regime.

2.2. Vase Life Parameters

Each treatment contained 10 stems, individually tagged and treated as single replicates. The vase life was the number of days from harvest to the day when petals were wilting or falling off.

Flower diameters were measured at the stage of full development and given as the average of two measurements on each flower, made at right angles to each other.

2.3. Water Balance Parameters

Stems of peony ‘Charles Binder’ were trimmed to 55 cm with one pair of leaves left and placed individually into calibrated cylinders (volume 0.5 dm$^3$) and treated as separate replications, with 10 stems in each treatment. Water uptake was measured daily and expressed in gram per stem per day. Flowering stem weight was taken each day after the solutions had been replenished. The relative fresh weight (RFW) of flowers was calculated as RFW (%) = (FWt/FWt 0) × 100, where FWt is the fresh weight of a flower (g) at t = days 0, 1, 2, etc., and FWt 0 is the fresh weight of a flower (g) at t = day 0 [38]. The transpiration rate was expressed as a difference between the water uptake and the weight change, and expressed as gram per stem per day.

2.4. Bacterial Levels in Holding Solutions

To determine the levels of bacterial populations in holding solutions, we took 0.5 mL aliquots in triplicate from each holding solution on the last day of the vase life of peony ‘Charles Binder’. These samples were serially diluted with 0.9% sterile normal saline (NaCl), and 0.1 mL aliquots were spread over agar plates and incubated at 30 °C for 24 h. Colony counts were expressed as colony-forming units per milliliter (CFU mL$^{-1}$).

2.5. Anatomical Studies of Stem Blockages

For microscopic observation of stem cross sections, we collected 1 cm long basal stem sections and compared them to those from freshly harvested stems (day 0). Stems from each treatment were sampled twice, on day 3 and 10 after harvest. Stem fragments were collected from 10 stems in each treatment on each sampling date. Specimens were fixed in 5% glutaraldehyde and 4% paraformaldehyde solution in 0.1 mol L$^{-1}$ sodium cacodylate buffer (pH 7.2–7.3) at 0.8 atm at 20 ± 2 °C, and rinsed with the same buffer. Observations were made under a scanning electron microscope (SEM) FEI QUANTA 200 ESEM, at the Analytical Centre, Warsaw University of Life Sciences.

2.6. Biochemical Analyses

Batches of 20 flowers from cultivars ‘Charles Binder’, ‘Festiva Maxima’, and ‘Gayborder June’ held tested solutions were used for biochemical analyses. Samples were collected on day 0 (immediately after harvest), days 3–4, and day 10 after harvest. For each analysis, we took 6 flowers from each treatment. Flower petals were finely cut and mixed, and 3 samples of 0.5 g each were taken. Three extracts were made from each sample, and 3 readings were made for each extract, giving a total of 9 readings for each data point. For the dry weight measurements, we took 3 samples and dried them at 105 °C until constant weight.

The reducing sugar content was measured by the Somogyi method, as modified by Nelson [39] and expressed in milligram glucose per g$^{-1}$ dry weight (DW). The material was homogenized in 80% ethanol. The extracts were incubated for 20 min in a boiling water bath with the copper reagent; the molybdenum arsenic reagent 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 soluble protein and free amino acid contents were determined according to Bradford [40] and Rosen [41], respectively; calculated from previously plotted standard curves; and expressed in milligram of albumin bovine serum or milligram leucine, respectively, per gram of dry weight (DW). The free proline content was determined according to Bates et al. [42] by measuring the quantity of a colored reaction product of proline with ninhydric acid. The absorbance was read at 520 nm. The amount of proline was calculated from a previously plotted standard curve and expressed in milligram g\(^{-1}\) of dry weight.

2.7. Oxidative Stress

Samples were collected from three cultivars, ‘Albert Crousse’, ‘Gayborder June’, and ‘Ursynów’, on day 0 (immediately after harvest), days 3–4, and days 9–10 after harvest. The plant material was prepared as described in Section 2.6. The petal hydrogen peroxide (H\(_2\)O\(_2\)) content was measured spectrophotometrically after the reaction with potassium iodide (KI), as described by Jedrzejuk et al. [30], and expressed at 390 nm as microgram of hydrogen peroxide per gram on a dry weight (DW) basis. The catalase (CAT) activity (EC 1.11.1.6) was determined spectrophotometrically (Shimadzu UV-1800, Kyoto, Japan) as the rate of H\(_2\)O\(_2\) disappearance at 405 nm according to Goth [43] and expressed as mkatal per gram dry weight (DW).

2.8. Statistical Analysis

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

3. Results

3.1. Vase Life and Flower Diameter

The vase life of cut flowers of the 14 cultivars held in water ranged between 7 and 20 days (Table 1). The longest vase life was in ‘Graziella’ (almost 20 days) and 13–14 days for ‘Albert Crousse’, ‘Charles Binder’, and ‘Vogue’. The shortest longevity (7–9 days) was in ‘Duchesse de Nemours’, ‘Festiva Maxima’, ‘Ursynów’, and ‘Wiesbaden’. Only 5 of 14 tested cultivars positively responded to the tested preservatives: ‘Duchesse de Nemorous’, ‘Hania’, ‘Königin Wilhelmina’, ‘Ursynów’, and ‘Wiesbaden’. The preservative composed of 8-HQC and S prolonged the vase life of only one cultivar—‘Wiesbaden’—by 32% (more than 2 days) relative to water control. More effective was the NS + S solution: four cultivars lasted longer than the control (by 17, 15, 22, and 35%, in ‘Duchesse de Nemorous’, ‘Hania’, ‘Ursynów’ (Figure 1B), and ‘Wiesbaden’, respectively) (Table 1). The commercial preservative Chrysal sachet prolonged the vase life of only two cultivars—‘Königin Wilhelmina’ and ‘Ursynów’ (Figure 1B)—by approximately 2 days (Table 1); the other, Floralife 300, was effective only in ‘Hania’.

Diameters of cut peony flowers held in water differed among the cultivars, and for most cultivars it was 10–11 cm. The biggest were ‘Duchesse de Nemours’ and ‘Laura Dessert’; the smallest was ‘Graziella’ (Table 2). Nine cultivars responded to the preservatives with increases of the flower diameter. The most effective was 8-HQC + S, which increased the flower diameter in five cultivars: ‘Albert Crousse’ (Figure 1A), ‘Graziella’, ‘Kabata’, ‘Laura Dessert’, and ‘Ursynów’ (Figure 1B) by 21, 57, 17, 15 and 27%, respectively (Table 2). NS + S affected the flower diameter in four cultivars: ‘Albert Crousse’ (Figure 1A), ‘Duchesse de Nemorous’, ‘Kabata’, and ‘Laura Dessert’. Of the commercial preservatives, Chrysal sachet increased the flower diameter in ‘Gayborder June’, while Floralife 300 increased the flower diameter in ‘Königin Wilhelmina’ and ‘Vogue’.
Table 1. The effect of preservatives on the vase life of cut peony flowers.

| Cultivar                 | Vase Life (Days) | Water | 8-HQC + S | NS + S | Chrysal Sachet | Florafile 300 |
|--------------------------|------------------|-------|-----------|--------|----------------|---------------|
| Albert Crousse           | BC 1 13.1 b 2    | 12.6 a| 11.0 a    | 11.8 a | 13.5 b         |               |
| Charles Binder           | IC 14.6 bc       | 13.8 b| 15.6 c    | 11.4 a | 13.2 b         |               |
| Duchesse de Nemours      | A 8.8 a          | 9.3 a | 10.3 b    | -      | -              |               |
| Festiva Maxima           | C 8.8 a          | 9.0 a | 10.2 a    | 8.4 a  | 9.2 a          |               |
| Gayborder June           | AB 10.0 b        | 8.6 a | 10.3 b    | 9.2 a  | 9.1 a          |               |
| Grazella                 | D 19.9 ab        | 21.9 b| -         | 18.0 a | 20.1 b         |               |
| Hania                    | AB 9.7 a         | 10.8 ab| 11.2 b   | 11.0 a | 11.2 b         |               |
| Jadwiga                  | B 11.0 a         | 11.0 a| 11.0 a    | 11.0 a | 11.0 a         |               |
| Kabata                   | B 11.3 a         | 9.7 a | 11.3 a    | 10.9 a | 10.8 a         |               |
| Königin Wilhelmina       | AB 10.4 a        | 9.8 a | -         | 12.3 b | 11.4 a         |               |
| Laura Dessert            | AB 9.7 a         | 10.7 a| 9.5 a     | 9.6 a  | 10.8 a         |               |
| Ursynów                  | A 8.2 a          | 8.7 ab| 10.0 bc   | 10.5 c | 9.0 ab         |               |
| Vogue                    | BC 12.6 b        | 12.0 ab| -        | 11.4 a | 11.8 ab        |               |
| Wiesbaden                | A 6.9 a          | 9.1 b | 9.3 b     | -      | -              |               |

1 Means in the first column followed by the same capital letter did not differ significantly at α = 0.05. Analyses were performed for flowers placed into water. 2 Means in each line followed by the same lower-case letter did not differ significantly at α = 0.05. Analyses were performed separately for each cultivar. -: not tested.

Figure 1. Appearance of cut peony ‘Albert Crousse’ (A) and ‘Ursynów’ (B) flowers placed into water and preservatives 10 days after harvest. 1—water, 2—8-hydroxyquinoline citrate (8-HQC) + sucrose (S), 3—Floralife 300, 4—Chrysal sachet, 5—nanosilver (NS) + sucrose (S).
Table 2. The effect of preservatives on the flower diameter of cut peony flowers.

| Cultivar                | Water | 8-HQC + S | NS + S | Chrysal Sachet | Florafile 300 |
|-------------------------|-------|-----------|--------|----------------|---------------|
| Albert Crousse          | CD    | 10.7 a    | 12.8 b | 11.8 ab        | 11.7 ab       |
| Charles Binder          | C     | 10.3 a    | 9.8 a  | 9.7 a          | 10.7 a        |
| Duchesse de Nemours     | E     | 15.8 ab   | 16.5 b | -              | -             |
| Festiva Maxima          | BC    | 9.7 a     | 8.8 a  | 7.8 a          | 9.2 a         |
| Gayborder June          | CD    | 11.1 a    | 11.8 ab| 10.5 a         | 13.0 b        |
| Graziella               | A     | 6.8 b     | 10.7 b | -              | 6.7 a         |
| Kabata                  | C     | 10.3 a    | 12.0 bc| 13.0 c         | 10.8 ab       |
| König Wilhelmina        | CD    | 10.7 a    | 11.1 a | -              | 9.0 a         |
| Laura Dessert           | E     | 13.7 a    | 15.8 b | 16.5 b         | -             |
| Ursynów                 | CD    | 11.0 a    | 14.0 b | 11.2 a         | 12.0 a        |
| Vogue                   | AB    | 7.2 a     | 8.7 ab | -              | 8.2 ab        |
| Wiesbaden               | D     | 11.5 ab   | 11.2 ab| 11.7 b         | -             |

1 Means in the first column followed by the same capital letter did not differ significantly at $\alpha = 0.05$. 2 Means in each line followed by the same lower-case letter did not differ significantly at $\alpha = 0.05$. Analyses were performed separately for each cultivar. -: not tested.

3.2. Water Balance

Water balance was conducted on ‘Charles Binder’ and the commercial preservatives were excluded. The solution uptake in this tested cultivar showed gradual reduction, with the exception of days 3 and 5, where a rapid increase was observed, especially in flowers held in water (Figure 2A). On average, during the course of 13 days, the smallest amount of absorbed solution was for NS + S and the largest for the water control (Table 3). Similarly, the transpiration intensity was the lowest in the NS + S solution and the highest in the water control (Table 3). In the controls, the transpiration intensity increased, especially on day 5 (Table 2), coincidental to the solution uptake rate. During the first 7 days of the experiment, the transpiration rate for various preservatives was similar; from day 8 on it was higher in the flowers placed into 8-HQC + S than those held in NS + S.

![Figure 2](https://example.com/figure2.png)

Figure 2. The effect of preservatives on water balance in cut peony ‘Charles Binder’ flowers. Water uptake (A), transpiration rate (B), and relative fresh weight (C) of cut peony flowers. Values are expressed as the mean ± SD. Vertical bars represent standard deviations of the means.
Table 3. The effect of preservatives on the water balance parameters in cut peony ‘Charles Binder’ flowers.

| Treatments  | Water Uptake (g stem\(^{-1}\) day\(^{-1}\)) | Transpiration Rate (g stem\(^{-1}\) day\(^{-1}\)) | Relative Fresh Weight (%) | Number of Bacteria (CFU mL\(^{-1}\)) |
|-------------|---------------------------------------------|-----------------------------------------------|---------------------------|--------------------------------------|
| water       | 16.95 ± 4.05 b \(^1\)                       | 16.76 ± 3.05 b \(^1\)                         | 123.32 ± 12.25 a \(^1\)   | 2.00·10\(^{-5}\) a \(^2\)            |
| 8-HQC + S   | 13.72 ± 3.23 ab                             | 12.30 ± 2.89 ab                               | 154.55 ± 14.35 b          | 2.90·10\(^{-5}\) b                   |
| NS + S      | 11.15 ± 3.92 a                              | 10.20 ± 3.83 a                               | 133.37 ± 14.70 a          | 2.20·10\(^{-5}\) a                   |

\(^1\) Means in each column followed by the same letter did not differ significantly at \(\alpha = 0.05\). Values are expressed as the mean of 13 days after harvesting ± SD. 
\(^2\) Bacterial populations in holding solutions were assessed on the last day of the vase life. Values are expressed as the mean ± SD.

The highest water uptake concomitant with the highest transpiration resulted in the lowest RFW of flowers held in water (Table 3). In NS + S, the lowest uptake and the lowest transpiration resulted in the average RFW value, comparable with that of the control (Table 3). However, the analysis of water balance on individual days showed that from day 9, the RFW was significantly higher for NS + S than the control (Figure 2C). The highest RFW was observed in flowers placed into 8-HQC + S (Table 3, Figure 2C), although the values for the uptake and transpiration were at average levels.

Bacterial populations were higher in the 8-HQC + S than in water or NS + S (Table 3).

Analyses performed immediately after harvest (Figure 3A,B) show the presence of crystals (druses) in the shoots of cv. ‘Charles Binder’ (Figure 3B). On day 3, blockages were in shoots placed in water in the form of numerous tyloses (Figure 3C,D). Vascular blockages also appeared in 8-HQC + S in the form of bacterial deposits (Figure 3E,F). No blockages were observed in shoots held in NS + S (Figure 3G,H). However, the natural presence of callose, which is not considered as a blocking factor, was evident (Figure 3H). On day 10, callose in shoots held in NS + S disappeared, and no blockages were evident in this treatment (Figure 4E,F). Blockages in the form of numerous tyloses occurred only in stems from the water control (Figure 4A,B).

Figure 3. Cont.
Analyses performed immediately after harvest (Figure 3A,B) show the presence of crystals (druses) in the shoots of cv. ‘Charles Binder’ (Figure 3B). On day 3, blockages were evident (Figure 3H). On day 10, callose in shoots held in NS + S disappeared, and no factor, was evident (Figure 3G,H). However, the natural presence of callose, which is not considered as a blocking agent, was evident (Figure 3H). On day 10, the sugar level of reducing sugars in ‘Festiva Maxima’ in 8-HQC + S was the highest sugar level, almost three times higher than the initial content, and by 32% relative to the initial level (Figure 5A,C,E). In ‘Gayborder’, the sugar level was held in NS + S on days 4 and 10, by 188% and 316%, respectively (Figure 5C). In ‘Gayborder’, the sugar level was held in NS + S on days 4 and 10, by 188% and 316%, respectively (Figure 5C). In ‘Gayborder’, the sugar level was held in NS + S on days 4 and 10, by 188% and 316%, respectively (Figure 5C). In ‘Gayborder’, the sugar level was held in NS + S on days 4 and 10, by 188% and 316%, respectively (Figure 5C). In ‘Gayborder’, the sugar level was held in NS + S on days 4 and 10, by 188% and 316%, respectively (Figure 5C).

Figure 3. Cross-section (A, C, E, G) and longitudinal section (B, D, F, H) through the stem of peony ‘Charles Binder’: A, B—immediately after harvest; C, D—kept in water for 3 days; E, F—kept in 8-HQC + S for 3 days; G, H—kept in NS + S for 3 days. druse; tylose; bacterial deposits; callose.

Figure 4. Cont.
Analyses performed immediately after harvest (Figure 3A,B) show the presence of crystals; druses; tyloses; bacterial deposits; callose.

were 2.5-, 3.9-, and 2.2-fold lower than in controls, respectively. The free amino acid contents in
Figure 5D). In this cultivar the highest protein content was detected in flowers from the 8-HQC + S (Figure 5B). The largest amounts of proteins were detected in flowers from the 8-HQC + S where a twofold increase relative to the initial level occurred during the vase life. In flowers in the NS solution, the soluble protein level lower than those in controls (Table 4)—on days 4 and 10 by 17% and 20%, respectively (Figure 5D). In flowers of ‘Gayborder June’ held in water, the soluble protein content increased steadily to 54% higher on day 10 than on the day of harvest (Figure 5F). This rise in proteins was the most pronounced in flowers kept in 8-HQC + S where a twofold increase relative to the initial value occurred during the vase life. In flowers in the NS solution, the soluble protein content was comparable to that of controls (Figure 5E, Table 4).

In flowers of all three cultivars kept in water, the free amino acid content increased several-fold over the initial level (Figure 6A,C,E). Both preservative 8-HQC + S and NS + S mitigated this rise (Table 4), especially on day 10, with the 8-HQC + S being more effective. On day 10, the free amino acid contents in flowers of ‘Charles Binder’, ‘Festiva Maxima’, and ‘Gayborder June’ held in 8-HQC + S were 2.5-, 3.9-, and 2.2-fold lower than in controls, respectively. The free amino acid contents in

3.3. Biochemical Changes

During the vase life, the contents of reducing sugars in flowers held in water increased in all cultivars tested (‘Charles Binder’, ‘Festiva Maxima’, and ‘Gayborder June’) by 108%, 161%, and 197%, respectively, relative to the initial level (Figure 5A,C,E). In ‘Charles Binder’ on day 10, the sugar level in flowers held in the 8-HQC + S solution was higher by 14% than that in the control. On day 10, the highest sugar level was in NS + S, almost three times higher than the initial content, and by 32% higher than in controls (Figure 5A). The content of reducing sugars in ‘Festiva Maxima’ in 8-HQC + S was comparable to that in the control (Table 4). The increase in reducing sugars was the highest in flowers held in NS + S on days 4 and 10, by 188% and 316%, respectively (Figure 5C). In ‘Gayborder June’, no significant effect of the holding solutions on the reducing sugar content was detected (Figure 5E, Table 4).

Soluble proteins in the ‘Charles Binder’ flowers held in water initially increased by 30% on day 3, but on day 10 dropped slightly to 12% below that of day 3 but still 16% higher than that on day 0 (Figure 5B). The largest amounts of proteins were detected in flowers from the 8-HQC + S solution (Table 4)—whereas on day 3 they were comparable to controls, on day 10 they were higher by 15% than in controls. The soluble protein content in flowers held in NS + S was comparable to the levels in flowers from 8-HQC + S on day 3, but on day 10 it dropped to the level observed in water control (Figure 5B). The soluble protein contents in the ‘Festiva Maxima’ flowers held in water increased 68% during the first 4 days of vase life relative to the initial level, and then it dropped by almost 30% (Figure 5D). In this cultivar the highest protein content was detected in flowers from the 8-HQC + S solution (Table 4), where on day 10 it was higher by 29% than in controls (Figure 5D). Flowers held in NS + S solution had the soluble protein level lower than those in controls (Table 4)—on days 4 and 10 by 17% and 20%, respectively (Figure 5D). In flowers of ‘Gayborder June’ held in water, the soluble protein content increased steadily to 54% higher on day 10 than on the day of harvest (Figure 5F). This rise in proteins was the most pronounced in flowers kept in 8-HQC + S where a twofold increase relative to the initial value occurred during the vase life. In flowers in the NS solution, the soluble protein content was comparable to that of controls (Figure 5E, Table 4).

In flowers of all three cultivars kept in water, the free amino acid content increased several-fold over the initial level (Figure 6A,C,E). Both preservative 8-HQC + S and NS + S mitigated this rise (Table 4), especially on day 10, with the 8-HQC + S being more effective. On day 10, the free amino acid contents in flowers of ‘Charles Binder’, ‘Festiva Maxima’, and ‘Gayborder June’ held in 8-HQC + S were 2.5-, 3.9-, and 2.2-fold lower than in controls, respectively. The free amino acid contents in

Figure 4. Cross-section (A,C,E) and longitudinal section (B,D,F) through the stem of peony ‘Charles Binder’: A,B—kept in water for 10 days; C,D—kept in 8-HQC + S for 10 days; E,F—kept in NS + S for 10 days; tylose.
flowers of all cultivars kept in NS + S were slightly higher than those in flowers held in 8-HQC + S, but significantly lower than in controls (Table 4).

**Figure 5.** Reducing sugar content (A,C,E) and soluble protein content (B,D,F) in cut flowers of peony ‘Charles Binder’ (A,B), ‘Festiva Maxima’ (C,D), and ‘Gayborder June’ (E,F). Values are expressed as the mean ± SD. Vertical bars represent standard deviations of the means.
Table 4. The effect of preservatives on biochemical changes in cut peony flowers.

| Cultivar       | Treatments | Reducing Sugars (mg g\(^{-1}\)DW) | Soluble Proteins (mg g\(^{-1}\)DW) | Free Amino Acids (mg g\(^{-1}\)DW) | Free Proline (mg g\(^{-1}\)DW) |
|----------------|------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------|
| Charles Binder | water      | 229.58 a\(^1\)                   | 20.87 a\(^1\)                    | 28.60 c\(^1\)                    | 0.53 b\(^1\)                 |
|                | 8-HQC + S  | 239.15 a                          | 21.89 b                          | 14.04 a                          | 0.18 a                       |
|                | NS + S     | 251.73 a                          | 20.55 a                          | 18.67 b                          | 0.24 a                       |
| Festiva Maxima | water      | 202.00 a                          | 18.96 b                          | 38.36 c                          | 0.90 c                       |
|                | 8-HQC + S  | 205.58 a                          | 20.14 c                          | 15.60 a                          | 0.23 a                       |
|                | NS + S     | 261.15 b                          | 16.32 a                          | 20.92 b                          | 0.57 b                       |
| Gayborder June | water      | 213.63 a                          | 17.89 a                          | 29.08 c                          | 0.20 b                       |
|                | 8-HQC + S  | 202.76 a                          | 19.12 b                          | 19.42 a                          | 0.15 a                       |
|                | NS + S     | 204.31 a                          | 17.82 a                          | 19.97 b                          | 0.15 a                       |

\(^1\) Means in each column followed by the same letter did not differ significantly at \(\alpha = 0.05\). Values are expressed as the mean of 10 days after harvesting. Analyses were performed separately for each cultivar.

Figure 6. Free amino acid contents (A,C,E) and free proline contents (B,D,F) in cut flowers of peony ‘Charles Binder’ (A,B), ‘Festiva Maxima’ (C,D), and ‘Gayborder June’ (E,F). Values are expressed as the mean ± SD. Vertical bars represent standard deviations of the means.
The free proline content increased in control flowers of all three cultivars: 7-, 10-, and 2-fold in ‘Charles Binder’, ‘Festiva Maxima’, and ‘Gayborder June’, respectively (Figure 6B,D,F). In ‘Charles Binder’ and ‘Gayborder June’, flowers from both preservative solutions (8-HQC + S and NS + S) reduced these rises (Figure 6B,F, Table 4). On day 10, the free proline contents in flowers of ‘Charles Binder’ and ‘Gayborder June’ kept in 8-HQC + S and NS + S were fourfold and almost twofold lower than in controls, respectively. In ‘Festiva Maxima’, the 8-HQC + S was more effective than NS + S in reducing the free proline level (Figure 6D, Table 4). On day 10, the 8-HQC + S reduced it by 73% relative to the water control while reduction in NS + S was by 43% (Figure 6D).

3.4. Oxidative Stress

In ‘Albert Crousse’ kept in water, the H$_2$O$_2$ content increased significantly at the end of the vase life by 23% on day 10 relative to the initial level (Figure 7A). However, in both preservatives, the H$_2$O$_2$ content increased on day 4 by 58% relative to that immediately after harvest, and by 65% relative to control. On day 10, the H$_2$O$_2$ content dropped to the level similar to that of the water control. In the water control of ‘Gayborder June’, we observed a small rise (Figure 7C). Flowers in NS + S contained much more H$_2$O$_2$ than control flowers, while in 8-HQC + S, its level was similar to the control (Table 5). Relative to the initial level, the H$_2$O$_2$ content on day 10 in the control treatment and in 8-HQC + S was approximately 10% higher, while in NS + S it was almost 30% higher. A similar situation was observed in the ‘Ursynów’ flowers (Figure 7E), where the H$_2$O$_2$ content in control flowers and in flowers in 8-HQC + S was comparable, with the highest level observed in NS + S (Table 5), but only on day 3 (Figure 7E).

| Cultivar        | Treatments | Hydrogen Peroxide (µg g$^{-1}$ DW) | Catalase Activity (mkatals g$^{-1}$ DW) |
|-----------------|------------|------------------------------------|----------------------------------------|
| Albert Crousse  | water      | 114.10 a                           | 2.84 a                                 |
|                 | 8-HQC + S  | 141.67 b                           | 3.63 ab                                 |
|                 | NS + S     | 140.67 b                           | 4.54 b                                 |
| Gayborder June  | water      | 137.20 a                           | 3.69 b                                 |
|                 | 8-HQC + S  | 128.77 a                           | 3.15 a                                 |
|                 | NS + S     | 151.20 b                           | 3.21 a                                 |
| Ursynów         | water      | 103.80 a                           | 0.80 a                                 |
|                 | 8-HQC + S  | 104.90 a                           | 0.83 a                                 |
|                 | NS + S     | 116.33 b                           | 0.80 a                                 |

1 Means in each column followed by the same letter did not differ significantly at α = 0.05. Values are expressed as the mean of 9 (‘Ursynów’) or 10 days (‘Alberto Crousse’, ‘Gayborder June’) after harvesting. Analyses were performed separately for each cultivar.

The catalase activity in ‘Albert Crousse’ flowers in water dropped almost twofold by day 4 and remained at around that level until the end of the vase life (Figure 7B). In 8-HQC + S, after a similar drop, an increase on day 10 was observed, but to the level 23% lower than that on the day of harvest. The highest CAT activity was observed in ‘Albert Crousse’ in NS + S (Table 5), whereas on days 4 and 10 it was almost 2 and 3.5 times higher than in the control, respectively. In ‘Gayborder June’ in water, a steady increase in the CAT activity was observed (Figure 7D). Both preservatives reduced this rise (Table 5), reaching the level 30% lower than in the control on day 10. On the other hand, in ‘Ursynów’, the CAT activity in control flowers dropped significantly to almost zero on day 9 (Figure 7F). Reductions were also observed in the preservatives, but not as dramatic as in the control. It is worthy to notice that the initial CAT activity in ‘Ursynów’ was two and three times lower than in ‘Gayborder June’ and ‘Albert Crousse’, respectively (Figure 7B,D,F).
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Figure 7. Hydrogen peroxide contents (A,C,E) and catalase activities (B,D,F) in cut flowers of peony ‘Albert Crousse’ (A,B), ‘Gayborder June’ (C,D), and ‘Ursynów’ (E,F). Values are expressed as the mean ± SD. Vertical bars represent standard deviations of the means.

4. Discussion

The vase life and flower diameter of peony cultivars show a wide variation [37]. In this study, the vase life of cut flowers of 14 peony cultivars held in water ranged between 7 and 20 days, and the flower diameter between 7 and 14 cm, with 10–11 cm for most cultivars.

To prolong the postharvest longevity, cut flowers are commonly kept in preservative solutions, usually containing a biocide and a sugar. The biocide inhibits the growth of microorganism in the vase water and in the vessels, and stimulates water absorption necessary to maintain the flower turgidity; sugars provide a respiratory substrate [18] and improve water balance by increasing the concentration of osmolytes [13,44]. Of the four preservatives tested here, the two commercial formulations were the least effective in the initial experiments and were removed from more detailed experiments.
Responses to preservatives in this study were highly cultivar-specific—only 5 of the 14 cultivars tested showed positive responses. NS + S was more effective than the standard preservative 8-HQC + S. NS has emerged as a new, safe biocide, which prolongs the vase life of many cut flowers [3,24,26–28,30,32–35]. For peony, Zhao et al. [3] recommended 30 mg L\(^{-1}\) NS applied as a pulse treatment of 36 h. Here, a much lower concentration was effective (1 mg L\(^{-1}\)), but the flowers were kept in the NS solution continuously, from harvest to fading. While the effect of preservatives of flower longevity was modest, an interesting side effect was observed—in a majority of cultivars tested, the preservatives increased the flower diameter and improved the general flower appearance (the aesthetic value). The effect was different for different cultivars and the preservative solution. Here, 8-HQC + S increased the flower size of five cultivars while NS + S affected the flower diameter in four. While no specific metric was used to measure this, the preservatives appeared to intensify petal pigmentation and improved the general appearance. A similar effect was reported by Lin et al. [45], where in spray carnations, NS + S promoted flower opening and enhanced the bright pink color of flower petals, thereby markedly improving their display quality.

Owing to the detachment from the mother plant, cut flowers are usually under water stress caused by improper water balance [18]. Such stems, even when held in water, are unable to absorb sufficient amounts of water to compensate for the transpiration losses. Moreover, their vessels become obstructed by tyloses and microorganisms [46], even though at harvest, xylem vessels of many stems are free from such obstructions [34,47–49]. This was true here for peony, where at harvest, vessels showed only the presence of crystals (druses), which occur naturally and cannot be considered a blocking factor. According to Liu et al. [50], there are three types of stem end blockages: microbial due to living bacteria and decay products; physiological (wound-induced) [38,51]; and physical, in the form of air emboli [52,53]. The vessels became progressively obstructed during the postharvest life. In peony flowers kept in water, numerous tyloses appeared in stems but no bacteria were observed, even though bacteria were present in the vase water. Usually, the build-up of stem obstructions can be slowed down by certain preservatives. The NS + S used here reduced the build-up of tylose, which confirms its anti-tylose action, as in snapdragon flowers [34]. NS is believed to act as an effective antimicrobial agent [29,54–56]. In spray carnations, observations by scanning electron microscopy revealed that the NS alleviated vascular occlusion by inhibiting bacterial colonization and biofilm formation both on the stem end surfaces and inside xylem vessels [45]. Here, NS + S prevented the presence of bacteria in the vessels but it did not prevent the presence of bacteria in vase solutions. This is deeply surprising, given the known antibacterial properties of NS; perhaps this was caused by sucrose in the vase solution. Presence of sucrose in vase solution—increasing its concentration—could have also impeded its uptake relative to clear water, seen especially on day 5. On the other hand, a sugar in the vase solution is indispensable as it provides a respiratory substrate and increases osmoticum, thus improving water uptake [44,54]. In shoots held in 8HQC + S, no tyloses were presence but bacterial deposits clearly blocked the vessels. Higher bacteria population was present in the vase solution, suggesting that perhaps for the given sucrose concentration, the concentration of 8-HQC was too low to prevent bacteria growth. NS may be a more potent biocide than 8-HQC in limiting xylem blockages, as is the case in garden cosmos [35]. Unfortunately, a reduction in stem blockages in peony did not translate into better parameters of water balance. The highest water uptake concomitant with the highest transpiration resulted in the lowest RFW of flowers held in water. In NS + S, the lowest uptake and the lowest transpiration resulted in an average RFW value, comparable with the control. The highest RFW was observed in flowers in 8-HQC + S. These differences did not translate into improved flower longevity of the tested cultivar ‘Charles Binder’. Here, the longevity of flowers held in water was comparable to those kept in either of the preservative solutions. This generated a difference between the effects of preservatives on the longevity and on the water-related parameter. This calls for a closer look at the relationship between the water balance and longevity of cut flowers, generally inferred in other species [9,54].
The sugar in the preservative solution is translocated to the petals, contributing to the accumulation of carbohydrates, mainly in the form of reducing sugars [18]. These sugars serve as osmoticum, improving water influx into petals [54]. Here, the biggest accumulation of carbohydrates was observed in flowers held in the NS + S solutions, where in the two cultivars tested, the reducing sugar content was 32% and 59% higher than in water controls. The same effect was observed in cut snapdragon and clematis, where supplementing biocide solutions with sucrose doubled the carbohydrate levels relative to the controls [34,57]. According to Pun and Ichimura [11], flower longevity is related to its sugar content. Generally, the vase life of flowers with higher sugar contents is longer than that of flowers with lower sugar levels. However, Eason et al. [58] claims that the petal sugar content does not always correlate with flower longevity. Similarly, the high sugar levels were not always associated with longer vase lives of clematis flowers [57]. Here, a higher carbohydrate pool in peonies placed into NS + S did not guarantee longer vase life; longevity of flowers of ‘Charles Binder’ and ‘Festiva Maxima’ kept in preservative solutions was comparable to that of controls. Perhaps the pool of carbohydrates in peony flowers is sufficient for bud opening, and thus supplementation with sugar in the preservative solution is not necessary. Walton et al. [59] estimated that fresh cut peony flowers contained enough carbohydrates to provide a total vase life of 14 days, only 2 days less than stems still attached to the mother plant. The role of carbohydrates in the postharvest handling of peony flowers is still being examined [60].

In cut flowers, protein levels decrease rapidly after harvest, suggesting a role as respiratory substrates [61]. The relationship between flower senescence and protein degradation has been reported in several cut flowers [3,34,57,62–65]. Zhao et al. [3] noticed that the soluble protein content in cut peony ‘Hongyan Zhenghui’ increased early in the vase life, and then dropped. Here, a similar pattern was observed in three cultivars in water. In flowers kept in 8-HQC + S, the protein contents in each of the three cultivars tested (‘Charles Binder’, ‘Festiva Maxima’, and ‘Gayborder June’) on day 10 were higher than in control flowers by 15, 29, and 23%. According to Olley et al. [61], the initial increase in soluble protein concentration suggests increased protein synthesis during the rapid bud development phase, but this appears to be true for attached and not for detached flowers.

In the peony flowers kept in water, free amino acids and the free proline contents increased several-fold above the initial level. The rise in the proline content during senescence may have been triggered by a lower water potential such as in rose petals [66]. This appears to be related to senescence [57,67]. Increases of the free proline content depend on species and even on cultivar. In the peony ‘Charles Binder’ a sevenfold rise was observed; in ‘Festiva Maxima’ it was 10-fold, while in ‘Gayborder June’ it only doubled. In clematis, no more than a 1.5-fold increase relative to the initial value was observed [57], but in cut roses it was 14-fold [68]. It has not yet been fully explored as to whether increased proline content contributes to flower senescence or whether it a consequence of senescence. Evidence that supports utilization of proline during senescence was provided by treating flowers with substances that prolong their vase life [67]. Reductions of the free proline content in flowers placed in senescence-delaying solutions have been reported in carnation, lisianthus, and clematis [57,69,70]. Here, preservative solutions reduced the rise in free amino acids, including free proline, especially on day 10. The most effective was 8-HQC + S—on day 10, the free amino acid content was 2–4 times lower than in controls. Similarly, on day 10, the free proline contents in 8-HQC + S were 5, 6.5, and almost 2 times lower than in control ‘Charles Binder’, ‘Festiva Maxima’, and ‘Gayborder June’, respectively. The free amino acids and free proline contents in flowers of ‘Charles Binder’ and ‘Festiva Maxima’ kept in NS + S were slightly higher than those in 8-HQC + S, but still significantly lower than in controls.

During senescence there is accumulation of the intermediates of the membrane lipid peroxidation [3]. According to Zhao et al. [3], free radical contents can be considered important inducers of the damage severity in senescing plants. Senescence of many cut flowers is related to an increase in the H₂O₂ content, such as daylily [71], orchids [72], and also peony [3]. In cut daylily flowers, this increase occurred even before the flowers fully developed [71]. Here, in all cultivars kept in water, the H₂O₂ content increased during vase life. Preservatives not only did not prevent this rise
but often intensified it, mostly during the first days of the vase life. Finally, on day 10, the level of H$_2$O$_2$ in the preservative solutions was comparable with controls or even slightly higher. In the NS-treated peony ‘Hongyan Zhenghui’, during the first 8 days after harvest, the levels were significantly lower than in the control, reaching a level similar to that of the control only after 12 days [3]. In rose, the H$_2$O$_2$ level dropped in response to NS [73]; in orchids, AgNO$_3$ reduced the increase of H$_2$O$_2$ level [72]; in cosmos, only the 8-HQC solution significantly limited the accumulation of H$_2$O$_2$ [35]. Pure NS and preservatives with sucrose based on NS and 8-HQC were ineffective [35].

Plants protect their cells against reactive oxygen species through antioxidant defense systems [74]. The activity of protective enzymes increases during senescence. However, Panavas and Rubinstein [71] showed a reduction in the CAT activity during the senescence of cut lily flowers. Here, the CAT activity also dropped in ‘Albert Crousse’ flowers in water, especially during the first 4 days, and remained at a lower level until the end of the vase life. The same pattern was observed in ‘Ursynów’, where the CAT activity in control flowers significantly decreased, reaching the lowest level on day 9, close to zero. On the other hand, in ‘Gayborder June’, the steady increase of the CAT activity was evident. It may be worth pointing out that the initial CAT activity in ‘Ursynów’, with the shortest vase life in water (8 days) was two and three times lower than that in ‘Albert Crousse’ and ‘Gayborder June’, respectively (vase life—13 and 10 days, respectively). In ‘Hongyan Zhenghui’ flowers, the activity levels of all protective enzymes increased at first and then dropped during the late vase period [3]. The same was true in chrysanthemum—the activity of CAT kept increasing until full bloom and later fell dramatically [74]. As for ‘Albert Crousse’, in preservative solutions, with the highest H$_2$O$_2$ content, the CAT activity was also significantly higher than in control flowers. This may have been because the protective enzymes are at first activated to protect flowers from damage when stress conditions induce the ROS production [3]. Moreover, in ‘Ursynów’, flowers in preservatives had CAT activity higher than in controls, which is in line with observations of Zhao et al. [3], where cut peony flowers under the NS treatment showed higher activities of protective enzymes. This may be an indication of their greater ability to eliminate hydrogen peroxide.

5. Conclusions

The incidence of xylem blockage in peony could be modified by holding solutions but the effects of these solutions on vascular occlusions and water balance, as well as on several senescence-related processes, were not directly associated with the flower postharvest longevity. While the preservatives tested here on a set of 14 peony cultivars did not affect their vase life, in a majority of cases they increased flower diameter, intensified petal pigmentation, and improved the general flower appearance. The reaction varied by the cultivar and the preservative. The results show that the postharvest treatment in peony must be individually tailored for each cultivar. As NS gave comparable or at times better results than 8-HQC, it is recommended as a component of preservatives for cut peony flowers.

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References

1. Holloway, P.S.; Buchholz, K. The state of the Alaska peony industry 2012. AFES. Misc. Pub. 2013, 3, 1–8.
2. Xue, J.; Tang, Y.; Wang, S.; Yang, R.; Xue, Y.; Wu, C.; Zhang, X. Assessment of vase quality and transcriptional regulation of sucrose transporter and invertase genes in cut peony (Paeonia lactiflora ‘Yang Fei Chu Yu’) treated by exogenous sucrose. Postharvest Biol. Technol. 2018, 143, 92–101. [CrossRef]
3. Zhao, D.; Cheng, M.; Tang, W.; Liu, D.; Zhou, S.; Meng, J.; Tao, J. Nano-silver modifies the vase life of cut herbaceous peony (Paeonia lactiflora Pall.) flowers. Protoplasma 2018, 255, 1001–1013. [CrossRef]
4. Heuser, C.W.; Evensen, K.B. Cut flower longevity of peony. *J. Am. Soc. Hortic. Sci*. 1986, 111, 896–899.

5. Gast, K.; McLaren, J.; Kampjes, R. Identification of bud maturity indicators for fresh-cut peony flowers. *Acta Hortic*. 2001, 543, 317–325. [CrossRef]

6. Kamenetsky, R.; Barzilay, A.; Erez, A.; Halevy, A.H. Temperature requirements for floral development of herbaceous peony cv. ‘Sarah Bernhardt’. *Sci. Hortic*. 2003, 97, 309–320. [CrossRef]

7. Holloway, P. Peonies as field grown cut flowers in Alaska. *Chronica Hortic*. 2019, 59, 25–29.

8. Halevy, A.; Mayak, S. Senescence and postharvest physiology of cut flowers, part 1. *Hortic. Rev*. 1979, 1, 204–236.

9. van Doorn, W.G. Water relations of cut flowers. *Hortic. Rev*. 1997, 18, 1–85.

10. Mayak, S.; Halevy, A.H. *Senescence in Plants*; CRC Press: Boca Raton, FL, USA, 1980.

11. Pun, U.K.; Ichimura, K. Role of sugars in senescence and biosynthesis of ethylene in cut flowers. *JARQ* 2003, 37, 219–224. [CrossRef]

12. Van Doorn, W.G. Is petal senescence due to sugar starvation? *Plant Physiol.* 2004, 134, 35–42. [CrossRef]

13. Reid, M.S.; Jiang, C.Z. Postharvest biology and technology of cut flowers and potted plants. *Hort. Rev.* 2012, 40, 1–54.

14. da Silva, J.A.T. The cut flower: Postharvest considerations. *J. Biol. Sci.* 2003, 3, 406–442.

15. van Doorn, W.G.; Abadie, P.; Belde, P. Alkylethoxylate surfactants for rehydration of roses and Bouvardia flowers. *Postharvest Biol. Technol.* 2001, 24, 327–333. [CrossRef]

16. Seyed, H.; Farokhzad, A.; Ghasemi, C. Using of preservative solutions to improve postharvest life of Rosa Hybrid cv. Black Magic. *J. Agric. Technol.* 2012, 8, 1801–1810.

17. Redman, P.B.; Dole, J.M.; Maness, N.O.; Anderson, J.A. Postharvest handling of nine specialty cut flower species. *Sci. Hortic.* 2002, 92, 293–303. [CrossRef]

18. Halevy, A.H.; Mayak, S. Senescence and postharvest physiology of cut flowers, part 2. *Hortic. Rev.* 1981, 3, 59–143.

19. Rai, M.; Yadav, A.; Gade, A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.* 2009, 27, 76–83. [CrossRef]

20. Furno, F.; Morley, K.S.; Wong, B.; Sharp, B.L.; Arnold, P.L.; Howdle, S.M.; Bayston, R.; Brown, P.D.; Winship, P.D.; Reid, H.J. Silver nanoparticles and polymeric medical devices, a new approach to prevention of infection. *J. Antimicrob. Chemother.* 2004, 54, 1019–1024. [CrossRef]

21. Sok, N.; Ho, C.M.; Chen, R.; He, Q.Y.; Yu, W.Y.; Sun, H.Z.; Tam, P.K.H.; Chiu, J.F.; Che, C.M. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *J. Proteome Res.* 2006, 5, 916–924. [CrossRef]

22. Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.Y.; et al. Antimicrobial effects of silver nanoparticles. *Nanomed. Nanotechnol. Biol. Med.* 2007, 3, 95–101. [CrossRef]

23. Tran, Q.H.; Nguyen, V.Q.; Le, A.T. Silver nanoparticles: Synthesis, properties, toxicology, applications and perspectives. *Adv. Nat. Sci. Nanos. Nanotechnol.* 2013, 4, 1–20. [CrossRef]

24. Kim, J.H.; Lee, A.K.; Suh, J.K. Effect of certain pre-treatment sub-stances on vase life and physiological character in lily (*Lilium spp.*) *Acta Hortic.* 2005, 673, 307–314. [CrossRef]

25. Solgi, M.; Kafi, M.; Taghavi, T.S.; Naderi, R. Essential oils and silver nanoparticles (SNP) as novel agents to extend vase-life of gerbera (*Gerbera jamesonii* cv. ‘Dune’) flowers. *Postharvest Biol. Technol.* 2009, 53, 155–158. [CrossRef]

26. Lü, P.; He, S.; Li, H.; Cao, J.; Xu, H.L. Effects of nano-silver treatment on vase life of cut rose cv. Movie Star flowers. *J. Food Agric. Environ.* 2010, 8, 1118–1122.

27. Kader, H.H.A. Effects of nanosilver holding and pulse treatments, in comparison with traditional silver nitrate pulse on water relations and vase life and quality of the cut flowers of *Rosa hybrida* L. cv. ‘Tineke’. *World Appl. Sci. J.* 2012, 20, 130–137.

28. Kazemi, M.; Ameri, A. Postharvest life of cut gerbera flowers as affected by nano-silver and acetylsalicylic acid. *Asian J. Biochem.* 2012, 7, 106–111. [CrossRef]

29. Liu, J.P.; Ratnayake, K.; Joyce, D.C.; He, S.G.; Zhang, Z.Q. Effects of three different nano-silver formulations on cut *Acacia holosericea* vase life. *Postharvest Biol. Technol.* 2012, 66, 8–15. [CrossRef]

30. Bahremand, S.; Razmjoo, J.; Farahmand, H. Effects of nano-silver and sucrose applications on cut flower longevity and quality of tuberose (*Polianthus tuberosa*). *Int. J. Hortic. Sci. Technol.* 2014, 1, 67–77.

31. Hashemabadi, D. The role of silver nano-particles and silver thiosulfate on the longevity of cut carnation (*Dianthus caryophyllus*) flowers. *J. Environ. Biol.* 2014, 35, 661–666.
32. Jedrzejuk, A.; Rabiza-Świder, J.; Skutnik, E.; Łukaszewska, A. Some factors affecting longevity of cut lilacs. *Postharvest Biol. Technol.* 2016, 111, 247–255. [CrossRef]

33. Naing, A.H.; Win, N.M.; Hang, J.S.; Lim, K.B.; Kim, C.K. Role of nano-silver and the bacterial strain *Enterobacter cloacae* in increasing vase life of cut carnation ‘Omea’. *Front. Plant Sci.* 2017, 8, 1590. [CrossRef] [PubMed]

34. Rabiza-Świder, J.; Skutnik, E.; Jedrzejuk, A.; Rochala-Wojciechowska, J. Nanosilver and sucrose delay the senescence of cut snapdragon flowers. *Postharvest Biol. Technol.* 2020, 165, 111165. [CrossRef]

35. Skutnik, E.; Jedrzejuk, A.; Rabiza-Świder, J.; Rochala-Wojciechowska, J.; Łatkowska, M.; Łukaszewska, A. Nanosilver as a novel biocide for control of senescence in garden cosmos. *Sci. Rep.* 2020, 10, 10274. [CrossRef]

36. Eason, J.; Pinkney, T.; Heyes, J.; Brash, D.; Bycroft, B. Effect of storage temperature and harvest bud maturity on bud opening and vase life of *Paonia lactiflora* cultivars. *N. Z. J. Crop Hortic. Sci.* 2002, 30, 61–67. [CrossRef]

37. Yu, X.-N.; Guo, P.-P.; Lu, G.-P.; Zhang, Q.-X. Optimum harvesting time of herbaceous peony buds for cutting flowers. *J. For. Res.* 2011, 22, 137–140. [CrossRef]

38. He, S.; Joyce, D.C.; Irving, D.E.; Faragher, J.D. Stem end blockage in cut Grevillea ‘Crimson Yul-lo’ inflorescences. *Postharvest Biol. Technol.* 2006, 41, 78–84. [CrossRef]

39. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 1944, 153, 357–380.

40. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976, 72, 248–254.

41. Rosen, H. A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.* 1957, 67, 10–15. [CrossRef]

42. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Physiol. Plant.* 1973, 39, 205–207. [CrossRef]

43. Goth, L. A simple method for determination of serum catalase activity and revision of reference range. *Clin. Chim. Acta* 1991, 196, 143–151. [CrossRef]

44. van Doorn, W.G.; Woltering, E.J. Physiology and molecular biology of petal senescence. *J. Exp. Bot.* 2008, 59, 453–480. [CrossRef] [PubMed]

45. Lin, H.; Li, H.; Lin, S.; Xu, M.; Liu, J.; Li, Y.; He, S. Improving the postharvest performance of cut spray ‘Prince’ carnations by vase treatments with nano-silver and sucrose. *J. Hortic. Sci. Biotechnol.* 2019, 94, 513–521. [CrossRef]

46. van Doorn, W.G. Vascular occlusion in cut flowers I. General principles and recent advances. *Acta Hortic.* 1999, 482, 59–64. [CrossRef]

47. Jedrzejuk, A.; Rochala, J.; Zakrzewski, J.; Rabiza-Świder, J. Identification of xylem occlusions occurring in cut clematis (*Clematis L.*, fam. *Ranunculaceae* Juss.) stems during their vase life. *Sci. World J.* 2012, 2012, 749281.

48. Li, H.; Huang, X.; Li, J.; Liu, J.; Joyce, D.; He, S. Efficacy of nano-silver in alleviating bacteria-related blockage in cut rose cv. Movie Star stems. *Postharvest Biol. Technol.* 2012, 74, 36–41. [CrossRef]

49. Rabiza-Świder, J.; Skutnik, E.; Jedrzejuk, A. The effect of preservatives on water balance in cut clematis flowers. *J. Horticul. Sci. Biotechnol.* 2017, 92, 270–278. [CrossRef]

50. Liu, J.; He, S.; Zhang, Z.; Cao, J.; Lv, P.; He, S.; Cheng, G.; Joyce, D.C. Nano-silver pulse treatments inhibit stem-end bacteria on cut gerbera cv. Ruikou flowers. *Postharvest Biol. Technol.* 2009, 54, 59–62. [CrossRef]

51. Loubaud, M.; van Doorn, W.G. Wound-induced and bacteria-induced xylem blockage in rose, *Astilbe*, and *Viburnum*. *Postharvest Biol. Technol.* 2004, 32, 281–288. [CrossRef]

52. van Meeteren, U. Role of air embolism and low water temperature in water balance of cut chrysanthemum flowers. *Sci. Hort.* 1992, 51, 275–284. [CrossRef]

53. van Doorn, W.G.; Reid, M.S. Vascular occlusion in stems of cut rose flowers exposed to air. Role of xylem anatomy and rates of transpiration. *Physiol. Plant.* 1995, 93, 624–629. [CrossRef]

54. van Doorn, W.G. Water relations of cut flowers: An update. *Hortic. Rev.* 2002, 40, 55–106.

55. Liu, J.P.; Zhang, Z.Q.; Li, H.M.; Xian, X.J.; Huang, X.M.; He, S.G. Nano-silver treatments alleviated bacterial blockage in cut carnation stems. *Acta Hortic. Sin.* 2014, 41, 131–138.

56. Li, H.B.; Li, H.M.; Liu, J.P.; Luo, Z.H.; Joyce, D.C.; He, S.G. Nano-silver treatments reduced bacterial colonization and biofilm formation at the stem-ends of cut gladiolus ‘Eerde’ spikes. *Postharvest Biol. Technol.* 2017, 123, 102–111. [CrossRef]
57. Rabiza-Świder, J.; Skutnik, E.; Jedrzejuk, A. The effect of a sugar-containing preservative on senescence-related processes in cut clematis flowers. Not. Bot. Horti Agrobot. Cluj Napoca 2019, 47, 432–440. [CrossRef]
58. Eason, J.R.; de Vréré, L.A.; Somerfield, S.D.; Heyes, J.A. Physiological changes associated with Sandersonia aurantiaca flower senescence in response to sugar. Postharvest Biol. Technol. 1997, 12, 43–50. [CrossRef]
59. Walton, E.F.; Boldingh, H.L.; McLaren, G.F.; Williams, M.H.; Jackman, R. The dynamics of starch and sugar utilization in cut peony (Paeonia lactiflora Pall.) stems during storage and vase life. Postharvest Biol. Technol. 2010, 58, 142–146. [CrossRef]
60. Kamenetsky, R.; Dole, J. Herbaceous peony (Paeonia): Genetics, physiology and cut flower production. Floricult. Ornam. Biotechnol. 2012, 6, 62–77.
61. Olley, C.M.; Joyce, D.C.; Irving, D.E. Changes in sugar, protein, respiration, and ethylene in developing and harvested Geraldton waxflower (Chamaelaurium uncinatum) flowers. N. Z. J. Crop Hortic. Sci. 1996, 24, 143–150. [CrossRef]
62. Stephenson, P.; Rubinstein, B. Characterization of proteolytic activity during senescence in daylilies. Physiol. Plant. 1998, 104, 463–473. [CrossRef]
63. Pak, C.; van Doorn, W.G. Delay of Iris flower senescence by protease inhibitors. New Phytol. 2005, 165, 473–480. [CrossRef][PubMed]
64. Azeez, A.; Sane, A.P.; Bhatnagar, D.; Nath, P. Enhanced expression of serine proteases during floral senescence in Gladiolus. Phytochemistry 2007, 68, 1352–1357. [CrossRef][PubMed]
65. Lerslerwong, L.; Ketsa, S.; van Doorn, W.G. Protein degradation and peptidase activity during petal senescence in Dendrobium cv. Khao Sanan. Postharvest Biol. Technol. 2009, 52, 84–90. [CrossRef]
66. Zhang, L.; Becker, D.F. Connecting proline metabolism and signaling pathways in plant senescence. Front. Plant Sci. 2015, 6, 1–8. [CrossRef]
67. Kumar, N.; Pal, M.; Srivastava, G.C. Proline metabolism in senescing rose petals (Rosa hybrida L. ‘First Red’). J. Hortic. Sci. Biotechnol. 2009, 84, 536–540. [CrossRef]
68. Kazemi, M.; Aran, M.; Zamani, S. Extending the vase life of lisianthus (Eustoma grandiflorum Mariachi cv. Blue) with different preservative. Am. J. Plant Physiol. 2011, 6, 167–175. [CrossRef]
69. Hassan, F.A.S.; Ali, E.F.; El-Deeb, B. Improvement of postharvest quality of cut rose cv. ‘First Red’ by biologically synthesized silver nanoparticles. Sci. Hortic. 2014, 179, 340–348. [CrossRef]
70. Chakrabarty, D.; Chatterjee, J.; Datta, S.K. Oxidative stress and antioxidant activity as the basis of senescence in chrysanthemum florets. Plant Growth Regul. 2007, 53, 107–115. [CrossRef]