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Postharvest treatments to optimize long-term storage of cut peony flowers

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Abstract: Cut peonies have limited time during which they can be harvested and commercialized. Interest in extending this period through long-term storage, both in dry and wet conditions, to obtain more profits is constantly growing. Sixty cut peony stems (cv. Alertie) were pulse-treated 24 h after harvest with 10 µM thidiazuron, 500 µL L^{-1} 1-methylcyclopropene, 10 mM glycerol, and a combination of them (All) in a refrigerated cell at 4 °C, where the flowers were stored with wet preservation for 15 and 30 days. After these periods, flowers were maintained at 20 °C, and their quality was assessed through *in vivo* and destructive analyses at 0, 4, 12, and 14 days. Thirty days of storage strongly inhibited flower opening, in contrast to the shorter preservation period. After 15 days of storage and during preservation at room temperature, flowers treated with glycerol showed less water loss and senescence symptoms. Nitrate and phenol concentrations increased in all treatments after storage. In conclusion, wet storage at 4 °C for 15 days was more suitable for the cultivar tested in the present study and it also allowed the identification of treatments that involved the use of glycerol as promising for long-term preservation of peonies and their vase-life.

Keywords: *Paeonia lactiflora* Pall.; 1-methylcyclopropene; thidiazuron; glycerol; preservation.

1. Introduction

Peonies are well known for their ornamental value and medicinal properties. The *Paeonia* genus (*Paeoniaceae*) comprises 35 species, divided into woody and herbaceous types. Since the 1980s, herbaceous peonies, mostly derived from *Peonia lactiflora* Pall., have become one of the most expensive and widely produced cut flowers because of their desirable characteristics (Song et al., 2021; Sun et al., 2022). The Netherlands is the main producer of herbaceous peonies, followed by Italy, Israel, and France (Kamenetsky-Goldstein and Yu, 2022). Herbaceous peonies are perennial deciduous plants native to Asia but are now cultivated worldwide, producing large, showy flowers in a range of colors, including white, pink, red, and purple (Zhang, 2021; Zhou et al., 2021). Peonies are one of the 20 most important cut flower sell all over the world (Meir and Philosoph-Hadas, 2021).

Despite their great importance in the flower trade, peonies have a limited window of time during which they can be harvested and sold in the flower market. Flowers are typically at their peak from late spring to early summer (Kamenetsky-Goldstein and Yu, 2022), and they have a relatively short vase life of only a few days, typically lasting only seven days after the exit from the supply chain (depending on the cultivar, the stage of harvesting, and the environment) (Rabiza-Świder et al., 2020).

Currently, the interest in extending the flowering season through long-term storage to obtain more profits is constantly growing. Depending on the variety, peonies have shown a vase life of up to 8 days

after 4 weeks of storage, whereas longer periods negatively affect bud opening and flower size (Kamenetsky and Dole, 2012). Nonetheless, other parameters must be considered to extend the vase life after long low-temperature handling. Several studies have been performed considering not only the time of storage, but also different temperatures along with the condition of preservation (dry or wet preservation) (Jahnke et al., 2020; Xue et al., 2019)

The storage of carbohydrates in peony buds, particularly starch accumulated during development, represents a pivotal supply of energy that plays a key role in controlling flowering time and vase life (Walton et al., 2010). Dehydration caused by embolized xylem vessels or bacteria can also negatively affect the flower opening of peonies and cause excessive water loss and flower wilting (da Silva, 2003). Ethylene is the most important plant hormone associated with senescence, although peonies appear to be less sensitive to it than other cut flower species. However, it is advisable to avoid high ethylene concentration during preservation (Kamenetsky and Dole, 2012).

Research on new methods to improve the storage, potential flower market, and vase life of herbaceous peonies is an area of enormous interest. In the present study, several treatments were applied, combined with two different long-term cold storage under wet conditions (15 and 30 days). Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea), a substituted phenylurea with high cytokinin-like activity that prevents leaf yellowing (Macnish et al., 2010; Ferrante et al., 2012; Bulgari et al., 2015), 1-methylcyclopropene, a non-toxic gaseous compound that inhibits ethylene action by binding to hormone receptors in an irreversible manner, thus preventing senescence (Ferrante and Francini, 2006; Naing et al., 2022), and glycerol, an anti-transpirant agent that enhances cell osmotic potential, reducing water loss (Shanan and Shalaby, 2011; Bulgari et al., 2015), were utilized as pulsed treatments, alone or in combination, to investigate their action in prolonging the preservation and quality of cut peonies.

2. Materials and Methods

2.1. Plant material

Cut peony flowers (*Peonia lactiflora* Pall., "Alertie") were provided by Floorcoop Sanremo (Taggia, IM, Italy) and then they were wet transported at 4 °C to University of Milan and treated in the 24 hours (h) after harvest as described below.

2.1.1. Postharvest flower treatments

Sixty stems were chosen for uniform growth and development at tight bud (TB) stage, according to Sun et al. (2022) (Figure 1), then they were cut (1 cm above the previous cutting) and divided in five groups, pulsed treated for 24 h, at 4 °C as follows: distilled water (control), 10 µM thidiazuron (TDZ 10 µM), 500 µL L^{-1} 1-methylcyclopropene (1-MCP), 10 mM glycerol (Gly 10 mM), and a combination of the different treatments (All). TDZ was dissolved in 0.1 mM KOH and then pH adjusted at 6 with 36% HCl. The 1-MCP was applied by fumigation in an airtight container, dissolving 1.6 g of powder in 250 mL of distilled water. Concentrations were chosen based on previous studies (Ferrante et al., 2012; Bulgari et al., 2015). Flowers were separated in several glass bottles (1.5 L) containing different solutions according to the treatment (distilled water, TDZ 10 µM, Gly 10 mM, and a combination of both) and were wet stored in dark conditions at 4 °C for 15 and 30 days, each group composed of 30 stems, six stems for each treatment. Once out of cold room, flowers were recut and divided into two sub-groups (15 stems, three stems for each treatment): one was utilized to perform non-destructive analyses, and the other was used for destructive analyses. Cut flowers were kept in a controlled environment (temperature 20 °C, relative humidity 60-70%, and light intensity 10-15 µmol m^{-2} s^{-1} PPFD for 12 h per day using white, fluorescent tube lamps) for up to 14 days.

Cut flowers quality was assessed every day by visually determining in every replicate of each treatment the presence or absence of senescence symptoms; final values were expressed as the mean number of days between the exit from the storage and the appearance of leaf yellowing, flower senescence, and

first petal loss. Data were recorded for every replicate of each treatment (n = 6). The diameter of every single flower for each treatment was measured daily with a digital caliper (n = 6).

2.2. Non-destructive analyses

2.2.1. Loss of fresh weight

Fresh weight (FW) of the stems for each condition was measured immediately after the exit from cold storage and then after 4, 12, and 14 days. FW loss was estimated as follows:

$$\frac{FW_{t0} - FW_{tx}}{FW_{t0}} \times 100$$

Where "t0" refers to the FW measured after 15 or 30 days of cold storage, and "tx" to all the other time points considered (n = 3).

2.2.2. Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured using a hand-portable fluorimeter (Handy-PEA, Hansatech Instruments) by randomly choosing three leaves in the middle portion of the stems for each condition (n = 3). Before measurement, leaves were dark-adapted with leaf clips (diameter 4 mm) for 30-40 min and then exposed to a saturating light (3000 μmol m^{-2} s^{-1}) provided by an array of three high-intensity light-emitting diodes for 1 s. Information about the structural and functional status of the photosynthetic apparatus was provided by the measured parameters and those derived from the JIP test calculation: the maximum quantum efficiency of photosystem II (F_v/F_m) and the performance index (PI). Analyses were performed prior to the treatments (ti) and at 0, 4, 12 and 14 days after 15 and 30 days of cold storage.

2.3. Destructive analyses

2.3.1. Chlorophyll concentration

Chlorophyll was extracted from leaf tissues using 5 mL of 99.9% (v/v) methanol. Three leaf disc samples (5 mm diameter, 30 mg FW), obtained from random leaves taken from the middle portion of the stem of each treatment, were kept in a dark room for 24 h at 4 °C. After that, absorbance readings were measured with a spectrophotometer (Thermo Italy) at 665.2 and 652.4 nm, and pigment levels were calculated by Lichtenthaler's formula. The results were expressed as μg of pigments g^{-1} FW (Lichtenthaler, 1987). The samplings were performed prior to the treatments (ti) and at 0, 4, 12, and 14 d after 15 and 30 days of cold storage. The analysis was conducted in biological triplicates (n = 3).

2.3.2. Phenolic index and anthocyanins concentration

Total phenolics and total anthocyanin were determined from leaf disc samples (5 mm diameter, 30 mg FW) obtained from random leaves taken from the middle portion of the stem of each treatment. Leaf samples were transferred to a tube containing 3 mL of methanol acidified with hydrochloric acid (1% v/v) and were kept in dark room for 24 h at 4 °C. Absorbance readings were taken with a spectrophotometer at 320 nm for total phenols (Ke and Saltveit, 1989), and at 535 nm for anthocyanin (Klein and Hagen, 1961). Total phenolics are expressed as phenolic index ABS_{320nm} g^{-1} FW. Anthocyanins concentrations are expressed in mg cyanidin-3-glucoside equivalents 100 g^{-1} FW using a molar extinction coefficient (ε) of 29,600 L M^{-1} cm^{-1}. The samplings were performed prior to treatments (ti) and at 0, 4, 12, and 14 days after 15 and 30 days of cold storage. The analyses were conducted in biological triplicates (n = 3).

2.3.3. Nitrate and total sugar concentration

Fresh leaf tissue was homogenized in distilled water (1 g of fresh tissue per 5 mL of distilled

water). The homogenate was centrifuged at 4000 rpm for 15 min at 20 °C, and the recovered supernatant was used for colorimetric analysis. Twenty µL of the extract were added to 80 µL of 5% (w/v) salicylic acid in concentrated H_2SO_4. Subsequently, 3 mL 1.5 N NaOH was added. The samples were cooled to room temperature, and the absorbance at 410 nm was measured using a spectrophotometer. Nitrate concentration was calculated using a KNO_3 standard calibration curve. The nitrate concentration was expressed as mg of NO_3-N per kg of fresh weight (Cataldo et al., 1975). The samplings were performed prior to the treatments (ti) and at 0, 4, 12, and 14 days after 15 and 30 days of cold storage. The analyses were conducted in biological triplicate (n = 3).

Total sugar concentration was determined using the anthrone method. Anthrone (200 mg) were melted in 100 mL of H_2SO_4 and shaken for 30-40 min. Subsequently, 200 µL of sample extract were added to 1 mL of anthrone solution. The samples were incubated at 95 °C for 5 min and then cooled on ice. Absorbance readings were measured at 620 nm and a calibration curve was constructed using glucose standards (Yemm and Willis, 1954). The samplings were performed prior to the treatments (ti) and at 0, 4, 12, and 14 d after 15 and 30 d of cold storage. Analysis was conducted in biological triplicate (n = 3).

2.4. Statistical analysis

Data are reported as the mean of the replicates. Statistical analyses were performed using GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, California, USA, www.graphpad.com). Two-way ANOVA was performed, followed by Tukey's post-hoc test ($p < 0.05$), considering the variables of treatment, time, and their interaction. Additional information has been reported in the figure legends.

3. Results

3.1. Loss of FW, stems quality and development of inflorescences

Loss of FW was measured at 4, 12, and 14 days of vase-life, after 15 and 30 days of cold storage. After 15 days of cold storage (Figure 2A), the interaction Time × Treatment was significant ($p < 0.05$). In all the treatments, the loss of FW increased over time; however, the best results were always recorded in the 10 mM Gly and in the combined treatment (All), although this was significantly different from the control only after 14 days. After 30 days (Figure 2B), the interaction was not significant. The loss of FW increased as the experiment progressed. Differences were observed for each treatment at different time points.

Regarding quality, the only two senescence symptoms recognisable were flower wilting and petal browning (Figure 3B) both after 15 and 30 days of storage. After 15 days of storage, flowers treated with TDZ and 1-MCP showed flower wilting and petal browning after 7-8 days, similar to the control (Table 1). Treatment with 10 mM Gly and All maintained a better quality: flower wilting and petal browning appeared after 11 days (Table 1), and 50% and 30% of the flowers, respectively for 10 mM Gly and All, did not show any senescence symptoms until 14 days (Figure 3A). After 30 days of storage, all treatments showed signs of senescence from day 7 onwards (data not shown). The development of flowers was not affected by different treatments (data not shown). Nevertheless, 30 days of storage severely affected flower opening, which was strongly inhibited compared to 15 days of storage (approximately -70% for each sampling point) (Figure 4). Considering the negative impact of the longest preservation period on complete flower opening, only the results for 15 days of storage will be further considered.

3.2. Chlorophyll a fluorescence

Chlorophyll *a* fluorescence was measured at different time points (0, 4, 12, and 14 days) after 15 days of cold storage to determine the health status of the leaves on the cut flower stems. Among the different parameters, F_v/F_m (whose optimal value is around 0.83 in non-stressed plants) (Maxwell and Johnson, 2000) and PI (which indicates the capability of plants to tolerate environmental adversities)

(Kalaji et al., 2011) were considered for evaluating treatment effects. While no differences were found in the functionality of photosystem II (Figure 5A), PI values were always higher in the leaves of flowers treated with 10 µM TDZ (Figure 5B). Until day 4, all the treatments had PI values greater than the control (although not all were significantly greater), but from day 12 values of 1-MCP and 10 mM Gly tended to strongly decrease, reaching final results similar to the control condition.

3.3. Destructive analyses on leaves

The total chlorophyll concentration (Figure 6A) at the beginning of the experiment was around 1.5 µg mg^{-1} FW. All treatments appeared to maintain the same level during the 14 days considered, and no significant results were found.

Regarding the phenolic index (Figure 6B), the interaction Time × Treatment was not significant. All treatments showed an increase in phenolic compounds after 15 days of cold storage (although the increase was not always significant), and the concentration remained constant during the period at room temperature. The Time × Treatment interaction was significant for anthocyanin concentration ($p < 0.05$) (Figure 6C). Leaves under the control conditions showed a higher concentration of anthocyanins immediately after the end of the first cold storage period, which tended to decrease over time. In contrast, no changes were observed in any of the other treatments.

The total sugar (Figure 7A) and nitrate (Figure 7B) concentrations were also measured. While no significant differences were found for the former, the interaction Time × Treatment was significant in the latter. In general, all treatments showed higher concentrations of nitrates at the end of the experiment compared to the beginning (ti). The highest value was recorded in the leaves of control plants after 4 days at 20 °C. It is interesting to note that flowers under the combination of various treatments (All) showed an increase in nitrate accumulation after the end of cold storage (0 days after 15 days of storage), but then the level tended to decrease as the experiment progressed (Figure 7B).

Table 1. Days before the appearance of petal browning and flower wilting in every flower of each treatment (expressed as mean values) stored for 15 days at 4 °C, which were the only main symptoms of senescence recognizable. Different letters indicate significant differences among treatments after one-way ANOVA ($p < 0.05$) ($n = 6$).

	Petal browning and flower wilting				
	Control	10 µM TDZ	1-MCP	10 mM Gly	All
Days	7.5[b]	7.8[b]	8.2[b]	11.0[a]	10.8[a]

Figure 1. Plant material. Flowers (TB stage) in refrigerator cell (4 °C).

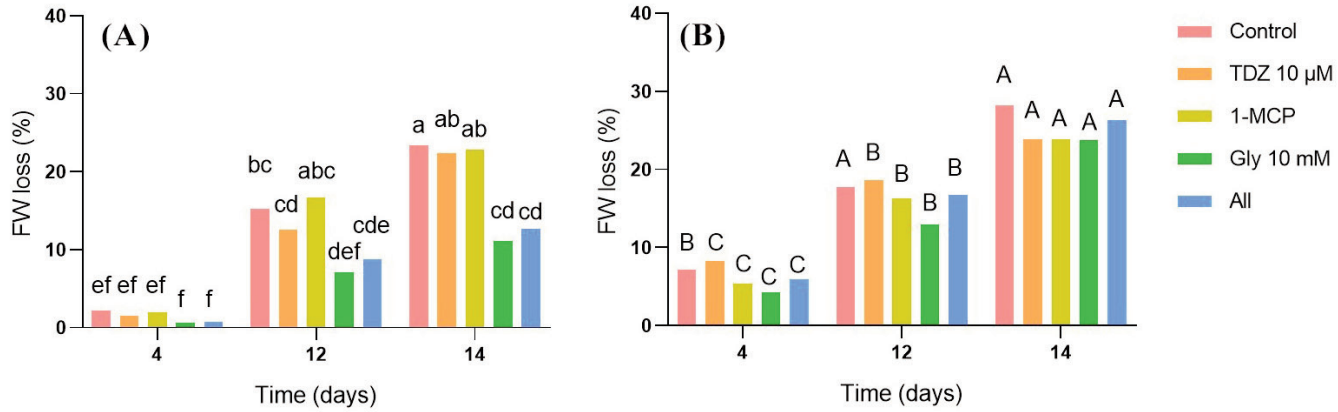

Figure 2. Percentage of loss of FW after 15 (A) and 30 (B) days of cold wet storage at 4 °C followed by up to 14 days at ambient temperature. After 15 days of cold storage the interaction Time × Treatment was significant. Different letters indicate significant differences after two-way ANOVA ($p < 0.05$) ($n = 3$). After 30 days of cold storage the interaction Time × Treatment was not significant. Different uppercase letters indicate significant differences between the time points for each treatment after two-way ANOVA ($p < 0.05$) ($n = 3$).

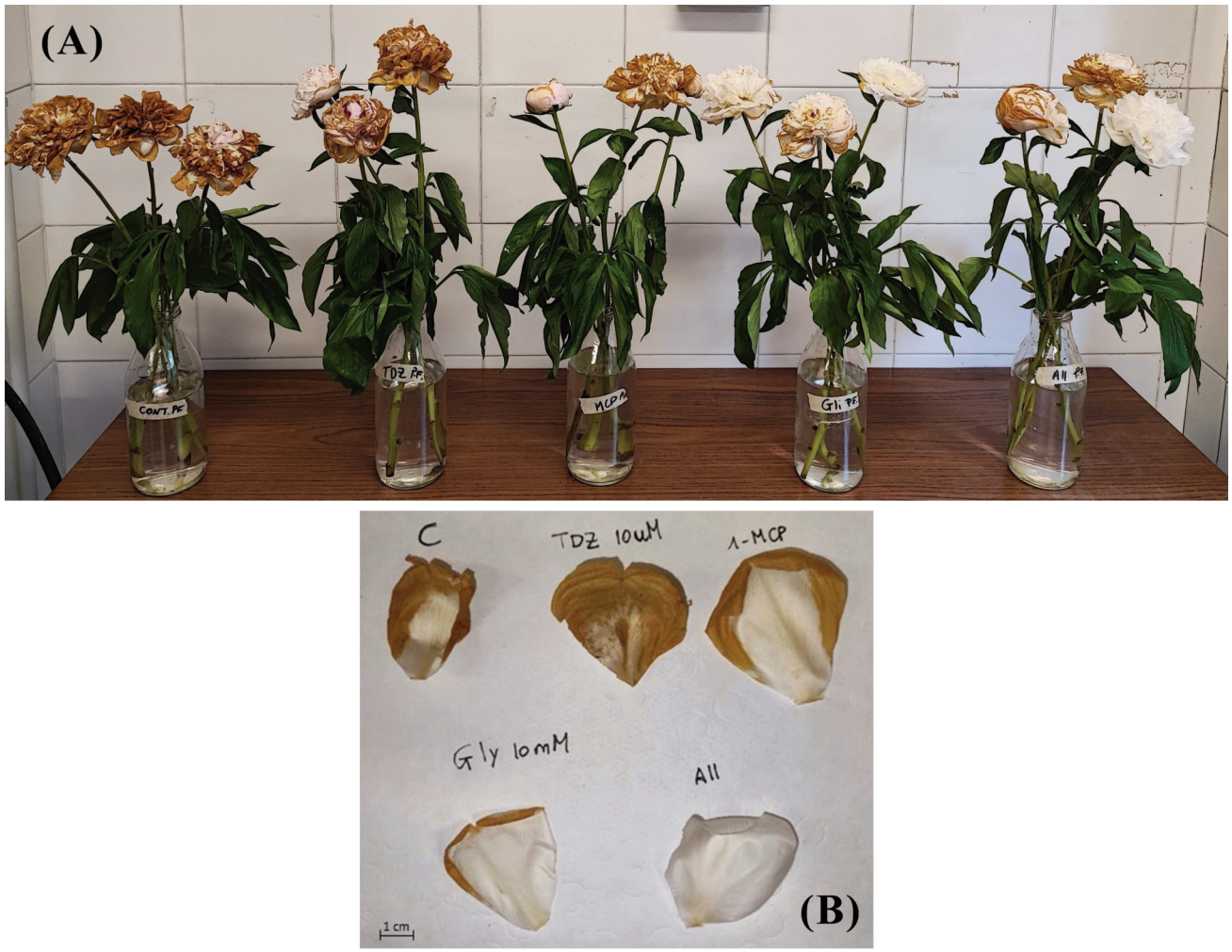

Figure 3. Quality of peony cut flowers after 15 days storage (A). Treatments (from left to right): control, 10 µM TDZ, 500 µL L-1 1-MCP, 10 mM Gly, and All (combination of treatments). External petals (B) after 14 days at 20 °C.

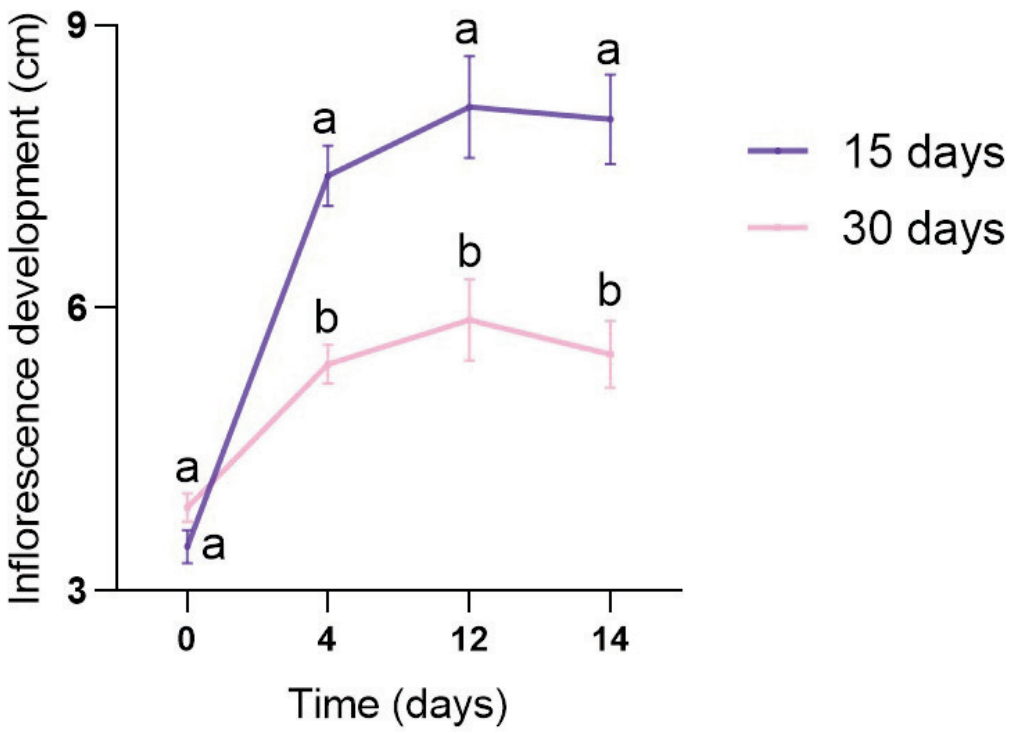

Figure 4. Flower opening. Development of inflorescences across time between long storage periods (15 days – blue line - 30 days – pink line). Considering the non- significant results obtained among treatments, results are expressed as the mean value of the records of all treatments of same storage and time point. Different letters indicate significant differences among the two cold storage periods at each timepoint after one-way ANOVA ($p < 0.05$) ($n = 15$).

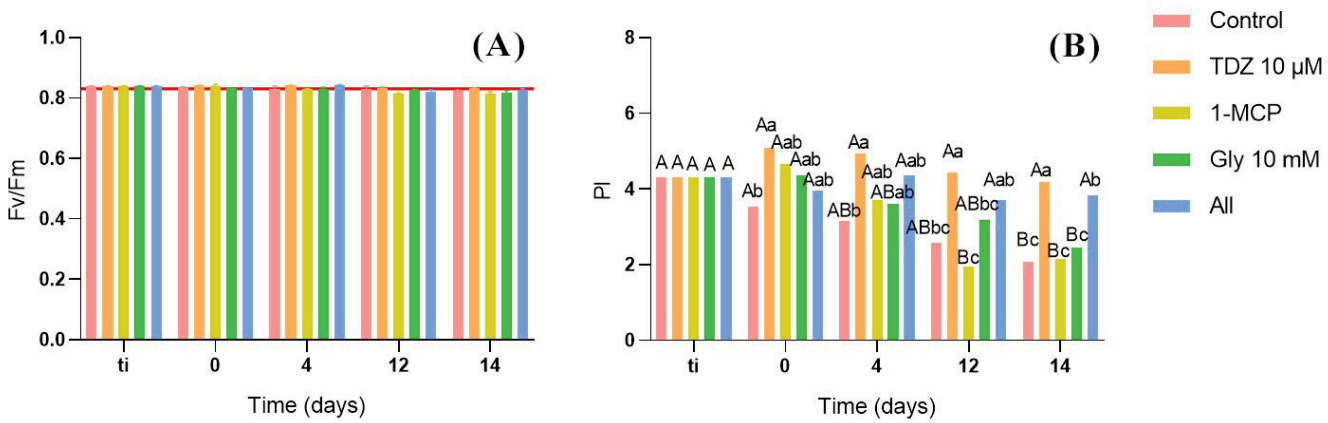

Figure 5. Maximum quantum efficiency of photosystem II (Fv/Fm) (A) and performance index (PI) (B) prior to treatments (ti) and after 15 days cold storage (0, 4, 12, and 14 days at 20 °C). The red line indicates the optimal threshold of 0.83 for Fv/Fm. The interaction Time × Treatment was not significant. In PI (b) Different uppercase letters indicate significant differences between the time points for each treatment after two-way ANOVA ($p < 0.05$) ($n = 3$). Different lowercase letters indicate significant differences among treatments at the same timepoint after two-way ANOVA ($p < 0.05$) ($n = 3$).

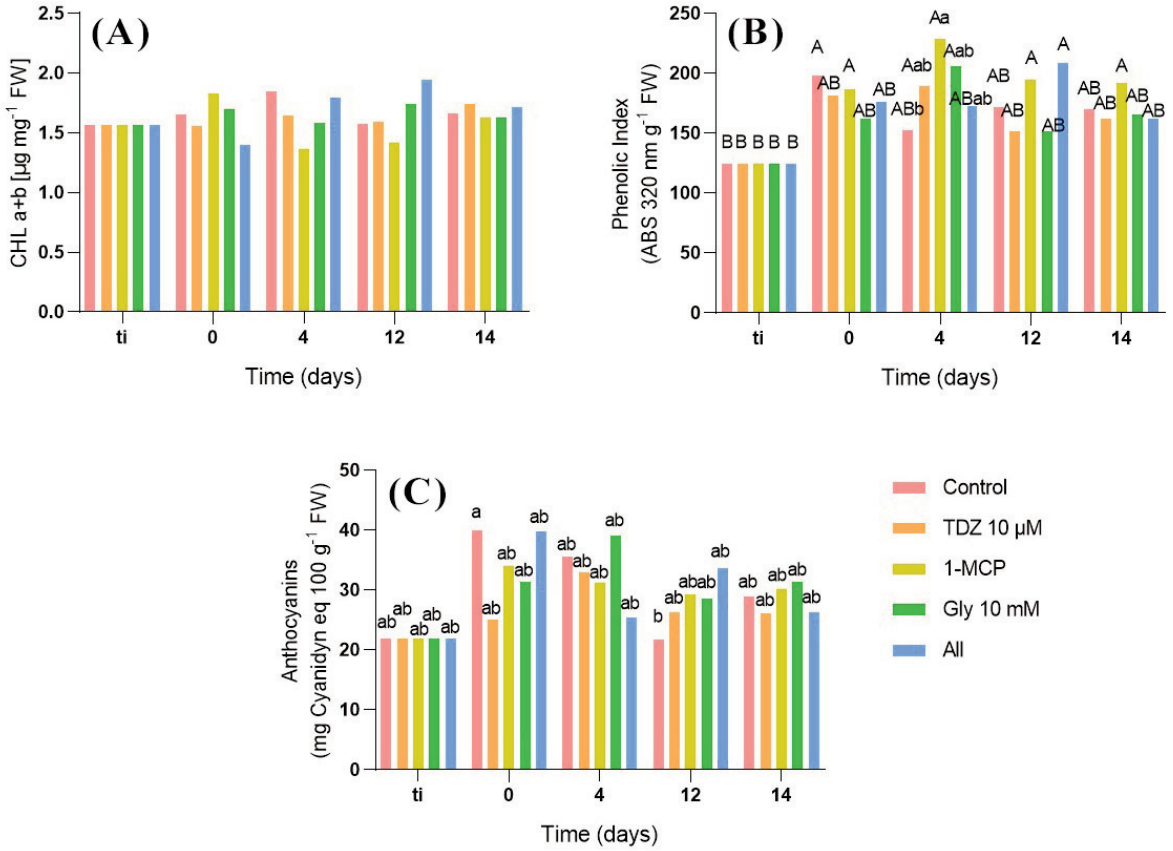

Figure 6. The concentration of chlorophyll a+b (A), phenolic index (B), and anthocyanins (C) in leaves of the different treatments after 15 days of cold storage. Results are shown previous to the treatment (ti) and after 0, 4, 12, and 14 days at 20 °C. No significant results were found for chlorophyll concentration (A). In the phenolic index (B) the interaction Time × Treatment was not significant. Different uppercase letters indicate significant differences between the sampling points for each treatment after two-way ANOVA ($p < 0.05$); Different lowercase letters indicate significant differences among treatments at the same sampling point after two-way ANOVA ($p < 0.05$) ($n = 3$). In anthocyanins (C) the interaction Time × Treatment was significant. Different letters indicate significant differences after two weeks of cold storage after two-way ANOVA ($p < 0.05$) ($n = 3$).

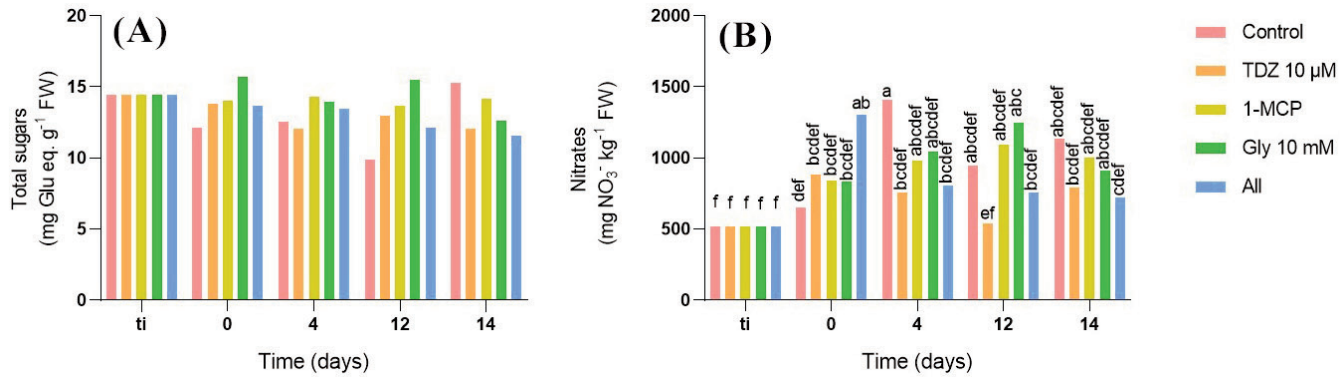

Figure 7. The concentration of total sugars (A) and nitrates (B) in leaves of the different treatments after 15 days of cold storage. Results are shown previous to the treatment (ti) and after 0, 4, 12, and 14 days at 20 °C. No significant results were found for total sugars concentration (A). In nitrates (B) the interaction Time × Treatment was significant. Different letters indicate significant differences after two weeks of cold storage after two-way ANOVA ($p < 0.05$) ($n = 3$).

4. Discussion

Peonies are among the most economically important cut flowers, but their short lifespan in the market poses a problem. Therefore, the search for new preservation methods to prolong the availability of these flowers is of fundamental importance (Shahri and Tahir, 2011; Jahnke et al., 2020; Sun et al., 2022). Chemical preservatives typically used for extending peony preservation include water, sugar, fungicides, inorganic salts, organic acids, plant growth regulators, and other beneficial substances (Kamenetsky-Goldstein and Yu, 2022). In this study, several pulse treatments were combined with cold storage under wet conditions for 15 and 30 days. The results showed that 30 days of preservation strongly inhibited flower opening, in contrast to the shorter period (15 days) in which all flowers reached the final stage of development before the appearance of senescence symptoms, regardless of the treatment application. This appears to be different from another study in which flower size was reduced or incomplete after only 8 weeks of storage compared to fresh-cut peonies (Walton et al., 2010). This might be due to the different temperature chosen in the two experiments and thus its effect on sugars metabolism. In particular, in the study carried out by Walton et al. (2010) peony flowers were stored at a lower temperature (0 °C) than that chosen in our experiment (4 °C). Moreover, the different behavior observed might also be related to the diverse tested cultivars.

Interesting results regarding water losses were also achieved after 15 days of preservation as stems treated with 10 mM Gly and All showed lower FW losses during the entire experiment. Glycerol plays a key role in enhancing plant osmotic potential and reducing and preventing water loss (Chen and Jiang, 2010). This could also explain the delay (4 days) in the appearance of petal browning in treatments that included Gly. Glycerol, together with other polyols such as sugars, salts, and quaternary ammonium compounds, is an important humectant which maintains treated plants in a plasticized state (Joyce and Faragher, 2012). The effectiveness of glycerol added to the preservation solution has been demonstrated in different cut flowers and foliage, such as ruscus, eucalyptus, *Monstera deliciosa*, and chrysanthemum (Bulgari et al., 2015; Campbell et al., 2000; Shanan and Shalaby, 2011; Amin, 2017).

Even if flowers treated with TDZ showed higher PI values, petals started to show severe senescence symptoms after seven days (like control and 1-MCP). This result on peony appears in contrast to a recent study performed on cut ranunculus stem (*Ranunculus asiaticus* L.), in which the effectiveness of TDZ and 1-MCP in prolonging vase life has been demonstrated (Cavallaro et al., 2023). Even if ethylene levels were not measured, we can speculate that this plant hormone does not play a key role in senescence symptoms (Kamenetsky and Dole, 2012).

Nitrate accumulation after preservation could be explained by the lower metabolic activity occurring during long cold storage. Cold storage limits transpiration, flower opening, and consumption of storage compounds (i.e., starch) (Shahri et al., 2011; Skutnik et al., 2020), causing a slowdown in metabolism and the key enzymes involved in this process (i.e., nitrate reductase) (Ferrante and Reid, 2006). All treatments showed an increase in nitrate concentration after 15 days of cold storage. However, with the exception of the control plants which showed a higher concentration of nitrates even after 4 days from 15 days of storage, all other treatments maintained a stable level of nitrates during preservation at room temperature.

Phenolic compounds play a vital role in plant defense mechanisms against various biotic and abiotic stresses and are particularly fundamental in the response to oxidative damage that occurs during flower senescence. The application of postharvest conditions, such as long storage and low temperature, stimulates the production of these phenolic compounds, even if there are few changes in antioxidant composition due to cold treatment (López-Martínez et al., 2020). All treatments showed an increase in phenolic compounds after storage, which remained stable during the experimental period.

5. Conclusions

Our findings suggest that in the tested cultivar (*P. lactifloria*, "Alertie") thirty days of cold wet stor-

age strongly inhibited the metabolism, resulting in a failure or incomplete flower opening during the subsequent period at room temperature. Cold wet storage for 15 days, instead, allowed for the identification of treatments that involved the use of glycerol, which showed to be promising for the long-term preservation of peonies and their vase life. Therefore, it is extremely important to consider the specific cultivar, together with the appropriate period of preservation and treatment.

In the future, it would be interesting to perform more specific analyses to obtain additional information about the senescence process (color of flower, leaf gas exchanges, senescence-associated enzymes, etc.) to better understand the contribution of glycerol in prolonging cut peony flowers vase life.

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Conflicts of Interest: The authors declare no conflict of interest.

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