agronomy

MDPI

Article

Extending Cut *Paeonia Lactiflora* Pall. Storage Duration Using Sub-Zero Storage Temperatures

Nathan J. Jahnke [1,*], John M. Dole [1,*], Ben A. Bergmann [1], Guoying Ma [2] and Penelope Perkins-Veazie [3]

[1] Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, USA; bergmann@ncsu.edu
[2] Plants for Human Health Institute, North Carolina Research Campus, Kannapolis, NC 28081, USA; guoying_ma@ncsu.edu
[3] Department of Horticultural Science, Plants for Human Health Institute, North Carolina Research Campus, Kannapolis, NC 28081, USA; penelope_perkins@ncsu.edu
* Correspondence: njjahnke@ncsu.edu (N.J.J.); jmdole@ncsu.edu (J.M.D.); Tel.: +1-701-390-3818 (N.J.J.); +1-909-515-2614 (J.M.D.)

Received: 16 September 2020; Accepted: 30 October 2020; Published: 1 November 2020

Abstract: Cut peonies (*Paeonia lactiflora* Pall.) have a relatively short vase life and limited availability due to seasonal production. Cultivars Festiva Maxima (FM), Monsieur Jules Elie (MJE), and Sarah Bernhardt (SB) stored at 0.7 °C had a longer flower open time at 12 weeks of storage compared to those held at −3.1 or 3.5 °C, while the flower bud time was unaffected. The flower open time of FM and MJE was no different for stems stored at a sub-zero temperature of −0.6 °C for 16 weeks compared to non-stored stems. Flower quality, opening, and lack of deformity was reduced at 16 weeks of storage in comparison to non-stored flowers, but higher for stems stored at −0.6 °C compared to 0.7 °C. Pre-treating stems before storage with pulses of a commercial hydrator solution or a 200 $g \cdot L^{-1}$ sucrose solution for 2 h at 4 °C had little commercial significance compared to non-pulsed control stems. The total phenolic content, malondialdehyde, and superoxide dismutase were not effective indicators of open time or quality loss. This study is the first to demonstrate the successful use of a non-freezing, sub-zero storage temperature for peony, and the first to store cut peonies for 16 weeks, despite an increased risk of reduced flower quality.

Keywords: peony; long-term storage; flower deformity; total phenolic content; malondialdehyde; superoxide dismutase

1. Introduction

Herbaceous peonies, primarily hybrids of *Paeonia lactiflora* Pall., have been produced as cut flowers for centuries and continue to grow in popularity and demand. Seasonal production and a short vase life of 5 to 9 d are two issues restricting availability and customer satisfaction. Peonies bloom once a year and the harvest window is limited to 2 to 4 weeks depending on the cultivar, plant age and size, and environmental factors such as temperature and moisture in a given location. Near year-round market availability is currently contingent on global production during the spring seasons of the Northern and Southern Hemispheres [1]. Growers primarily rely on the selection of early, mid, and late blooming cultivars to maximize cut flower production and cold storage is used to extend availability.

Cut peonies are stored as unopened buds at storage temperatures ranging from about 0.6 to 4.0 °C for up to 8 weeks, but vase life and cut flower quality of stored stems are reduced as storage duration increases [2,3]. Standard methods to improve peony vase life through carbohydrate additives and commercial hydrator solutions prior to storage or shipping have been unsuccessful or inconsistent

across the large number of cultivars [4–6]. These factors limit the storage life of cut peonies and leave a 1 to 3-month availability gap following August.

Cooling technology revolutionized the postharvest handling of perishable crops such as cut flowers. Holding flowers in cold storage preserves vase life and quality by reducing respiration [7], carbohydrate loss [3], and flower development [8,9], and limits ethylene production and sensitivity during storage [7,10]. Cut flowers historically fall into two storage temperature classes: Chilling sensitive flowers, consisting of many tropical and sub-tropical species that should be held no colder than 12 or 2 °C, respectively, and those that should be held no lower than 0 °C [11]. The industry standard temperature for cut peony storage has been determined to be between 0 and 2 °C with over 25 cultivars tested in storage for 4 to 12 weeks [3,4,8,12,13]. The vase life of stems stored for 4 weeks near 0 °C is reduced by 1 to 2 d when compared to freshly cut stems [14]. An additional day or two is lost as storage durations increase to 10 or 12 weeks of storage along with an increase in disease development [3,4]. Dry storage, where cut stem ends are not held in water, preserves the bud stage and subsequent vase life of peony and is the standard method of holding stems in cold storage [13].

Near-freezing storage temperatures (<0 °C) have gained recent interest in edible crops for improved storage length. Apricots (*Prunus armeniaca* L.) [15,16], cherries (*Prunus avium* L.) [17], and nectarines (*Prunus persica* L.) [18] were stored 14 to 30 d longer when using near-freezing storage temperatures (<0 °C) compared to ≥ 0 °C; near-freezing storage better maintained firmness, color, aroma, and sugar content. Authors also reported lower ethylene production [15,18] and respiration rate [15,17]. Sub-zero temperatures are not currently utilized for cut flower storage due to the limited knowledge of how these temperatures affect cut flower vase life and quality and the potential injury caused by ice formation. Depending on the specific temperature and the amount of time below 0 °C, freezing can be avoided and quality preserved longer than possible at above 0 °C temperatures. Post and Fischer [12] reported a higher vase life for cut *Chrysanthemum* spp., *Narcissus* spp., *Iris germanica* L., *Convallaria majalis* L., and *Tulipa* spp. when stored at −0.6 °C compared to 0.6 °C. Minimal vase life improvements were reported for cut *Narcissus* 'Carlton' when comparing −0.6 and 0.6 °C for short-term storage (10 d), but Nichols and Wallis [19] stated −0.6 °C could be more beneficial when considering long-term storage.

Cut peonies exhibit multiple characteristics of tolerating sub-zero temperatures. Stems, leaves, and already fully-differentiated floral tissue [20] frequently experience sub-zero events prior to spring flowering in North Carolina (personal observation). Peonies are harvested in a tight bud stage and would likely tolerate a lower temperature than fully developed flowers [21]. Sepal tissue of tree peony (*P. suffriticosa* Andr.) 'Luoyanhong' and 'Fengdanbai' did not freeze until exposed to −3.8 and −4.0 °C, respectively [21]. Developing peony buds are high in starch [22], which could indicate a low tolerance of sub-zero temperatures and freezing [23–25]. However, hydrolysis of starch to soluble carbohydrates such as fructose, glucose, and later sucrose occurs within buds after 2 weeks of storage at 0 °C [3], which could improve sub-zero tolerance by increasing solute concentration [26,27]. Floral preservatives containing soluble carbohydrates may also be used to improve sub-zero tolerance of cut peonies. Sucrose pulses of 200 $g \cdot L^{-1}$ decreased freezing points of cut carnation by 2.6 °C [28]. Peonies are an excellent candidate for testing the efficacy of sub-zero storage temperatures due to their popularity, high value, seasonality, bud harvest stage, short vase life, and ability to tolerate dry storage.

The available literature describes the effects of above 0 °C storage on peony vase life, but not the impact of storage on quality. The quality criteria of cut peonies consists of a long stem length, large bud size at harvest, and buds that fully open and not deformed or discolored. Cut peonies are graded prior to sale and peonies with the longest stems and largest buds generally have the highest value. Peonies are marketed in a tight bud stage and flower opening is vital for customer satisfaction. Failure to open, a disfigured shape, or smaller flower size reduces value and customer satisfaction. Short cold storage durations generally improve peony bud opening [3,8]. However, long storage durations inhibit the bud opening of other cut flower species such as Dutch iris that are bud harvested [29,30]. Bud opening has been linked to water movement into petals causing cell expansion [31] and has been improved through

adding soluble carbohydrates into pulse solutions and vase water. Highly concentrated sucrose pulses (>100 g·L^{-1}) improved vase life by opening new florets of *Gladiolus* spp. [32], lilies [33], and sweet pea (*Lathyrus* spp.) [34]. *Dianthus caryophyllus* L. (carnation) pulsed with a 200 g·L^{-1} sucrose solution prior to storage for 10 d at −4 °C opened while un-treated stems failed to open [28].

Phenotypic data are often coupled with physiological stress indices to facilitate inferences on stress tolerance. Total phenolic content (TPC) and specific antioxidant tests are two methods of determining plants' ability to mitigate stress [35–37] in the form of reactive oxygenating species (ROS) that cause cellular damage. ROS and antioxidant concentration impact the senescence of day lily (*Hemerocallis* spp.) [38], rose (*Rosa hybrida* L.) [39], and carnation [36,40] flowers. Petals contain numerous phenolic compounds including antioxidants and anthocyanins that have antioxidant properties to reduce the impact of ROS [41,42]. The total phenolic content (TPC) is a general measurement of the bioactive compounds correlated to antioxidant activity [35]. Superoxide dismutase (SOD) is one of the more prevalent antioxidant enzymes and has associated with the initial stages of flower senescence [36,39,40] and potentially flower longevity. A common stage of senescence, also influenced by stressors such as temperature and ROS, is cell membrane dysfunction and injury. Studies indicate that lipid peroxidation, measured as malondialdehyde (MDA) [43], increases as floral tissue senescence [41], following freeze tests [44] and periods of drought simulated on marigold (*Tagetes erecta* L.) species [45]. Peony flowers [46] and roots [47] have been used medicinally for centuries due to the number of bioactive compounds [48]. The high total phenolic content, >30 ascorbic acid equivalent [46], and 26.8 mg·g^{-1} gallic acid equivalent (GAE) [47] could indicate tolerance to stress induced by sub-zero temperatures and prolonged dry storage where stems are stored without water.

Two experiments were implemented to study the effects of sub-zero temperatures on the storage and vase life of cut peonies. The objectives were to (i) determine the effects of sub-zero storage temperatures compared to temperatures currently used in the industry, (ii) evaluate the use of pre-storage pulses; and (iii) determine if antioxidant assays are useful stress indicators of peony vase life and quality in response to storage duration, temperature, and pre-storage pulses.

2. Materials and Methods

2.1. Plant Material

Cut flowers of three peony cultivars: Festiva Maxima (FM), Monsieur Jules Elie (MJE), and Sarah Bernhardt (SB) were obtained from a local commercial grower and transported dry to North Carolina State University within 3 h of harvest. Exp1 FM and MJE were harvested on 26 April 2018 and SB on 3 May 2018. Exp2 FM and MJE were harvested on 24 April and SB on 29 April 2019. All stems had one apical bud. Peony stems were processed by cutting to a length of 50 cm from the bud tip to stem end. All but the three uppermost lobed leaves were removed. Bud stages were primarily at stage 2 as defined by Eason et al. [14], but did include some stems with buds at stages 1.5 and 2.5. Stems were assigned to each treatment ensuring that all treatments had the same number of stems at each bud stage.

2.2. Exp1—Broad Temperature Range Storage

Treatment combinations of three storage temperatures and 12 storage durations contained 15 replicates (n = 15) plus a non-stored control resulted in a total of 37 groups of each cultivar. The control group of each cultivar, which experienced no storage temperature or storage duration, was placed directly into room-temperature tap water for postharvest evaluation following processing. Storage groups were completely wrapped in dry newspaper and placed horizontally into cardboard boxes. Boxes were lined with polyvinyl wrap to reduce dehydration. A box of each cultivar was held at three storage temperatures: −3.1 ± 0.2, 0.7 ± 0.7, or 3.5 ± 0.9 °C. The relative humidity (RH) for each temperature was 89, 94, and 93%, respectively. Temperatures experienced by cut stems were based on the average of three data loggers (RHT10, Extech Instruments, Waltham, MA, USA) located in each

chamber. Each data logger was in a separate box, one for each cultivar, surrounded by the groups of cut stems. Groups of 15 stems of every cultivar were removed from each storage temperature every week for 12 weeks for a total of 12 storage durations.

2.3. Exp2—Near-Freezing Temperature Storage

Treatment combinations of three pre-storage pulses, two storage temperatures, and eight storage durations contained 13 replicates, 10 for postharvest evaluation ($n = 10$) and three for destructive physiological indices ($n = 3$), for a total of 48 groups for each cultivar. Groups were treated with pulses that consisted of a 2-h treatment at 4 °C where stem ends were placed in either a commercial hydrator solution (Chrysal #1 Hydration Solution, Chrysal Americas, Dora, FL, USA), 200 $g \cdot L^{-1}$ sucrose solution, or held dry with no pulse solution. Control groups for each pulse treatment, which experienced no storage temperature or storage duration, were placed directly into room-temperature tap water for postharvest evaluation following processing. Storage groups were completely wrapped in dry newspaper and placed horizontally into cardboard boxes. Boxes were lined with polyvinyl wrap to reduce dehydration. A box of each cultivar was held dry at each of the two storage temperatures: 0.7 ± 0.2 or −0.6 ± 0.2 °C. The RH for each temperature was 93 and 96%, respectively. Temperatures experienced by cut stems were based on the average of three data loggers (RHT10, Extech Instruments, Waltham, MA, USA) in each chamber. Each data logger was placed into a separate box, one for each cultivar, in the middle of the groups of cut stems. Groups of 13 stems of every cultivar were removed from each storage temperature every 2 weeks for 16 weeks for a total of eight storage durations.

2.4. Post-Storage Evaluation

Following the processing of non-stored controls and removal from storage, stems were recut, removing the lower 2.5 cm to remove the dehydrated tissue per industry practices. Individual stems were placed into their own vase filled with 400 mL of tap water. Flowers were evaluated in a climate-controlled room held at 22.8 ± 1.5 °C, 40 to 60% RH, and a 12-h photoperiod at 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Fresh weight lost (FWL) was calculated as a percentage using the following equation:

$$FWL = (Initial_{FW} - Post\text{-}storage_{FW})/Initial_{FW} \times 100 \quad (1)$$

Post-storage FW was measured following stem removal from storage and before recutting. Freeze injury on petal tissue was rated 24 h after removing stems from storage using a scale of 0 to 5 where 0 = no water-soaked spotting; 1 = 1 to 20%; 2 = 21 to 40%; 3 = 41 to 60%; 4 = 61 to 80%; and 5 = 81 to 100%. The number of days as a bud (bud time) and the number of days flowers were open (open time) were recorded and the summation was used to compare the total vase life to previously published literature. Reasons for termination included the following petal drop, disease covering >10% of the bud, >50% necrotic petals, >50% wilted petals, stem collapse, or failure to open (FTO). The flower diameter was measured before termination and flower openness was scored as either FTO, partially open, or fully open. A flower was considered partially open when it reached stage 5 and fully open when it reached stage 6 (14). Flowers were considered deformed if petals did not fully expand, were missing, wrinkled, or exhibited some type of disfigurement.

2.5. Indices of Physiological Stress and Antioxidant Activity

Following storage in Exp2, three buds of each cultivar were cut from their stems, including 1 cm of stem tissue, and flash frozen in liquid nitrogen for 30 s. Samples were stored in −80 °C until processed for malondialdehyde (MDA), superoxide dismutase (SOD), and total phenolic content (TPC). Petals from the outer edges (4 to 5 petals) of the bud were collected and used to determine MDA and TPC for all three cultivars including the effect of pre-storage pulse treatments, storage temperature, and storage duration. The thiobarbituric acid reactive substances (TBARS) method from Heath and Packer [49] was used to determine the MDA concentration. Outer petals were removed from the frozen bud and

0.4 g of tissue was placed into 2 mL sealing vials with 0.1 mL ethanol and four steel beads (4 mm diam). Samples were homogenized for 1 min using a bead mill (BeadBug, D1030, Sigma Aldrich, St. Louis, MO, USA). The puree was incubated on ice for 10 min and then centrifuged at 11,500× *g* for 15 min at 4 °C. The supernatant was collected and 100 uL was added to 900 uL of a 10% trichloroacetic acid solution including 0.67% thiobarbituric acid (TBA). The sample was incubated at 95 °C for 25 min and then cooled on ice for 10 min. The sample was centrifuged at 11,500× *g* for 10 min at 4 °C and the absorbance of the supernatant was measured at 700, 600, 532, 450, and 440 nm on a spectrophotometer (Shimazdu 160A, Columbia, MD, USA). The equation used to calculate the MDA concentration was:

$$\begin{gathered}\text{MDA equivalents (nmol/mL)} = (\text{A}-\text{B})/157{,}000 \times 1{,}000{,}000 \\ \text{A} = [(\text{Abs}_{532} + \text{TBA}) - (\text{Abs}_{600} + \text{TBA})] - (\text{Abs}_{532} - \text{TBA}) - (\text{Abs}_{600} - \text{TBA}) \\ \text{B} = [(\text{Abs}_{440} + \text{TBA}) - (\text{Abs}_{600} + \text{TBA}) \times 0.0571]\end{gathered} \tag{2}$$

The total phenolic content (TPC) was determined using the Folin-Ciocalteau (F-C) reagent based on the methods described by Singleton et al. [50]. The outer peony petal tissue ground with a bead mill (0.1 g) was extracted three times with 14 mL of methanol:water:formic acid (60:37:3). Samples were vortexed for 1 min and sonicated for 10 min. Supernatants were collected after each extraction by centrifuging for 20 min at 4 °C and combined. Twenty microliters of solvent-extracted supernatant were added to 20 uL of 0.25 F-C reagent (0.25 N) followed by 20 uL of sodium bicarbonate (1 N), and 120 uL of H_2O into a microplate. The microplate was shaken for 60 s and held at room temperature for 6 min. Samples were incubated for 1 h at room temperature covered in foil to exclude light. Following incubation, absorbance was read at 765 nm on a microplate reader (Epoch 2, Biotek-Agilent, MD, USA). Gallic acid was used as the standard and values were recorded as GAE in mg· kg^{-1} FW.

SOD was measured using the EnzyX™ kit (Assay Biotech, San Francisco, CA, USA) in MJE and SB petals from stems stored for 0, 2, 4, 8, and 16 weeks of storage at −0.6 °C. About 0.2 g of inner petals were removed from the frozen buds stored at −20 °C, refrozen in liquid nitrogen for 30 s, and ground using 2.8 mm steel beads in a ball mill (Genogrinder, SPEX, MA, USA) for 45 s at 12,000 rpm. Cold buffer (1.3 mL) [0.1 M Tris–HCl (pH 7.8), 0.1 mM EDTA] was added to 0.2 g of ground peony petals and vortexed for 1 min. Samples were centrifuged for 30 min at 14,000 rpm at 4 °C before the supernatant was collected. The supernatants (extracts) were stored at −80 °C and thawed on ice before assaying. The SOD standard curve was made with the concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 U·mL^{-1}. For the SOD activity assay, 50 uL of sample, control, or standard were added into a microplate well along with 25 uL of dye reagent and 25 uL of the working solution. The plate was incubated at room temperature for 60 min and absorbance at 560 nm was recorded. The SOD activity was calculated based on the standard curve and expressed as U·g^{-1} FW.

2.6. Experimental Design and Statistics

A completely randomized design was used for both Experiments 1 and 2. Cultivars were analyzed separately due to the different time of entry into the experiments. Percent FWL, flower diameter, flower open time, and petal necrosis responses were subjected to ANOVA using the GLIMMIX procedure (SAS version 9.4; SAS Institute, Cary, NC, USA). The bud time was not analyzed due to the non-normal distribution and lack of equal variance. Post hoc mean separation was implemented using Tukey's honest significant differences (HSD) with $p < 0.05$. The SLICE statement was used to implement a partitioned analysis or simple effect analysis when a significant interaction with storage duration occurred. This allowed for a simple explanation of treatment effects at specific levels of storage temperature or pre-storage pulse treatments at a specific storage duration and vice versa. Reported values were the least squared means to account for missing samples, such as flowers that failed to open (FTO), which were not used in flower diameter and vase life calculations. Percent FWL, and flower diameter were also regressed (GraphPad Prism 8.3.1, San Diego, CA, USA) where the model with the best r^2 value was reported in supplementary tables.

Binary data measuring a yes or no response for FTO and flower deformity were analyzed and regressed using PROC LOGISTIC, which had a generalized linear model parameterization. Logistic models, odds ratios, and comparisons are reported in the supplemental materials. Odds ratios were used to explain the influence storage temperature or storage duration had on FTO or deformity while keeping other variables constant. Partially open and fully open flowers were combined for FTO regression. Comparisons were adjusted with Tukey's HSD. Only significant terms with $p < 0.05$ were included in the reported models.

3. Results and Discussion

3.1. Vase Life (Bud and Open Time)

3.1.1. Exp1—Broad Temperature Range Storage

Peony vase life is the summation of the number of days buds remained unopen (bud time) and the time flowers were open (open time). Non-stored stems, storage duration of 0, were used as the control and the open time of these stems' flowers is the amount of time from stage 5 until they were terminated during postharvest evaluation. Following storage for 1 or more weeks, the open time declined for all cultivars (Table 1). In comparison to the open time of the non-stored control, MJE was the only cultivar that did not see a significant decline in vase life when stored for 1 week (Table 1). At 9 or more weeks of storage, the open time of FM stored at −3.1 °C was significantly shorter than stems stored at 3.5 or 0.7 °C. In contrast, the open time of MJE was generally similar among each storage temperature with the exception of 1, 5, 9, 10, and 11 weeks of storage. The reaction of SB was between that of FM and MJE where the open time was usually lowest for stems stored at −3.1 but no different than stems stored at 3.5 °C, which was no different than stems stored at 0.7 °C. Across cultivars, the open time was longest for stems stored at 0.7 °C at 12 weeks of storage.

Table 1. Mean flower open time (d) in response to storage temperature (ST) and storage duration for *Paeonia lactiflora* cultivars Festiva Maxima (FM), Monsieur Jules Elie (MJE), and Sarah Bernhardt (SB) peonies, analyzed separately in Exp1. Grand mean bud time for FM, MJE, and SB was 1.2, 1.3, and 1.2 d, respectively.

Cultivar		**FM**			**MJE**			**SB**		
		Open Time (d)			**Open Time (d)**			**Open Time (d)**		
ST (°C)		−3.1	0.7	3.5	−3.1	0.7	3.5	−3.1	0.7	3.5
Storage duration (weeks)	1	2.9 * ns	2.6 * ns	3.3 * ns	5.5 * bs	6.4 * as	5.3 * bs	4.5 * ns	5.5 * ns	5.0 * ns
	2	2.0 * ns	2.6 * ns	2.3 * ns	4.0 * ns	4.3 * ns	4.0 * ns	3.5 * bs	5.1 * as	4.3 * ab
	3	2.0 * ns	2.9 * ns	2.5 * ns	4.1 * ns	3.9 * ns	4.4 * ns	2.8 * bs	4.4 * as	3.3 * bs
	4	1.5 * b[1]	2.6 * as	2.6 * as	3.9 * ns	4.5 * ns	4.2 * ns	3.0 * bs	4.8 * as	3.9 * ab
	5	2.0 * b[1]	3.4 * as	3.4 * as	4.0 * bs	4.5 * bs	5.1 s as	2.6 * bs	3.9 * as	3.7 * ab
	6	2.0 * b[1]	3.0 * as	3.0 * as	3.4 * ns	4.2 * ns	4.0 * ns	2.8 * ns	3.1 * ns	3.4 * ns
	7	0.9 * b[1]	2.2 * as	2.2 * as	2.9 * bs	4.0 * as	4.0 * as	2.7 * bs	4.0 * as	3.3 * ab
	8	1.2 * b[1]	3.3 * as	3.8 * as	3.9 * ns	4.6 * ns	3.9 * ns	2.0 * bs	3.9 * as	3.3 * ab
	9	0.0 [2,3] b*	3.0 * ss	1.8 * ss	2.4 * bs	4.0 * as	3.7 * as	0.0 * . s	3.3 * ss	3.0 * ss
	10	0.0 [2,3] b*	2.5 * ss	1.0 * ss	2.0 * bs	4.3 * as	3.8 * as	0.0 * . s	3.0 * ss	3.0 * ss
	11	0.0 [2,3] b*	2.4 * ss	1.0 * ss	1.9 * bs	4.3 * as	3.0 * bs	0.0 * . s	3.4 * ss	3.5 * ss
	12	0.0 [2,3] b*	3.0 * ss	0.0 * ss	1.0 * . s	3.6 * ss	2.3 * ss	0.0 * . s	3.4 * ss	3.0 * ss
Non-stored			4.6			5.5			7.0	

* Indicates that the least squared means within cultivar are significantly different than the non-stored, control when $p < 0.05$. [1] Least squared means followed by the same letter within each storage duration are not significantly different when adjusted with Tukey's honest significant difference test with $p < 0.05$ in a partitioned analysis of simple effects. [2] A value of 0.0 d was reported when no flowers opened during post-storage evaluation. [3] Values followed by a "." had three or fewer observations and were not statistically compared in the partitioned analysis. Total vase life = bud time plus open time.

The amount of time flowers stayed as a bud (bud time) following storage was not statistically compared due to the lack of and/or unequal variances (data not shown). Non-stored FM, MJE, and SB flowers had a bud time of 2.5, 1.9, and 1.3 d, respectively. Initially, the bud time decreased to 1.0 ± 0.2 d for FM at 3 weeks of storage and for MJE and SB at 2 weeks of storage regardless of storage temperature. In a similar fashion, all cultivars tested by Heuser and Evensen [8] had a bud time of 1 d after a storage period of 4 weeks. Faster opening following storage is likely caused by the hydrolysis of starch to soluble sugars [3] induced by cold temperatures [51]. However, long-term storage in the current study negatively affected bud time. Both FM and SB bud time increased and eventually buds failed to open when stored at −3.1 °C, which is reflected in the 0 d open time following 9 or more weeks of storage (Table 1) and likely caused by ice formation and vascular damage. Immature carnation buds also had a reduced ability to open at longer durations at various sub-zero storage temperatures [28].

The bud time increased for FM, MJE, and SB when held at 3.5 °C at 9, 12, and 10 weeks of storage, respectively (data not shown). The inability of buds to open following storage has been reported in cut *Iris* × *hollandica* Tub. [30] and carnations [29]. The cause has been linked to collapsed or blocked vascular tissue including air embolisms, dehydrated cells, and bacterial growth [31].

Total vase life (bud + open time) data for stems stored at 0.7 °C was similar to reports of peonies stored between 0 and 2 °C [4,8,52]. Effects of long-term storage at temperatures above and below this range showed different trends. Flower development and opening during peony forcing had a linear relationship with the production temperature [53]. This could explain a shorter vase life when cut stems were stored at 3.5 °C compared to 0.7 °C. Conversely, as storage duration at −3.1 °C increased, the bud time increased yet the total vase life decreased as storage duration increased (data not shown). Ice formation and damage to the vascular tissue, observed as stem collapse during the longest durations of storage (personal observation), likely reduced the ability of stems to maintain an open state. This is reflected in the vase life data of stems stored at −3.1 °C (Table 1). In all cultivars, the flower open time reached a value of 0 d earliest when stored at −3.1 °C. Cultivar differences were observable with MJE being the most tolerant cultivar of storage at −3.1 °C with a vase life of 1.9 d after 11 weeks of storage.

3.1.2. Exp2—Near-Freezing Temperature Storage

Negative sub-zero effects on vase life, specifically open time, were avoided by changing the sub-zero temperature to −0.6 °C and removing the warmest temperature of 3.5 °C. The open time was significantly longer for MJE when stored at −0.6 compared to 0.7 °C at 6 or more weeks of storage (Table 2). There was also no significant decline in the open time throughout the storage period when stored at −0.6 °C. Storage duration did not significantly impact open time (3.1 d) for FM. The open time of FM stems stored at −0.6 °C was on average significantly longer by 0.2 d compared to those stored at 0.7 °C. The stored SB open time was significantly ($p \leq 0.0001$) shortened from 4.0 to 2.9 d at 14 weeks of storage. Independent of storage duration, open time was significantly ($p \leq 0.0001$) shorter by 0.4 d at −0.6 °C compared to stems stored at 0.7 °C. While these differences were statistically significant, they likely have little commercial significance in comparison to the 0.6 to 1.4 d improvement seen with MJE when stored at −0.6 °C.

The storage life of cut peonies held at 0.6 °C was better compared to −0.6 °C [12], although the cultivar used and vase life data were not reported. The current study suggests that benefits of sub-zero storage may be cultivar specific, which would align with previous storage studies in other cut flower species [2,5,14]. These data may also indicate that cultivars with a long fresh-cut vase life, such as MJE, may benefit more from storage at −0.6 °C compared to those with a short fresh-cut vase life such as FM. The total vase life (bud time plus open time) of SB stored for 8 weeks in the current study was 1 to 2 d shorter than the vase life reported by Walton et al. [3] who used 0 °C, but similar to that of stems stored at 2 to 3 °C for 8 and 10 weeks [4]. The different response of SB compared to other studies may be from pre- and postharvest factors including temperature, rainfall, and duration of processing prior to the experiment. Specifically, the shorter open time in Exp2 compared to Exp1 may have been influenced by the environment prior to harvest seen in 2019. The average cumulative precipitation from two weather

stations [54] near the grower was over 2.5 cm less in 2019 compared to 2018. The average temperatures of the month and week prior to harvest were 2.4 and 4.7 °C warmer in 2019. On the day of harvest, the minimum temperature, prior to the morning harvest, was also warmer in 2019 by 3.2 °C.

Table 2. Exp2 bud time (d), flower open time (d), percentage of flowers that failed to open, and percentage of deformed flowers for *Paeonia lactiflora* cultivar Monsieur Jules Elie (MJE) in response to storage temperature (ST) and storage duration.

		Bud Time (d)		Open Time (d)		FTO (%)		Deformation (%)	
	ST (°C)	0.7	−0.6	0.7	−0.6	0.7	−0.6	0.7	−0.6
Storage duration (weeks)	2	1.1	1.0	3.3 * a [1]	3.3 * a [1]	00	0	00	00
	4	1.0	1.0	3.9 * a [1]	4.2 * a	00	0	00	00
	6	1.0	1.0	3.2 * b [1]	3.9 ** a	00	0	00	00
	8	1.0	1.1	3.5 * b [1]	4.1 * a	00	0	07	00
	10	1.1	1.1	3.8 ** b [1]	4.4 ** a	00	0	07	03
	12	1.1	1.0	4.0 ** b [1]	5.4 * a	00	0	10	00
	14	1.0	1.1	3.2 * b [1]	4.5 ** a	10	0	43	10
	16	1.0	1.0	3.6 * b [1]	4.3 * a	20	0	33	27
	Non-stored	1.2	1.2	4.1 b [1]	4.1 a	00	0	00	00

* Indicates that the least squared means within cultivar are significantly different than the non-stored, control when $p < 0.05$. [1] Least squared means followed by the same letter within each storage duration are not significantly different when adjusted with Tukey's honest significant difference test with $p < 0.05$ in a partitioned analysis of simple effects. Total vase life = bud time plus open time.

The open time of all cultivars was influenced by the pre-storage pulse treatment, but only FM was influenced by the interaction of pulse and storage duration treatments (Table 3). A partitioned analysis of the simple effects for the interaction depict a significant decline in FM open time by 16 weeks for both the non-pulsed control and hydrator-pulsed stems, but not sucrose-pulsed stems (Table 1). The open time of sucrose-pulsed stems was not significantly longer than non-pulsed stems or hydrator-pulsed stems for any storage duration. Hydrator-pulsed MJE stems lasted significantly longer (4.1 d) than sucrose-pulsed stems (3.9 d) and the non-pulsed SB open time (3.4 d) was significantly longer than sucrose-pulsed stems (3.0 d). Neither of these statistical differences are likely of commercial value.

Sucrose pulses have proven ineffective on other cut flower species such as tulips [55] and Exp2 results match previous reports on peonies [4–6]. In conjunction with storage, Loyola-López et al. [6] reported a lower vase life for 'Karl Rosenfield' when pulsing with 100 $g{\cdot}L^{-1}$ sucrose for 4 h at 1 °C after stems had been stored for 25 d. Peonies may have a reduced ability to uptake sucrose solutions at high sucrose concentrations. Gladiolus stems took up less solution during 24 h as the sucrose pulses concentration increased from 0 to 16% [56]. Upregulation of sucrose transporter genes have been reported in *P. lactiflora* 'Yang Fei Chu Yu' in response to a constant availability of sucrose in vase water [57]. Cut flower species and peony cultivars may differ in their ability to transport and use sucrose, which could inhibit vase life improvement.

The bud time of non-stored SB was between 1.1 and 1.8 d; it was the only cultivar to have a slightly longer bud time as storage duration reached 10 or more weeks. All MJE flowers opened within 1.0 to 1.2 d regardless of treatment and storage duration (Table 2). Non-stored flowers of FM opened within 1.6 to 2.8 d. Following 2 or more weeks of storage, the FM bud time was reduced to 1.1 to 1.9 d depending on the pulse treatment and storage temperature, but no trend was seen following 2 or more weeks of storage. These data reflect bud time results of Exp1 when stems were stored at 0.6 °C. Additionally, Nichols and Wallis [19] reported no difference in bud time when storing *Narcissus* 'Carlton' at either 0.6 or −0.6 °C.

Table 3. Mean flower open time (d) for *Paeonia lactiflora* cultivar Festiva Maxima (FM) in response to pre-storage pulse treatments and storage duration in Exp2 independent of storage temperature (0.7, −0.6 °C). Grand mean bud time was 1.4 d.

		Pre-Storage Pulse Treatment [1]		
		Non-Pulsed	**Hydrator**	**Sucrose**
			Open Time (d)	
Storage duration (weeks)	2	2.8 * a[2]	3.1 * a	2.8 * a
	4	3.5 * a[1]	3.2 * a	3.4 a
	6	3.0 * a[1]	2.8 * a	2.9 a
	8	3.0 * a[1]	3.0 * a	2.7 * a
	10	3.2 * ab	3.3 * a	2.6 * b
	12	3.1 b[1]	4.4 * a	3.0 b
	14	3.0 * a[1]	2.8 * a	2.8 * a
	16	2.9 * a[1]	2.9 * a	2.9 a
Non-stored		3.7 * a[1]	3.3 a	3.6 a

[1] Pre-storage pulse treatments were applied by placing cut stem ends in either a dry bucket (non-pulsed, control), commercial hydration solution (hydrator), or 200 g·L^{-1} sucrose solution for 2 h at 4 °C. * Indicates that the least squared means within cultivar are significantly different than the non-stored, control when $p < 0.05$. [2] Least squared means followed by the same letter within each storage duration are not significantly different when adjusted with Tukey's honest significant difference test with $p < 0.05$ in a partitioned analysis of simple effects. Total vase life = bud time plus open time.

Based on just open time, data from both Experiments 1 and 2 support the industry standard storage temperature of 0.6 °C. Data also indicate that the storage life of peonies could be extended to 16 weeks with minimal loss of open time. This would allow growers to supply peonies through months where production is limited or nonexistent. Mayak and Halevy [58] and Nichols and Wallis [19] suggested that the minimal improvement of vase life at −0.6 °C does not outweigh the potential injury and cost of incorporating such precise equipment into cut flower operations. Given the improvement of cooler systems, narrowing profit margins, and the variety of species applicable to storage at −0.6 °C [12], there could be increased interest in this storage temperature. The slightly longer open time seen in stems held at −0.6 °C in Exp2 may provide enough buffer for shipping long-term stored stems or using shipment methods such as sea freight.

3.2. Flower Quality

3.2.1. Exp1—Broad Temperature Range Storage

The percent of FWL increased linearly over the storage duration regardless of storage temperature (Figure 1) for MJE and FM, and for SB held at temperatures other than 0.6 °C. SB held at 0.6 °C fit a quadratic polynomial (Table S1) where FWL slowed between 8 and 12 weeks of storage. Across cultivars, regression slopes for the percent of FWL were the highest for stems held at 3.5 °C followed by 0.6 °C and the lowest for −3.1 °C. Upon the removal from storage, leaves were wilted, which progressed in severity as storage duration increased (personal observation). Leaves rehydrated within 24 h of placing stems in water, except when the storage duration was longer than 8 weeks at −3.1 °C. Wet storage can alleviate wilting, but is more commonly used for short-term storage or for cut species that fail to rehydrate following dry storage [57,59]. Storage at −3.1 °C also caused stems, leaves, and buds to freeze. Ice was observed at the cut stem ends (personal observation). This likely limited the amount of water that was able to evaporate during storage. Colder temperatures also limit molecule movement, including water evaporation. The continuous dehumidification of the chambers during cooling could explain the continued loss of FW across all treatments. Logistic regression models depict the increase in probability of buds failing to open (FTO) over the storage duration in response to the storage temperature (Figure 1D–F). Stems stored at 0.6 °C resulted in the lowest FTO probability followed by

−3.1 and 3.5 °C by week 12 of storage. This is reflected in the odds ratio comparisons located in Table S3. At 4 weeks of storage, there was no significant difference in FTO for FM among storage temperatures. However, at 8 and 12 weeks of storage, the FTO probability after storage at 0.6 °C was significantly lower than at −3.1 and 3.5 °C (Table S3). There was no significant duration and temperature interaction for MJE and SB meaning that the probability of MJE and SB FTO were significantly lower when stored at 0.6 °C compared to −3.1 and 3.5 °C (Table S2).

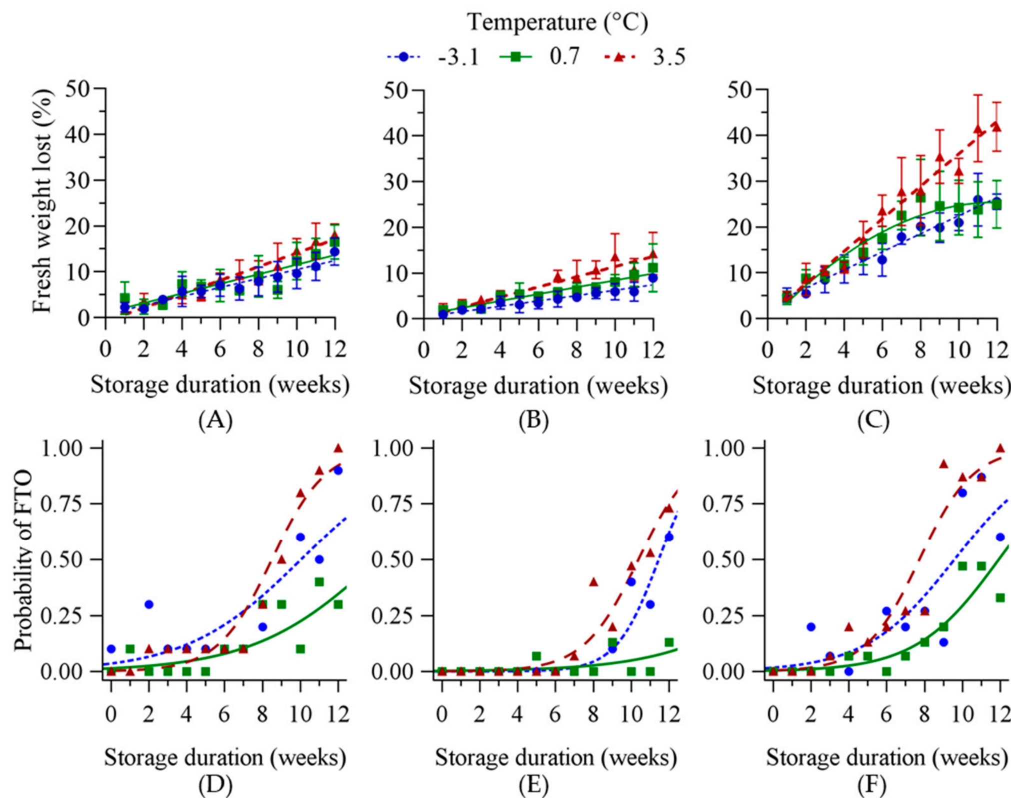

Figure 1. Exp1 fresh weight loss (%) (**A**–**C**) and probability of flowers that failed to open (FTO) (**D**–**F**) for *Paeonia lactiflora* cultivars Festiva Maxima (**A**,**D**), Monsieur Jules Elie (**B**,**E**), and Sarah Bernhardt (**C**,**F**) in response to three storage temperatures and thirteen storage durations. Buds that failed to open or flowers that partially opened and petals that did not complete reflex or unfold were labeled as FTO during post-storage evaluation. Flowers were considered deformed if petals did not fully expand, were missing, wrinkled, or exhibited some type of disfigurement. Markers for weight loss are the means along with ± SD ($n = 5$) with linear or quadratic regressions. Logistic regressions are the predicted FTO probability along with the observed percentage that did occur as a decimal ($n = 15$). Regression models and fit statistics are located in Tables S1–S3.

The inability of peony flowers to open following storage has been missing from a majority of cut peony literature, but has been related to the harvest bud maturity of stored stems [14]. The water movement into petal tissue is considered a primary factor in petal expansion and flower opening [31]. Dehydration and the inability to uptake water could reduce the ability of peony buds to open. Stems were recut following storage to aid in water uptake. However, the removal of 2.5 cm from the cut end may not have been enough to reach viable vascular tissue. Mayak and Halevy [58] reported that the lower water conductivity in *Iris* × *hollandica* stems was seen as high as 20.5 cm from the cut stem end after 4 d of dry storage. Overall, percent FWL was highest for stems held at 3.5 °C, which may relate to stems having the highest FTO percentage at this temperature. Stems held at −3.1 °C had a similar but slightly lower FTO percentage and had a similar percent FWL.

The diameter of all cultivars was significantly influenced by the interaction of storage temperature and storage duration. Non-stored stems of FM, MJE, and SB had flower diameters of 12.3, 12.0,

and 12.8 cm, respectively. Following storage, the flower diameter either decreased as a linear or quadratic function (Figure 2). The industry standard of 0.6 °C preserved the flower diameter longest in comparison to the other temperatures for both FM and SB. At this temperature, the flower diameter of FM was not statistically different at 12 weeks (9.0 cm) compared to 5 weeks of storage (10.0 cm). 'Sarah Bernhardt' stems stored for 12 weeks had a diameter of 8.8 cm, which was not statistically different from 10.5 cm at 3 weeks of storage. Both FM and SB had a diameter of 0 when stored at −3.1 or 3.5 °C between week 9 and 11.

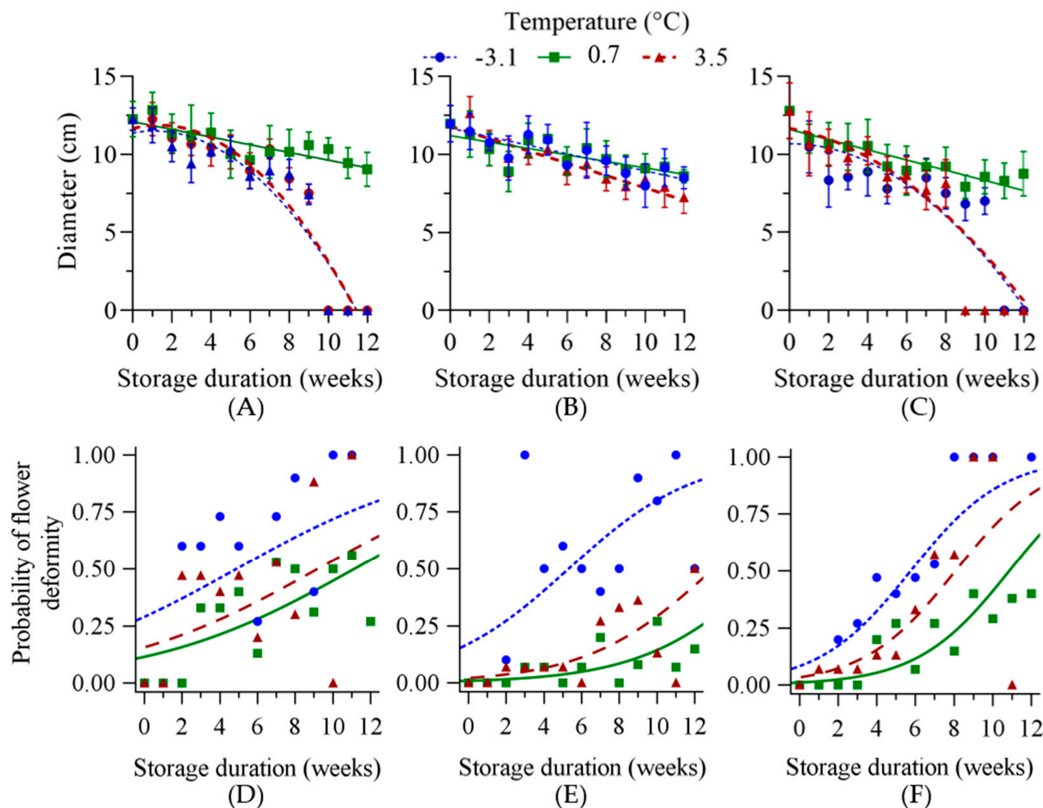

Figure 2. Exp1 diameter (**A**–**C**) and probability of deformed flowers (**D**–**F**) for *Paeonia lactiflora* cultivars Festiva Maxima (**A**,**D**), Monsieur Jules Elie (**B**,**E**), and Sarah Bernhardt (**C**,**F**) in response to three storage temperatures and thirteen storage durations. Buds that failed to open or flowers that partially opened and, petals that did not complete reflex or unfold were labeled as FTO during post-storage evaluation. Flowers were considered deformed if petals did not fully expand, were missing, were wrinkled, or exhibited some type of disfigurement. Markers for diameter are the least squared means along with ± SD ($n \leq 15$) with linear or quadratic regressions. Least squared means were used to account for missing samples when the flowers did not open. Logistic regressions are the predicted deformity probability along with the observed percentage as a decimal ($n \leq 15$). Regression models and fit statistics are located in Tables S4–S6.

MJE had measurable flower diameters at all temperatures and storage durations (Figure 2). Statistically, the diameter was not consistently higher at one storage temperature. The diameter was significantly lower for stems held at 3.5 °C compared to the diameter of flowers held at either both or one of −3.1 and 0.7 °C at 3, 4, 8, and 11 weeks of storage. While bud weight was not assessed, it is likely that water uptake into the bud and petals was reduced causing a loss of petals and minimized petal expansion. The loss of petals and increased occurrence of deformities, discussed in the following paragraph, could also have contributed to smaller flowers as storage duration increased. Low r^2 values may be explained by the variability caused by flowers that failed to open or partially opened and those that may have been deformed (Table S4).

Flower deformity was most prevalent in FM during early durations of storage. At 2 weeks of storage, flower deformities occurred in 60, 33, and 47% of flowers when held at −3.1, 0.7, and 3.5 °C, respectively. MJE was the only cultivar to never reach 100% deformity when held at 3.5 °C. MJE also had the lowest percentage of deformed flowers at 12 weeks of storage when held at 0.7 °C (Figure 2). Logistic regressions (Table S5) of the deformity probability indicate the highest probability of flowers being deformed when stored at −3.1 °C (Figure 2). Only SB had a significantly higher probability of deformity when comparing stems stored at 3.5 and 0.7 °C (Table S6). Flower deformities tended to increase as storage duration increased (personal observation) as evidenced by a loss of petals primarily in the center of the flower, curled, not fully expanded petals, or as center petals not opening similar to the outer petals (Figure 3). Non-stored flowers opened normally and were used to validate flower deformity. As stated earlier, deformity may have been caused by an inability of flowers to move water into petals for expansion.

Figure 3. Floral deformities in *Paeonia lactiflora* Pall. hybrids (**A**) 'Monsieur Jules Elie', (**B**) 'Festiva Maxima', and (**C**) 'Sarah Bernhardt'. Deformity severity increased as storage duration increased and was observed as a loss of petals primarily in the center of the flower, curled petals, not fully expanded petals, or center petals not opening similar to the outer petals.

Necrosis (Figure 4A,B) and water-soaked spotting of petals were observed (Figure 4C,D) on stored cut peonies. Cultivars FM and SB were most prone to develop petal necrosis (personal observation). The necrotic regions were primarily located on outer petals and were generally equal in size and shape. Water-soaked spotting was observed only on petals of stems stored at −3.1 °C indicating that injury was caused by ice formation. Water soaking occurs when plants thaw following being frozen due to the damage of cell membranes and leakage of content into the apoplast [60]. Although plants have been shown to recover from such injury [61], this was not observed in the current study.

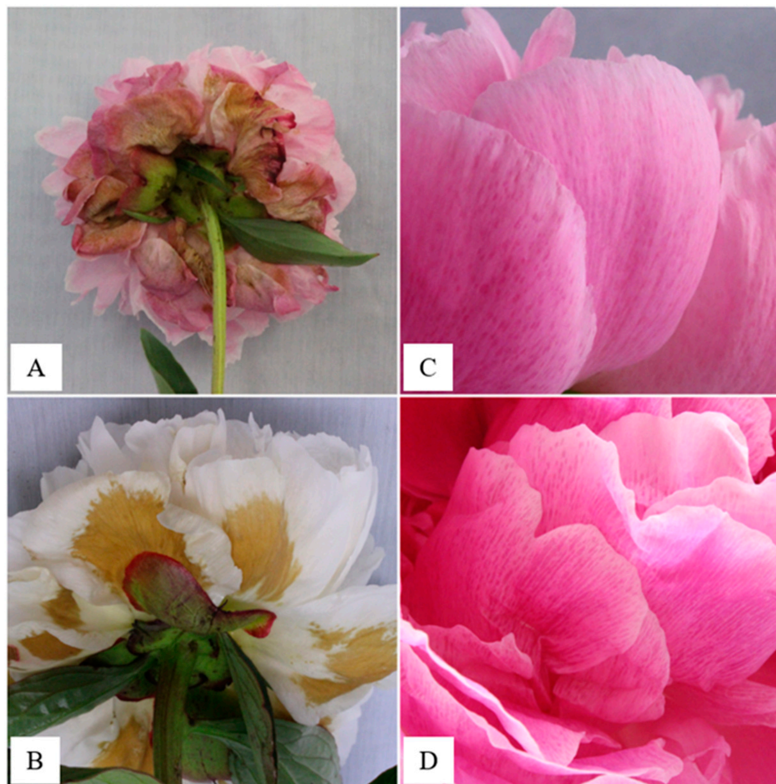

Figure 4. Petal injury in select *Paeonia lactiflora* cultivars. Necrosis was primarily observed on the outer petals of (**A**) 'Sarah Bernhardt' and (**B**) 'Festiva Maxima' when stored above 0 °C. Water-soaked spotting, pictured on (**C**) 'Monsieur Jules Elie' and (**D**) 'Sarah Bernhardt', developed on all cultivars following storage at −3.1 °C.

Ice formation in peony petal tissue was reported to occur between −3.8 and −4.0 °C for *P. suffruticosa* [21] and −1.7 °C [62] for *P. lactiflora*. No freeze injury was reported on cut peony stems of FM, 'Karl Rosenfield', and SB after 5 h at −2 °C [63]. One week of exposure to −3.1 °C likely influenced ice nucleation and caused injury. Prolonged storage at −3.1 °C did not cause an observable increase in water-soaked spotting, but likely manifested in other ways such as flower deformities and buds failing to open.

3.2.2. Exp2—Near-Freezing Temperature Storage

The FTO occurrence was low in both FM and MJE. Logistic regression models (Table S7) depict a slight increase in probability over the storage duration for FM (Figure 5A), but no significant difference was seen in the odds ratio comparison (Table S8). There was no FTO for MJE stored at −0.6 °C while a large number of MJE stored at 0.7 °C were diseased, which led to a high observance of FTO. SB was the only cultivar to have a significantly lower FTO probability when stored at −0.6 compared to 0.7 °C (Table S8). Overall, the FTO occurrence was less in Exp2 when comparing storage at 0.7 in Exp1. Raising the sub-zero temperature to −0.6 °C in Exp2 from −3.1 °C in Exp1 reduced the FTO percentage likely due to the avoidance of ice formation as none was observed throughout the 16 weeks of storage.

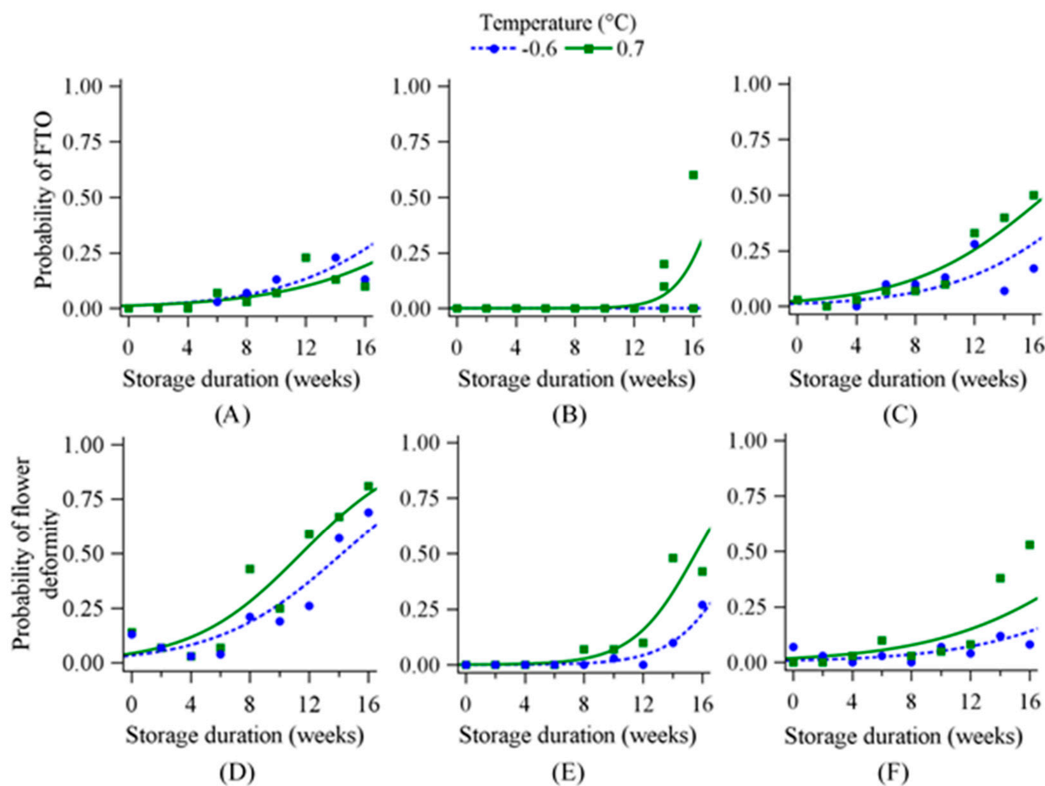

Figure 5. Exp2 probability of flowers that failed to open (FTO) (**A**–**C**) and probability of deformed flowers (**D**–**F**) for 'Festiva Maxima' (**A**,**D**), 'Monsieur Jules Elie' (**B**,**E**), and 'Sarah Bernhardt' (**C**,**F**) in response to significant effects of either storage temperature at nine storage durations. Buds that failed to open or flowers that partially opened and petals that did not complete reflex or unfold were labeled as FTO during post-storage evaluation. Flowers were considered deformed if petals did not fully expand, were missing, were wrinkled, or exhibited some type of disfigurement. Logistic regressions show the predicted FTO or deformity probability along with the percentage that did occur as a decimal ($n \leq 10$). Logistic models and associated statistics are located in Tables S7–S10.

The FM flower diameter significantly declined ($p \leq 0.0001$) from 14.4 cm for non-stored stems to 10.0 cm at 16 weeks of storage. FM stored at −0.6 °C were on average significantly ($p \leq 0.0001$) larger by 0.4 cm. Storage duration and temperature had similar significant effects on the MJE flower diameter, which decreased from 15.0 to 10.5 cm by 16 weeks of storage; diameters were on average 0.5 cm larger when stored at −0.6 °C. The SB flower diameter also declined as storage duration increased, and a significantly smaller diameter was measured when stored for 4 or more weeks (Table 4). Following 6 or more weeks of storage, the SB flower diameter was larger when stored at −0.6 °C, but was only significant at 10 or 14 weeks of storage (Table 4). The pulse treatment had no significant effect on SB, but hydrator-pulsed stems were significantly larger by 0.3 and 0.5 cm for FM and MJE, respectively, when compared to the non-pulsed control.

Logistic regression models depict the benefit of storing stems at −0.6 compared to 0.7 °C (Figure 5D–F). The probability of FM, MJE, and SB flowers being deformed were significantly lower when stored at −0.6 compared to 0.7 °C (Table S10). A higher percentage of SB flowers were deformed when pulsed with sucrose, but this was not statically significant. The deformity incidence in Exp2 was similar to Exp1. The extension of storage from 12 to 16 weeks in Exp2 increased the incidence of flower deformity in FM and MJE by 5 to 10%.

Table 4. Mean flower diameter (cm) for *Paeonia lactiflora* cultivar Sarah Bernhardt (SB) in response to storage temperature and storage duration in Exp2 independent of pre-storage pulses.

		Storage Temperature (°C)	
		0.7	−0.6
		Diameter (cm)	
Storage duration (weeks)	2	12.1 a[1]	12.1 a
	4	19.8 * a[1]	19.6 * a
	6	10.3 * a[1]	10.6 * a
	8	19.2 * a[1]	19.7 * a
	10	18.6 * b[1]	10.1 * a
	12	19.4 * a[1]	19.6 * a
	14	18.8 * b[1]	10.0 * a
	16	18.6 * a[1]	19.5 * a
Non-stored		13.3 a[1]	12.8 a

[1] Least squared means followed by the same letter within each storage duration are not significantly different when adjusted with Tukey's honest significant difference test with $p < 0.05$ in a partitioned analysis of simple effects. * Indicates that the least squared means within cultivar are significantly different than the non-stored control when $p < 0.05$.

Overall, better quality flowers were obtained when stored at −0.6 °C. This was primarily reflected in MJE where FTO did not occur when stems were stored at −0.6 °C compared to FTO occurring in 20% of stems stored at 0.7 °C (Table 2). Deformity occurrences were also lower by 33 and 6% at 14 and 16 weeks, respectively when stored at −0.6 °C. In some cases, differences between 0.7 and −0.6 °C were small, likely caused by the small (1 °C) difference in storage temperature treatments. In contrast, a difference of 1 to 2 °C lengthened the storage life of apricot [15], cherry [17], and nectarine [18] fruit by reducing the respiration rate, retaining weight, preserving color, and maintaining a carbohydrate content. Pre-storage pulses had a minimal effect on peony vase life and quality. A pre-storage hydrator treatment was statically beneficial for quality, but lacks commercial significance, meaning growers may decide to forego this step from their postharvest handling procedures [64]. The number of flowers terminated due to disease development was relatively low in both experiments. However, disease incidence and the number of flowers terminated due to disease increased as storage duration increased (data not shown). Leaves were the most commonly diseased tissue; removal prior to storage may be useful to minimize the spread of inoculum and preserve the aesthetic of the cut stem. Leaf removal following harvest increased vase life of cut *Euphorbia pulcherrima* Willd. [65] and *Hydrangea macrophylla* (Thunb.) Ser. [66], but had no effect on the vase life of *Lilium* 'Stargazer' [67]. It is unknown how leaf removal prior to storing cut peonies would affect the subsequent hydration of cut peony.

3.3. Stress Indices

The total phenolic content (TPC) GAE ranged between 31.08 and 59.90 mg· g^{-1} FW in peony petals and was generally higher at longer storage durations (Table 5). Independent of storage duration and temperature, petal TPC was lower for hydrator-pulsed stems compared to non-pulsed and sucrose pulsed stems by 1 to 4 mg· g^{-1} FW for MJE ($p \leq 0.0001$) and SB ($p \leq 0.0001$). Both sucrose- and hydrator-pulsed FM stems were significantly ($p = 0.0048$) lower than the non-pulsed control by 1 mg·g^{-1} FW. TPC values were between reports by Li et al. [42] who tested 24 cultivars where values ranged from 9.41 to 32.2 mg· g^{-1} DW and *P. lactiflora* petals containing 222.01 mg· g^{-1} DW [35]. They greatly exceeded that of strawberry (*Fragaria* × *ananassa* Duch.) (2470 mg· kg^{-1} FW) [68] and blackberry (*Rubus* sp.) (6818.3 mg· kg^{-1} DW) [69].

Table 5. Total phenolic content (TPC) gallic acid equivalent in petals of *Paeonia lactiflora* cultivars Festiva Maxima (FM), Monsieur Jules Elie (MJE), and 'Sarah Bernhardt' (SB) in response to storage duration independent of storage temperature and pre-storage pulses.

		FM	**MJE**	**SB**
		TPC (μg·kg^{-1} FW)		
Storage duration (weeks)	2	31.08 edd	38.83 e[1]d	38.89 cde
	4	34.06 ded	42.42 de[1]	39.61 cdd
	6	38.12 abc	42.91 de[1]	42.07 abc
	8	37.17 bcd	46.30 cd[1]	41.04 bcd
	10	36.33 bcd	47.50 cd[1]	41.39 bcd
	12	35.09 cdd	58.13 ad[1]	35.96 ded
	14	39.82 abd	59.90 ad[1]	47.35 add
	16	41.344 add	53.63 bd[1]	45.91 abd
Non-stored		34.81 cddg	38.53 e[1]d	33.92 edd
***p*-value**		<0.0001	<0.0001	<0.0001

[1] Least squared means followed by the same letter are not significantly different when adjusted with Tukey's HSD test with $p < 0.05$.

High TPC values may be explained by the numerous concentration of bioactive compounds. The F-C reagent reacted with many compounds other than polyphenols [70] including carbohydrates, thiols, vitamins, phenolic acids such as gallic acid contained in peony [35], and soluble protein. Anthocyanins may also attribute to a higher TPC given their reducing properties. Total anthocyanins of *P. lactiflora* ranged from 2 to 180 mg· g^{-1} [71] compared to 17.6 to 33.5 g·kg^{-1} in blackberry [70]. This may also explain the lower TPC values in peony roots (*P. lactiflora* 26.75 mg· g^{-1} DW [47] and *P. suffruticosa* 24.51 mg· g^{-1} DW) [47], which lack pigmentation compared to petals. In the current study, TPC may be related to flower color. MJE (pink) had the highest TPC followed by SB (pink) and finally FM (white). There was no indication of this trend by Li et al. [42], and anthocyanins were undetectable to near zero in white *P. lactiflora* petals [71,72].

Storage temperatures near 0 °C likely maintained TPC similar to that seen in strawberry [69], apricot [15], nectarine [18], and cherry fruit [17]. Peony petal TPC was relatively constant up to 10 or 12 weeks of storage and then rose by 9 to 10 mg· g^{-1}, which was far more compared to 1 to 2 mg· g^{-1} in blackberry fruit [70], which was only stored for 15 d. The compounds responsible for TPC increases in blackberries were gallic acid and flavanols. Phenolic acids were the primary cause for TPC increase in petunia leaves when exposed to chilling temperatures [73]. Similar compounds may have increased while storing cut peonies as they contain gallic acid, phenolic acids, and tannins which are derivatives of flavanols [48,71].

The MDA content remained relatively constant throughout storage with the exception of stems stored for 10 weeks (Table 6). On average, MJE petals contained about 4 and 2 times more MDA than FM and SB, respectively. The magnitude of the difference among cultivar MDA values may seem significant as smaller differences correlated with observable phenotypic differences following free-thaw tests on spinach leaves [61]. However, SB had less MDA than MJE, more than FM, and was the most impacted by storage duration losing approximately one full day of open time over the 16-week storage period. In comparison, by 16 weeks of storage, open time of FM and MJE was on average 0.6 and 0.2 d shorter, respectively. Petal MDA values were 10 to 100 times higher than those reported on peony roots when exposed to temperatures as low as −36 °C for 5 h [44]. However, no phenotypic data was provided limiting the inference of stress indices used by Wang et al. [44]. Given that petal tissue in the current study was largely uninjured after 16 weeks at −0.6 °C, MDA did not accurately reflect phenotypic damage.

Table 6. Malondialdehyde (MDA) content in petals of *Paeonia lactiflora* cultivars Festiva Maxima (FM), Monsieur Jules Elie (MJE), and Sarah Bernhardt (SB) in response to storage duration independent of storage temperature and pre-storage pulses.

		FM	MJE	SB
		MDA (nmol·g^{-1} FW)		
Storage duration (weeks)	2	0.40	6.20 bc	3.60
	4	0.40	6.88 bc	3.64
	6	0.40	7.64 ab	3.98
	8	0.40	7.64 ab	4.12
	10	0.40	9.43 ad	3.73
	12	0.40	7.95 ab	3.30
	14	0.40	4.79 cd	3.43
	16	0.40	7.06 bd	4.95
Non-stored		0.40	4.78 cd	3.09
p-value		NS	<0.0001	NS

[1] Least squared means followed by the same letter are not significantly different when adjusted with Tukey's HSD test with $p < 0.05$.

Based on the values, it seems that the pink pigment of MJE and SB may have impacted absorbance and increased the amount of MDA. However, no impact was seen in calculations based on equations used from either Heath and Packer [49] compared to that of Hodges et al. [43], who adjusted MDA calculations to account for interfering compounds such as anthocyanins and carbohydrates. The adjustment for interference was only tested using fruits or vegetative tissue not floral tissue. Peonies may still contain other compounds that influence the TBARS method and MDA measurements. Total soluble sugars are likely not an issue given peony buds maintain between 200 and 250 g·kg^{-1} throughout 10 weeks of storage [3]. In contrast, fresh blueberry fruit, one of the large anthocyanin containing fruit tested by Hodges et al. [43], can contain around 500 g·kg^{-1} total soluble sugars [74].

Only non-pulsed, control petals of MJE and SB were sampled for SOD after 0, 2, 4, 8, and 16 weeks of storage at 0.7 °C. No treatment significantly influenced the SOD content of non-pulsed control stems. Values ranged from 8000 to 10,573 U·g^{-1} FW for MJE and 7838 to 100,764 U·g^{-1} FW for SB. SOD values in petals were 20 to 25 times higher than those reported in 24 peony cultivars evaluated by [42] sometimes 100 times higher than values in peony roots [44]. Details on SOD kits and respective indicators are lacking; a variety of SOD forms such as Cu/Zn are used among kits, which can greatly affect SOD values in reports [36]. All three forms plus isoforms were identified in carnation petals [36], but peonies have not been studied. Correlations among storage duration and physiological indices: TPC, MDA, and SOD were all lower than 0.5 (data not shown). There is limited information that can be concluded when comparing physiological data to vase life and quality characteristics. In general, no trend was obvious among TPC, MDA, SOD and open time and quality traits such as diameter, deformity, and FTO. Stronger treatment effects may have been seen if MDA would have been measured during Exp1 where open time and quality data declined when stems were stored at 3.5 and −3.1 °C.

4. Conclusions

While the vase life of cut peonies remains relatively short in comparison to many other cut flower species, storage could be extended to 16 weeks with the acknowledgement that increased storage duration increases the risk of reduced flower quality. Across the three cultivars, cut peonies can be stored up to 12 weeks at 0.7 °C and up to 14 weeks at −0.6 °C, while still being readily marketable (≤25% FTO and deformity). Neither pre-storage pulses nor sub-zero temperatures improved the open time of cut peonies. Although TPC, MDA, and SOD values did not distinguish the phenotype response to treatment, the high antioxidant activity found could explain why cut peonies tolerate dry long-term storage compared to other cut flowers species. Throughout the post-storage evaluation, petals often

abscised from the receptacle long before petals wilted, developed necrotic lesions, or other termination criteria were met. Ultimately, the key to a longer vase life could be related to water movement into the petals and the inhibition of an abscission layer. Petal retention may improve the open time of peonies by 1 to 2 d, which would improve both grower and customer satisfaction, versatility as a cut flower, and provide more time to sell and store cut stems.

If sub-zero storage can be applicable to more species, storage life extension could also have positive implications on demand and labor by allowing growers to maintain uniform production and labor throughout the year through storing the product instead of scaling up production and labor prior to high demand holidays, events, or seasons. Adoption of this storage temperature by the industry could take time due to a number of factors such as cost of equipment sensitive enough to avoid large temperature fluctuations and the limited number of species known to be adaptable. Future research into more sub-zero tolerant cut flower species such as tulips and other spring flowering, seasonal crops would make this technology more attractive.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/11/1694/s1. Table S1: Percent weight loss linear regression models and fit statistics for Figure 1A–C; Table S2: Failure to open (FTO) logistic regression models for Figure 1D–F; Table S3: Failure to open (FTO) storage temperature treatment comparisons using least squares means and Tukey HSD for Figure 1D–F; Table S4: Flower diameter linear regression models and fit statistics for Figure 2A–C; Table S5: Flower deformity logistic regression models for Figure 2D–F; Table S6: Flower deformity storage temperature treatment comparisons using least squares means and Tukey's HSD for Figure 2D–F; Table S7: Failure to open (FTO) logistic regression models for Figure 5A–C; Table S8: Failure to open (FTO) storage temperature treatment comparisons using least squares means and Tukey's HSD for Figure 5A–C; Table S9: Flower deformity logistic regression models for Figure 5D–F; Table S10: Flower deformity storage temperature treatment comparisons using least squares means and Tukey's HSD for Figure 5D–F.

Author Contributions: Conceptualization, N.J.J., J.M.D. and B.A.B.; methodology, N.J.J., B.A.B. and G.M.; formal analysis, N.J.J.; investigation, N.J.J.; resources, J.M.D. and P.P.-V.; writing—original draft preparation, N.J.J.; writing—review and editing, J.M.D., N.J.J. and P.P.-V.; visualization, N.J.J.; supervision, J.M.D., B.A.B. and P.P.-V.; funding acquisition, B.A.B. and J.M.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the North Carolina Department of Agriculture and Consumer Services through the North Carolina Specialty Crop Block Grant Program. The grantee contract number is 17-182-4004.

Acknowledgments: The authors would like to thank Dual Venture Farms for their generous donation of plant material, the Perkins-Veazie lab including Erin Deaton and Joyce Edwards, and Ingram McCall for her help with the experiment setup and data collection.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Kamenetsky, R.; Dole, J. Herbaceous peony (*Paeonia*): Genetics, physiology, and cut flower production. *Floricult. Ornam. Biotechnol.* **2012**, *1*, 62–77.
2. Gast, K. *Fresh-Cut Peonies. Report of Progress 818*; Kansas State University: Manhattan, KS, USA, 1997; Available online: https://www.ksre.k-state.edu/historicpublications/pubs/SRP818.pdf (accessed on 31 October 2020).
3. 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]
4. Gast, K. *Fresh-Cut Peonies. Report of Progress 864*; Kansas State University: Manhattan, KS, USA, 1999; Available online: https://www.ksre.k-state.edu/historicpublications/pubs/SRP864.pdf (accessed on 31 October 2020).
5. Gast, K. *Fresh-Cut Peonies. Report of Progress 866*; Kansas State University: Manhattan, KS, USA, 2000; Available online: https://www.ksre.k-state.edu/historicpublications/pubs/SRP866.pdf (accessed on 31 October 2020).
6. Loyola-López, N.; Prieto-Labbé, C.; Villouta-Barr, B. Application of calcium, boron and sucrose on cut peony stems (*Paeonia lactiflora* Pall.) cv. Karl Rosenfield. *Agron. Colomb.* **2012**, *30*, 103–110.
7. Celikel, F.G.; Reid, M.S. Storage temperature affects the quality of cut flowers from the *Asteraceae*. *HortScience* **2002**, *37*, 148–150. [CrossRef] [PubMed]
8. Heuser, C.W.; Evensen, K.B. Cut flower longevity of peony. *J. Am. Soc. Hort. Sci.* **1986**, *111*, 896–899.

9. Prisa, D.; Burchi, G.; van Doorn, W.G. Effects of low temperature storage and sucrose pulsing on the vase life of Lilium cv. Brindisi inflorescences. *Postharvest Biol. Technol.* **2013**, *79*, 39–46. [CrossRef]
10. Faragher, J.D.; Mayak, S.; Tirosh, T. Physiological response of cut flowers to cold storage. *Physiol. Plant.* **1986**, *67*, 205–210. [CrossRef]
11. Halevy, A.H.; Mayak, S. Senescence and postharvest physiology of cut flowers-Part 2. In *Horticulture Reviews*; Janick, J., Ed.; Horti AVI Publishing Co. Inc.: Westport, CT, USA, 1981; pp. 59–143. [CrossRef]
12. Post, K.; Fischer, C.W., Jr. *Commercial Storage of Cut Flowers; Cornell Extension Bulletin 853*; Cornell University: Ithaca, NY, USA, 1952.
13. Xue, J.; Tang, Y.; Wang, S.; Xue, Y.; Liu, X.; Zhang, X. Evaluation of dry and wet storage on vase quality of cut peony based on the regulation of starch and sucrose metabolism. *Postharvest Bio. Technol.* **2019**, *155*, 11–19. [CrossRef]
14. Eason, J.; Pickney, T.; Heyes, J.; Brash, D.; Bycroft, B. Effect of storage temperature and harvest bud maturity on bud opening and vase life of *Paeonia lactiflora* cultivars. *N. Z. J. Crop Hort. Sci.* **2002**, *30*, 61–67. [CrossRef]
15. Fan, X.; Zhao, H.; Liu, B.; Cao, J.; Jiang, W. Improving fresh apricot (*Prunus armeniaca* L.) quality and antioxidant capacity by storage at near freezing temperature. *Sci. Hort.* **2018**, *231*, 1–10. [CrossRef]
16. Liu, B.; Jiao, W.; Wang, B.; Shen, J.; Zhao, H.; Jiang, W. Near freezing point storage compared with conventional low temperature storage on apricot fruit flavor quality (volatile, sugar, organic acid) promotion during storage and related shelf life. *Sci. Hort.* **2019**, *249*, 100–109. [CrossRef]
17. Zhao, H.; Wang, B.; Cui, K.; Cao, J.; Jiang, W. Improving postharvest quality and antioxidant capacity of sweet cherry fruit by storage at near-freezing temperature. *Sci. Hort.* **2019**, *246*, 68–78. [CrossRef]
18. Zhao, H.; Shu, C.; Fan, X.; Cao, J.; Jiang, W. Near-freezing temperature storage prolongs storage period and improves quality and antioxidant capacity of nectarines. *Sci. Hort.* **2018**, *228*, 196–203. [CrossRef]
19. Nichols, R.; Wallis, L.W. *Cool Storage of Cut Narcissus*; Agricultural Research Council: Kent, UK, 1972.
20. Barzilay, A.; Zemah, H.; Kamenetsky, R. Annual life cycle and floral development of 'Sarah Bernhardt' peony in Israel. *HortScience* **2002**, *37*, 300–303. [CrossRef]
21. Wang, W.; Zhang, X.; Shi, J.; Gao, S.; Wang, Z.; Shi, G. Changes of cold resistance of floral organs during sprout in different tree peony cultivars (In Chinese). *China Acad. J.* **2013**, *21*, 52–56.
22. Walton, E.F.; McLaren, G.F.; Boldingh, H.L. Seasonal patterns of starch and sugar accumulation in herbaceous peony (*Paeonia lactiflora* Pall.). *J. Hort. Sci. Biotechnol.* **2007**, *82*, 365–370. [CrossRef]
23. Galindo, F.G.; Sjöholm, I.; Rasmusson, A.G.; Widell, S.; Kaack, K. Plant stress physiology: Opportunities and challenges for the food industry. *Crit. Rev. Food Sci. Nutr.* **2007**, *46*, 749–763. [CrossRef]
24. Jones, K.S.; Paroschy, J.; McKersie, B.D.; Bowley, S.R. Carbohydrate composition and freezing tolerance of canes and buds in *Vitis Vinifera*. *J. Plant Physiol.* **1999**, *155*, 101–106. [CrossRef]
25. Patton, A.J.; Cunningham, S.M.; Volenec, J.J.; Reicher, Z.J. Differences in freeze tolerance of Zoysiagrasses: II. Carbohydrate and proline accumulation. *Crop Sci.* **2007**, *47*, 2170–2181. [CrossRef]
26. Ashworth, E.N.; Stirm, V.E.; Volenec, J.J. Seasonal variations in soluble sugars and starch within woody stems of *Cornus sericea* L. *Tree Physiol.* **1993**, *13*, 379–388. [CrossRef]
27. Yu, D.J.; Hwang, J.Y.; Chung, S.W.; Ohm, H.D.; Yun, S.K.; Lee, H.J. Changes in cold hardiness and carbohydrate content in peach (*Prunus persica*) trunk bark and wood tissues during cold acclimation and deacclimation. *Sci. Hort.* **2017**, *219*, 45–52. [CrossRef]
28. Heins, R.D.; Howell, G.S.; Wilkins, H.F. The influence of sucrose, ethanol and calcium nitrate on the freezing-point and long-term low-temperature storage of carnation flowers. *Sci. Hort.* **1981**, *14*, 269–275. [CrossRef]
29. Goszczyńska, D.; Rudnicki, R.M. Long-term storage and carnations cut at the green-bud stage. *Sci. Hort.* **1982**, *17*, 289–297. [CrossRef]
30. van Doorn, W.G.; Dole, I.; Celikel, F.G.; Harkema, H. Opening of cut *Iris x hollandica* flowers as affected by temperature, dry storage, and light. *Postharvest Biol. Technol.* **2014**, *89*, 40–43. [CrossRef]
31. van Meeteren, U.; Arévalo-Galarza, L.; van Doorn, W.G. Inhibition of water uptake after dry storage of cut flowers: Role of aspired air and wound-induced processes in Chrysanthemum. *Postharvest Biol. Technol.* **2006**, *41*, 70–77. [CrossRef]
32. Mayak, S.; Bravdo, B.; Gvilli, A.; Halevy, A.H. Improvement of opening of cut gladioli flowers by pretreatment with high sugar concentrations. *Sci. Hort.* **1973**, *1*, 357–365. [CrossRef]

33. Doi, M.; Reid, M.S. Sucrose improves the postharvest life of cut flowers of a hybrid *Limonium*. *HortScience* **1995**, *30*, 1058–1060. [CrossRef]
34. Mor, Y.; Reid, M.S.; Kofranek, A.M. Pulse treatments with silver thiosulfate and sucrose improve the vase life of sweet peas. *J. Am. Soc. Hort. Sci.* **1984**, *109*, 866–868.
35. Chen, G.-L.; Chen, S.-G.; Xie, Y.-Q.; Chen, F.; Zhao, Y.-Y.; Luo, C.-X.; Gao, Y.-Q. Total phenolic, flavonoid and antioxidant activity of 23 edible flowers subjected to in vitro digestion. *J. Func. Foods* **2015**, *17*, 243–259. [CrossRef]
36. Droillard, M.-J.; Bureau, D.; Paulin, A.; Daussant, J. Identification of different classes of superoxide dismutase in carnation petals. *Electrophoresis* **1989**, *10*, 46–48. [CrossRef]
37. Lee, S.C.; Kwon, Y.S.; Son, K.H.; Heo, M.Y. Antioxidative constituents form *Paeonia lactiflora*. *Arch. Pharm. Res.* **2005**, *28*, 775–783. [CrossRef]
38. Chakrabarty, D.; Verma, A.K.; Datta, S.K. Oxidative stress and antioxidant activity as the basis of senescence in *Hemerocallis* (day lily) flowers. *J. Hort. For.* **2009**, *1*, 113–119.
39. Kumar, N.; Srivastava, G.C.; Dixit, K. Rose of superoxide dismutases during petal senescence in rose (*Rosa* hybrid L.). *J. Hort. Sci. Biotechnol.* **2007**, *82*, 673–678. [CrossRef]
40. Mayak, S.; Legge, R.L.; Thompson, J.E. Superoxide radical production by microsomal membranes from senescing carnation flowers: An effect of membrane fluidity. *Phytochemistry* **1983**, *22*, 1375–1380. [CrossRef]
41. Bartoli, C.G.; Simontacchi, M.; Guimet, J.J.; Montaldi, E.; Puntarulo, S. Antioxidant enzymes and lipid peroxidation during aging of *Chrysanthemum morifolium* RAM petals. *Plant Sci.* **1995**, *104*, 161–168. [CrossRef]
42. Li, W.; Yang, S.; Cui, H.; Hua, Y.; Tao, J.; Zhou, C. Nutritional evaluation of herbaceous peony (*Paeonia lactiflora Pall.*) petals. *Emir. J. Food Agri.* **2017**, *29*, 518–531. [CrossRef]
43. Hodges, D.M.; DeLong, J.M.; Forney, C.F.; Prange, R.K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **1999**, *207*, 604–611. [CrossRef]
44. Wang, Q.; Cheng, T.; Yu, X.; Teixeira da Silva, J.A.; Byrne, D.H. Physiological and biochemical responses of six herbaceous peony cultivars to cold stress. *S. Afr. J. Bot.* **2014**, *94*, 140–148. [CrossRef]
45. Tian, Z.; Wang, F.; Zhang, W.; Liu, C.; Zhao, X. Antioxidant mechanism and lipid peroxidation patterns in leaves and petals of marigold in response to drought stress. *Hort. Environ. Biotechnol.* **2012**, *53*, 183–192. [CrossRef]
46. Fan, J.; Wenxue, Z.; Kang, H.; Ma, H.; Tao, G. Flavonoid constituents and antioxidant capacity in flowers of different Zhongyuan tree peony cultivars. *J. Funct. Foods* **2012**, *4*, 147–157. [CrossRef]
47. Li, H.-B.; Wong, C.-C.; Cheng, K.-W.; Chen, F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT-Food Sci. Technol.* **2008**, *41*, 385–390. [CrossRef]
48. Wu, S.-H.; Wu, D.-G.; Chen, Y.-W. Chemical constituents and bioactivities of plants from the genus *Paeonia*. *Chem. Biodivers.* **2010**, *7*, 90–104. [CrossRef] [PubMed]
49. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [CrossRef]
50. Singleton, V.L.; Otherofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
51. Miller, W.B.; Langhans, R.W. Low temperature alters carbohydrate metabolism in Easter lily bulbs. *HortScience* **1990**, *25*, 463–465. [CrossRef]
52. Rehman, S.I.; Qadir, Z.A.; Sheikh, M.Q.; Ahmed, Z. Effect of pulsing with sucrose and silver thiosulphate complex on keeping quality of cut peony (*Paeonia lactiflora* Pall.) cv. Sarah Bernhardt. *Afr. J. Agri. Res.* **2013**, *8*, 2360–2365. [CrossRef]
53. Hall, A.J.; Catley, J.L.; Walton, E.F. The effect of forcing temperature on peony shoot and flower development. *Sci. Hort.* **2007**, *133*, 188–195. [CrossRef]
54. CRONOS. *State Climate Office of North Carolina*; NC State University: Raleigh, NC, USA, 2019; Available online: http://climate.ncsu.edu/cronos/ (accessed on 22 November 2019).
55. van Doorn, W.G.; Perik, R.R.J.; Abadie, P.; Harkema, H. A treatment to improve the vase life of cut tulips: Effects on tepal senescence, tepal abscission, leaf yellowing and stem elongation. *Postharvest Bio. Technol.* **2011**, *61*, 56–63. [CrossRef]

56. Bravdo, B.; Mayak, S.; Gravrieli, Y. Sucrose and water uptake from concentrated sucrose solutions by gladiolus shoots and the effect of these treatments on floret life. *Can. J. Plant Sci.* **1974**, *52*, 1271–1281. [CrossRef]
57. Xue, J.; Tang, Y.; Wang, S.; Yang, R.; Xue, Y.; Wu, C.; Zang, 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 Bio. Technol.* **2018**, *143*, 92–101. [CrossRef]
58. Mayak, S.; Halevy, A.H. Water stress as the cause for failure of flower bud opening in iris. *J. Am. Soc. Hort. Sci.* **1971**, *96*, 482–483.
59. Cevallos, J.-C.; Reid, M.S. Effect of dry and wet storage at different temperatures on the vase life of cut flowers. *HortTechnology* **2001**, *11*, 199–202. [CrossRef]
60. Pearce, R.S. Plant freezing and damage. *Annu. Bot.* **2007**, *87*, 417–424. [CrossRef]
61. Min, K.; Chen, K.; Arora, R. Effect of short-term versus prolonged freezing on freeze-thaw injury and post-thaw recovery in spinach: Importance in laboratory freeze-thaw protocols. *Environ. Exp. Bot.* **2014**, *106*, 124–131. [CrossRef]
62. Wright, R.C. *The Freezing Temperatures of Some Fruits, Vegetables, and Florists' Stocks*; United States Department of Agriculture: Washington, DC, USA, 1942; p. 447. [CrossRef]
63. Jahnke, N.J.; Dole, J.M.; Livingston, D.P., III; Bergmann, B.A. Impacts of carbohydrate pulses and short-term sub-zero temperatures on vase life and quality of cut *Paeonia lactiflora* Pall. hybrids. *Postharvest Biol. Technol.* **2020**, *161*. [CrossRef]
64. Dole, J.; Stamps, B.; Carlson, A.; Ahmad, I.; Greer, L.; Laushman, J. *Postharvest Handling of Cut Flowers and Greens*; ASCFG Press: Oberlin, OH, USA, 2017.
65. Dole, J.M. Storage and simulated shipping of cut 'Renaissance Red' poinsettias. *Acta Hort.* **2005**, *683*, 103–110. [CrossRef]
66. Kitamura, Y.; Ueno, S. Inhibition of transpiration from the inflorescence extends the vase life of cut hydrangea flowers. *Hort. J.* **2015**, *84*, 156–160. [CrossRef]
67. Han, S.S. Role of sugar in the vase solution on postharvest flower and leaf quality of Oriental lily 'Stargazer'. *HortScience* **2003**, *38*, 412–416. [CrossRef]
68. Shin, Y.; Liu, R.H.; Nock, J.F.; Holliday, D.; Watkins, C.B. Temperature and relative humidity effects on quality, total ascorbic acid, phenolics and flavonoid concentrations, and antioxidant activity of strawberry. *Postharvest Biol. Technol.* **2007**, *45*, 349–357. [CrossRef]
69. Kim, M.J.; Perkins-Veazie, P.; Ma, G.; Fernandez, G. Shelf life and changes in phenolic compounds of organically grown blackberries during refrigerated storage. *Postharvest Biol. Technol.* **2015**, *110*, 257–263. [CrossRef]
70. Everette, J.D.; Bryant, Q.M.; Green, A.M.; Abbey, Y.A.; Wangila, G.W.; Walker, R.B. A thorough study of reactivity of various compound classes towards the Folin-Ciocalteu reagent. *J. Agric. Food Chem.* **2010**, *58*, 8139–8144. [CrossRef] [PubMed]
71. Bao, M.; Liu, M.; Zhang, Q.; Wang, T.; Sun, X. Factors affecting the color of herbaceous peony. *J. Am. Soc. Hort. Sci.* **2020**, *145*, 257–266. [CrossRef]
72. Jia, N.; Shu, Q.-Y.; Wang, D.-H.; Wang, L.-S.; Liu, Z.-A.; Ren, H.-X.; Xu, Y.-J.; Tian, D.-K.; Tilt, K.M. Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-mass spectrometry in herbaceous peony species. *J. Am. Hort. Sci.* **2008**, *133*, 418–426. [CrossRef]
73. Pennycooke, J.C.; Cox, S.; Stushnoff, C. Relationship of cold acclimation, total phenolic content and antioxidant capacity with chilling tolerance in petunia (*Petunia × hybrida*). *Environ. Exp. Bot.* **2005**, *53*, 225–232. [CrossRef]
74. Mikulic-Petkovsek, M.; Schmitzer, V.; Slatnar, A.; Stampar, F.; Veberic, R. Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *J. Food Sci.* **2012**, *77*, 1064–1070. [CrossRef]

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).