ABSTRACT

JAHNKE, NATHAN JOSEPH. Impacts and Use of Sub-zero Temperatures During Cut Flower Production and Postharvest Handling (Under the direction of Dr. John M. Dole and David P. Livingston III).

Sub-zero temperatures are often avoided during the production and postharvest handling of perishable cut flowers due to the potential of freezing and injury. However, previous research suggested that using sub-zero temperatures during storage could have a positive effect on the subsequent vase life and quality of cut flowers. Storage is a vital component of the cut flower supply chain because it allows for season extension of species with constricted production, flexibility in product supply, and potentially reduces production costs. Primarily, cut *Paeonia lactiflora* Pall. (peony) hybrids were used to explore the use of sub-zero temperatures for long-term storage due to their high value, seasonal but world-wide production, and tolerance to sub-zero temperatures and the ability to store cut stems without water (dry storage). Freezing was confirmed using infrared cameras and cut peonies were uninjured when stems were held -2 °C for 5 h. Using a storage temperature of -0.6 °C, there was a small improvement of cut peony vase life when compared to stems stored at the industry standard temperature of 0.7 °C. Quality of dry-stored cut peonies was higher in stems stored at -0.6 °C as determined by a decrease in the number of peony flowers that failed to open, a decrease in flower deformities, larger flower diameters, and a decrease in disease incidence. Storage life was extended to an unprecedented 16 weeks with minimal impact on the vase life. In a cooperative study with Washington State University, a sub-zero temperature of -1 °C minimized development of *Botrytis cinerea*, on inoculated cut peonies following both long-term storage and shipment of stems to North Carolina State University. Sub-zero temperature storage was tested on cut *Tulipa* (tulips) and *Iris × hollandica* (Dutch iris) stems in conjunction with various other postharvest handling techniques. Three improved handling methods were developed leading to a storage life of 6 weeks for tulips
and improved quality of Dutch iris following storage at -0.6 °C. Finally, sub-zero temperatures were used to simulate spring freeze events on whole peony plants to address yield losses occurring in NC peony growers’ fields. These data provide preliminary information on peony bud tolerance to freezing temperatures. This compilation of work sets forth information of how to improve postharvest handling guidelines of cut flowers using sub-zero temperatures while providing insight into cut flower physiology during production and postharvest handling.
Impacts and Use of Sub-zero Temperatures During Cut Flower Production and Postharvest Handling

by
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A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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DEDICATION

This dissertation is dedicated to my grandfather, Roland Robert Uran. Thank you for being a strong Christian influence in my life, showing me the beauty of nature, and how to respect creation.

June 6, 1937 - January 8, 2020
BIOGRAPHY

Nathan Joseph Jahnke was born on October 9, 1992 to Clark and Susan Jahnke in West Fargo, North Dakota. He owes his love of plants to many people, but especially his grandmother, Judy Uran, and father. Together, he and his father, built and operated Nathan’s Greenhouse for over 6 years selling vegetables and bedding plants. This experience inspired him to study horticulture at North Dakota State University where he completed his Bachelor’s degree in December of 2014. In his pursuit to always work with flowers, Nathan started his Master’s degree at North Carolina State University in August 2015 under Dr. John Dole. Since 2017, Nathan has pursued a Ph.D. under Dr. Dole studying postharvest handling of cut flowers.
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formed due to ice formation.
CHAPTER 1:

Impacts of carbohydrate pulses and short-term sub-zero temperatures on vase life and quality of cut Paeonia lactiflora Pall. hybrids

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Highlights

- Bud time of ‘Karl Rosenfield’ was reduced by >2 d after 5 h of -4 °C
- Carbohydrate pulses did not improve total vase life of any peony cultivar
- No freeze injury was observed when stems were kept dry prior to cold treatment
- Infrared video depicted supercooling and freezing within 2 to 5 h at -4 °C

Abstract

Flower quality of cut *Paeonia lactiflora* (peony) Pall. hybrids is best preserved between 0 and 1 °C. However, cut flower traits such as vase life and flower size often decline following 4 or more weeks of storage. While the use of sub-zero temperatures is avoided in the cut flower industry due to fears of freeze injury, sub-zero temperatures may allow extended storage of cut flowers. Peonies are a candidate for sub-zero storage due to their natural cold tolerance, exposure to spring freezes before harvest, and limited seasonal availability. Three cultivars: Karl Rosenfield, Monsieur Jules Elie, and Sarah Bernhardt were used to evaluate freeze tolerance of cut peonies by holding cut stems at three temperatures: 0, -2, -4 °C for 5 h. Pre-cold treatment pulses consisting of 24 h in either 100 g·L⁻¹ sucrose, 100 g·L⁻¹ fructose, or tap water did not improve total vase life, summation of the time spent as a bud and time open. Total vase life was 10.5, 7.1, and 9.3 d for ‘Karl Rosenfield’, ‘Monsieur Jules Elie’, and ‘Sarah Bernhardt’, respectively. Sucrose-pulsed stems of ‘Karl Rosenfield’ and ‘Sarah Bernhardt’ had the lowest total vase life. Pulses and cold-treatments decreased bud time for ‘Karl Rosenfield’ and ‘Monsieur Jules Elie’ by 2 to 3 d and 0.5 to 1 d, respectively. Petals were the only tissue to develop water-soaked spotting (freeze injury) following 5 h at -4 °C. Stems kept dry (not pulsed) prior to cold treatment were uninjured. Fructose-pulsed stems of ‘Karl Rosenfield’ and ‘Monsieur Jules Elie’ had the highest injury ratings when held at -4 °C. Carbohydrate-pulsing
did not influence injury ratings on ‘Sarah Bernhardt’. Supercooling and multiple freeze events were observed with infrared video in all tissues when held at -4 °C. Typically, ice nucleation started at the base of the cut stems and propagated throughout the stem, leaves, and bud within 3 to 5 min of initiation. Stems that were not pulsed remained in a supercooled state longer than those that were pulsed. These findings indicate that storage temperatures between 0 and -2 °C may be a good option for longer periods of dry storage for peonies and other cold tolerant cut flower species.
1.1 Introduction

*Paeonia lactiflora* (peony) Pall. remains a highly valued and desired cut flower after centuries of cultivation due to its large, lush, and often fragrant flowers. However, maintaining quality and supply are problematic due to the short vase life and seasonal nature of the crop. Wholesalers and florists must follow production around the world to buy flowers after their local season has ended (Kamenetsky and Dole, 2012). Growers can reliably use dry storage between 0 and 1 °C for up to 4 weeks with a small reduction in vase life (Gast, 1999, 2000; Walton et al., 2010). While this length of storage is beneficial, it does not extend the season long enough to bridge the availability gap of 2 to 3 months. Long-term storage of more than 8 weeks would extend peony availability through production gaps, as proposed by Kamenetsky and Dole (2012). However, storage at 0 to 1 °C for this duration causes a significant reduction in vase life for most cultivars (Gast, 2000; Walton et al., 2010) despite storage technique (Nowak and Rudnicki, 1990; Dole et al., 2017), preservative pulses (Gast, 2000), or harvest stage (Eason et al., 2002).

Traditionally, air temperatures between 0 and 10 °C and floral preservatives containing carbohydrates have been used to preserve and maintain cut flower vase life (Dole et al., 2017). Little research has been done to explore the combination of sub-zero temperatures and carbohydrate additives. Sub-zero temperatures are avoided because many species reportedly experience injury below 0 °C (Post and Fischer, 1952; Heins et al., 1981). However, carnations (*Dianthus caryophyllus* L.) have also been stored dry for extended periods of time (Goszczyńska and Rudnicki, 1982) and stems have been reported to survive 8 d of storage at -4 °C with 7.3 d of vase life (Heins et al., 1981). Cut peonies may tolerate storage at sub-zero temperatures because dormant underground crowns are considered cold hardy in most of the world and herbaceous shoots and reproductive buds often experience multiple sub-zero events prior to harvest. Peonies
are grown for cut flower production as far north as USDA plant hardiness zone 1, which has an average annual minimum temperature of -48.3 to -45.6 °C (Stern, 1946; Holloway and Buchholz, 2013). The industry-standard storage temperature is 0.6 °C, and the majority of literature has focused on temperatures at or just above 0 °C (Heuser and Evensen, 1986; Eason et al., 2002; Walton et al., 2010; Xue et al., 2019). Post and Fischer (1952) compared peony vase life after storing flowers at 0.6 and -0.6 °C, but reported no vase life or quality data when stating 0.6 °C preserved vase life better than -0.6 °C. To our knowledge no information is available on the performance of cut peony stems at sub-zero temperatures.

Ice formation and dehydration are the biggest concerns when holding plant material at sub-zero temperatures. Unlike edible produce that can retain value after freezing, cut flowers need to survive the freezing and thawing, while avoiding physical damage, retaining a marketable vase life, and maintaining the ability to open if stored as a bud. Keeping plant tissue in a supercooled state may increase the success of sub-zero storage. Dormant woody plant tissues frequently supercool during winter freeze events (Ashworth, 1990; Flinn and Ashworth, 1994). Free water and soluble carbohydrate concentrations are two components thought to affect supercooling ability (Pearce, 2001). Some species have been shown to have movement of free water outside of essential reproductive tissue where ice formation can occur in a separate space, termed extraorgan freezing (Ashworth et al., 1989; Flinn and Ashworth, 1994). Accumulating soluble carbohydrates prior to freezing has been correlated with reduced freezing points in cut carnation (Heins et al., 1981) and freezing tolerance of crowns of various peony cultivars (Wang et al., 2014). Increased solutes can also prevent dehydration stress by decreasing the amount of free water (Herman et al., 2006). High concentrations of carbohydrates such as starch have been reported in both field plants (Walton et al., 2007) and stored cut stems of peony (Walton et al.,
2010). Starch content readily hydrolyzes in field peony flowers prior to flower opening (Walton et al., 2007) and buds of cut peony stems during cold storage (Walton et al., 2010). Often called “low temperature sweetening”, the conversion of starch to soluble sugars may explain why peonies tolerate dry storage and lower temperatures better than many other cut flowers.

Cut flower preservatives are commonly used prior to or after shipping and storing cut flowers. They often include a carbohydrate component, most commonly sucrose and are formulated for specific purposes. Holding solutions are used for 2 to 3 d before sale, pulses can be used at any point before sale and are used for no longer than 24 h. Vase solutions are meant to be used by consumers as a consistent treatment. A 20 g·L⁻¹ sucrose vase solution (Xue et al., 2018) and a 200 g·L⁻¹ pulse (Sang et al., 1998) have increased vase life in different peony cultivars. Further information on sucrose effects on cut flower species has been reviewed by Pun and Ichimaru (2003). Carbohydrate solutions may improve tolerance to sub-zero temperatures and improve vase life. The combination of a sub-zero temperature and preservative pulses have not been evaluated on cut peonies. The following study had two objectives: 1) Determine the effect of carbohydrate pulses in combination with short-term sub-zero temperature exposure on vase life and flower quality and 2) Determine if ice formation occurs and visualize freezing of cut peony stems using infrared cameras. This study will provide foundational information for future work using sub-zero temperatures for the preservation of cut peonies during storage with the goal of extending this storage method to other cold-tolerant cut flower species.

1.2 Materials and Methods

Plant material

Cut flowers of three peony cultivars: Karl Rosenfield (KR), Monsieur Jules Elie (MJE), Sarah Bernhardt (SB), were obtained from a local commercial grower. For the 21 d prior to
harvest in 2018, the temperature averaged 15.1 °C for the 23 Apr. harvest and 15.8 °C for the 30 Apr. harvest and average daily temperatures ranged from a low of 2.6 °C to a high of 28.6 °C for the first harvest period and from 3.6 to 28.6 °C for the second period. There was 6.4 cm of rain during the first period and 6.5 cm during the second period (CRONOS, 2019). Stems were transported dry to North Carolina State University within 6 h of harvest. Flowers were processed by cutting stems to 40 cm and removing all but the three uppermost leaves. Stems were sorted into groups based on their bud stage. Using the largest group first, flowers were randomly assigned to a treatment ensuring each treatment had the same number of flowers at a particular bud stage.

**Pulse solutions and cold treatments**

After processing, stems were held upright at 1 °C for 24 h in either 9.5 L buckets with no solution (dry) or buckets containing 2 L of one of the following pulse treatments: 100 g·L⁻¹ fructose, 100 g·L⁻¹ sucrose, tap water. Each bucket contained 16 stems. Stems were wiped dry and wrapped in newspaper prior to placement into one of three upright freezers (Model 253.9260410, 20.3 cu. ft., Kenmore, Chicago, IL). Freezers were modified with additional cooling fans and FE Micro-controllers PXR4 (Fuji Electronic Systems Co., Ltd., Tokyo, Japan) with programmable ramp and soak capabilities. Stems were placed into freezers when the chamber temperature reached either 0, -2, or -4 °C. Stems were held at their respective temperature for 5 h starting after the chamber temperature was stable for 30 min. Following treatment, freezers warmed at a rate of 2 °C·h⁻¹. Stems were removed from freezers after 2 h at 4 °C. Each treatment contained 8 stems (n=8). An untreated control, which was not pulsed nor received any cold treatment, was placed directly into tap water for evaluation following processing.
Infrared thermography

A subset of 2 stems per cultivar and pulse treatment were monitored via infra-red camera (FLIR T620, FLIR Systems, Wilsonville, OR) (640 × 480 pixels) at -4 °C. Four thermocouples were placed in close proximity to peony buds. Thermocouples were calibrated at 0 °C with an ice-water slurry. Actual air temperatures were recorded every 10 seconds (CR10X, Campbell Scientific, Utah, USA). Stems were upright in plastic cone-tainers (3.8 cm diameter x 21 cm depth). Freezing was recorded with a computer and IR software (FLIR Systems, Wilsonville, OR) at 1 frames·s\(^{-1}\) and a continuous video recording with BandiCam software at 30 frames·s\(^{-1}\).

Post-storage evaluation

After the cold treatment, stems were recut, removing the lower 2.5 cm. Stems were placed into tap water and evaluated in a climate-controlled room at 22.1 ± 2.9 °C and 53 ± 7 % relative humidity recorded every 60 seconds (HOBO Pro Series, Onset Computer Corporation, Bourne, MA). Total vase life (TVL) was calculated as the days as a bud (bud time) and the days open until termination (open time). Flowers were counted as open when the bud maturity was a stage 5: petals mostly separated, but still curved inward (Eason et al., 2010). Reasons for termination included petal drop induced after a slight shake of the stem, fungal growth or disease within petals or on the receptacle, ≥50 % necrotic petals, ≥50 % of petals wilting, stem collapse, or a failure to open. Freeze injury was rated 48 h after treatment on a scale of 0 to 5: 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 %.

Statistics

A completely randomized design was used and treatments were a 4 x 4 factorial of three temperatures (0, -2, -4 °C) and the no cold control, and three carbohydrate pulses (100 g·L\(^{-1}\))
fructose, 100 g·L⁻¹ sucrose, tap water) and a no pulse control. The experiment was conducted twice. Data from each cultivar were separately subjected to analysis of variance using the GLIMMIX procedure with run as a random variable when appropriate. Tukey’s Honest Significant Difference was used when appropriate for post hoc mean separation tests with \( P \leq 0.05 \).

1.3. Results and Discussion

Vase life

Pulse and cold treatments reduced bud time to varying degrees for each cultivar. All ‘Karl Rosenfield’ flowers held at -4 °C opened 2 to 3 d earlier than the no-pulse, no-cold control (Figure 1.1). Sucrose-pulsed stems without cold treatment and those held at 0 °C, and fructose-pulsed stems held at -2 °C also opened faster than the no-pulse, no-cold control (Figure 1.1). All pulses without cold treatment significantly reduced bud time by approximately 1 d for MJE when compared to the no-pulse, no-cold control (Figure 1.2). Bud time was also significantly lower for all MJE held at -2 °C. Comparing KR and MJE, all treatments were relatively successful at reducing bud time, but cultivars differed in response to temperature and specific pulse and cold combinations. In contrast, no treatments significantly reduced SB bud time from 2.9 d. Growers have reported flowers treated with a short cold period, above 0 °C, open faster than freshly cut stems (R. Illingsworth, personal communication). A cold treatment longer than 5 h may be needed to open buds more quickly because the results of the current study indicate that a 5 h cold treatment near 0 °C did not reduce bud time. Only when cold treatment was combined with water and fructose pulses was bud time significantly reduced in KR and MJE, respectively. Five hours may not be long enough to promote flower opening. Eight weeks of storage at 0 °C reduced bud time by 2 d for SB (Walton et al., 2010). Bud maturity at harvest may explain the
differences among the three cultivars. Eason et al. (2002) reported that the more developed buds were going into storage the faster they opened following storage, but vase life of more developed buds was also reduced. In the current study, buds that opened faster did not have a lower vase life. We observed MJE to be the tightest bud stage, while buds of SB were the most developed. ‘Sarah Bernhardt’ buds may have been so far developed that pulse and cold treatments did not influence bud time.

The combination of 2-h, 100 g·L\(^{-1}\) sucrose pulses and 4 weeks of storage at 1 °C reduced bud time for ‘Jayhawker’, ‘Lora Dexheimer’, ‘Edulis Superba’, and ‘Mister Ed’ peony cultivars, but not MJE (Gast, 1999). In the current study, 100 g·L\(^{-1}\) sucrose alone significantly reduced MJE bud time. Sucrose pulses have improved flower opening in lisianthus (\textit{Eustoma} hybrids) (Huang and Chen, 2002), gladioli (\textit{Gladiolus} sp.) (Mayak et al., 1973), and sweet pea (\textit{Lathyrus odoratus} L.) (Mor et al., 1984), which are all harvested with some unopened flower buds. Among pulses, sucrose was the most consistent at reducing peony bud time.

Cold treatment may have promoted starch hydrolysis in peony buds. ‘Karl Rosenfield’ and MJE had lower bud time in response to cold treatment. Peony flower opening has been correlated with the hydrolysis of starch reserves in field peonies (Walton et al., 2007) and stored stems (Walton et al., 2010). Low-temperature sweetening is commonly associated with postharvest storage of root, tuber and bulb crops (Miller and Langhans, 1990; Novy et al., 2008) and woody plants during winter cold periods (Ashworth et al., 1993). This likely occurs in cut flower stems. Cut peony starch and sucrose concentrations decrease during long-term storage at 0 °C (Walton et al. (2010). Starch levels were almost undetectable following 2 weeks of storage at 0 °C; sucrose levels declined following 1 week of storage; glucose and fructose levels were steady following 3 weeks of storage. There are no known data on the effect sub-zero
temperatures have on bud time when used for long-term storage. A storage temperature between 0 and -2 °C may provide similar carbohydrate preservation to 0.6 °C while maintaining bud maturity and vase life.

The amount of time peonies stay open is a key component to vase life and customer satisfaction. No pulse nor cold treatment significantly improved the amount of time flowers stayed open. Water-, fructose- and sucrose-pulsed stems of KR flowers were open for 7.0, 6.4 and 6.2 d. The open time of sucrose-pulsed stems was significantly lower than the no-pulse control at 6.5 d \( (P \leq 0.0080) \). Sucrose-pulsed MJE were open for 5.8 d compared to 5.6 d for the no-pulse control. Similar results with 2-h 100 g·L\(^{-1}\) sucrose pulses were reported by Gast (1999) across eight different cultivars, including MJE which was open for 6.0 d. Cold treatments slightly improved vase life of KR and MJE by 0.1 to 0.4 d compared to no-cold treatment, but this was not significant.

There was no improvement of total vase life (TVL) for any cultivar compared to the non-treated control. ‘Karl Rosenfield’ TVL was highest for the no-pulse control at 11.0 d, which was only different from sucrose-pulsed stems at 9.7 d \( \text{(Figure 1.3)} \). Total vase life of water- and sucrose-pulsed SB stems significantly differed by 0.8 d, but neither were significantly different from the no-pulse control and stems pulsed with fructose, at 9.4 and 9.2 d, respectively. A decrease in TVL was also seen in ‘Festiva Supreme-White’ and ‘Ozark Beauty-Pink’ when pulsed with the same concentration of sucrose (Gast, 2000). Pulse and cold treatments did not significantly influence the total vase life MJE, which was 7.1. Carbohydrate treatments are not beneficial for all cut flower species (Clark et al., 2010; Redman et al., 2002) and high concentrations can reduce vase life (Redman et al., 2002). This is likely not the case for peony because a
concentration of 200 g·L\(^{-1}\) sucrose has increased vase life in different peony cultivars (Sang et al., 1998; Xue et al., 2018). Cultivars may differ in their ability to transport sucrose or reduce sucrose to fructose and glucose. Xue et al. (2018) identified various sucrose transport genes in *Paeonia lactiflora* ‘Yang Fei Chu Yu’ and their upregulation when stems were in a constant sucrose solution. Pulse treatments may not have been a long enough duration for stems to uptake sucrose and improve vase life or effect freezing tolerance. Sang et al. (1998) reported increased vase life on multiple peony cultivars using a 24-h, 200 g·L\(^{-1}\) sucrose pulse. High sucrose concentrations improved vase life of cut gladiolus, but primarily was improved through the opening of new florets (Mayak et al., 1973). There are three obstacles to the adoption of the concentrated sucrose pulse: the cost to create a concentrated pulse, the unreliability of vase life improvement across peony cultivars, and the development of peony buds during the pulse (Gast, 1999; Eason et al., 2002; Xue et al., 2019). Commercially-available preservatives are generally 10 times less concentrated, around 10 to 20 g·L\(^{-1}\) (Gast, 1997; Dole et al., 2017). Concentrated carbohydrate solutions might also promote bacterial growth, which decreases vase life by clogging of xylem tissue in the absence of biocides (Zagory and Reid, 1986; van Doorn et al., 1991). Bacterial growth is likely minimal in the current study because of limited time stems were pulsed and the low temperature during pulsing. Techniques for peony vase life improvement need to be implemented quickly if through water uptake or other techniques such as pre-harvest treatments need to be explored.

*Freeze injury*

Cold treatment only injured petal tissue. Regardless of temperature, untreated stems of KR and MJE kept dry were uninjured (*Table 1.1*). As expected, -4 °C injured petals more than warmer temperatures. Buds pulsed with water did not develop the highest injury in any cultivar.
This was unexpected because increased water content generally leads to a lower solute concentration and reduced ability to supercool. This was seen in water-pulsed carnations stored for 5 d at -4 °C, which had a vase life of 0 d (Heins et al., 1981). Generally, higher solute concentrations have been correlated with lower freezing points (Heins et al., 1981) and the ability to supercool in woody plants like dogwood (Ashworth et al., 1993). A 200 g·L⁻¹ sucrose pulse prior to storage protected carnations stored at -4 °C for 10 d, resulting in a vase life of 5 d compared to water-pulsed stems, which had a vase life of 0 d (Heins et al., 1981). In the current study injury was highest on stems pulsed with fructose and sucrose. A 2-h pulse of fructose and sucrose in peonies may have improved water uptake providing more free water for freezing. However, this is unlikely because water uptake is reported to be reduced during highly concentrated sucrose pulses (Bravdo et al., 1974).

Other factors along with bud maturity may influence freeze injury of peony petals. Receptacles of large, primary strawberry (Fragaria × ananassa Duch.) flowers were damaged at warmer sub-zero temperatures than smaller tertiary flowers (Ki and Warmund, 1992). Strawberry flowers’ reproductive organs also became more sensitive to chilling and freezing temperatures as development continued (Ariza et al., 2015). Tightly closed buds of blackberry flowers froze similarly to fully open flowers, suggesting that tight bud stages may not be more resistant to freeze injury (Takeda and Glenn, 2016). This is similar to the current study. Buds of KR and MJE were less developed and had a higher injury rating compared to SB. ‘Sarah Bernhardt’ had an average freeze injury rating of 0.4 when held at -4 °C, but was not significantly different from the un-treated control. Petals on buds held at warmer temperatures were uninjured.
Because peony flowers were not deformed after opening, it is likely that the stage of development is important for freezing resistance or that the duration of freezing was not long enough to cause major cellular damage. Peony floral development is generally completed before shoots emerge and growth is primarily achieved through cell elongation (Barzilay et al., 2002). If stems are harvested before flower opening, ice formation within the stem, leaves, and flower bud may cause little deformity when tissue needs to only to expand. Details on how herbaceous peony tissue resists freeze injury is limited, but may be attributed to the concentration of carbohydrates (Walton et al., 2010) and total phenolic compounds that act as antioxidants (Wang et al., 2014).

Applying this research to long term storage of cut flowers will involve the duration flowers are held at sub-zero temperatures. Increasing durations of freezing changed the ability of spinach to recover after freezing (Min et al., 2014). Other factors including acclimation to sub-zero temperatures and deacclimation were not tested during this experiment. Precooling stems may improve petal tolerance and supercooling ability by causing starch to hydrolyze before sub-zero storage. Supercooling is a common mechanism for avoiding ice nucleation and injury (Ashworth et al., 1989; Ki and Warmund, 1992; Salazar-Gutiérrez et al., 2016). Similar to precooling, a slow thawing period at temperatures just above 0 °C may allow ice to melt slowly and water be reabsorbed back into cells. Water-soaking was observed to be less severe as time elapsed during post-treatment evaluation (personal observation). This may mean cellular damage was not enough to hinder water reabsorption. An acclimation period or longer deacclimation period may further improve or be beneficial to adapting sub-zero temperatures for cut flower storage.


Infrared Thermography

Infrared video of stems held at -4 °C for 5 h confirmed that all stems, leaves, and buds experienced freeze events. These events can be seen in detail by viewing the supplemental video (https://go.ncsu.edu/jahnkeonyfreeze). Location and timing of ice nucleation was variable, but primarily occurred from the base of the cut shoot and upwards into the leaves, sepals and petals (Figure 1.4A-F). Frames A-C illustrates freezing from the bottom up in the right stem. Freezing from the top down occasionally occurred and is seen in Frames D-F in the stem on the left. Ice propagated through tissue in approximately 4 to 5 min. If nucleation occurred from the exposed xylem tissue of the cut stem, ice did not always or immediately propagate into bud tissue. Frame C, shows ice formation in sepal tissue of the right bud approximately 30 min following ice nucleation and propagation in the leaf of Frame B. Ice propagated smoothly from bud tissue into the stem and leaves when nucleation occurred from the top down. Readers are encouraged to view this in supplemental video where multiple stems freeze from the top down.

Additional freeze events occurred in petal and sepal tissue throughout the cold treatment, as seen in Frame F in the right bud. This may be evidence of ice barriers in peony receptacle. From the starting point of the video viewers should note the light coloration of the peony buds depicting the bud tissue being a higher temperature and retained heat longer than stem and leaf tissue. The main peony cultivars grown for cut flowers are mostly semi-doubles and doubles. Within a small bud there are hundreds of tightly compacted petals, which may improve heat retention. All shoots, leaves, and flower buds remained supercooled for various lengths of time. Freeze events were not observable in MJE 2 h into the cold treatment. Freeze events were observable in SB bud tissue throughout the entire 5-h cold treatment until the chamber began the warming program. Thawing occurred slowly as temperatures increased in the chamber. All tissue
slightly wilted and then regained turgidity as the ice melted. This suggests that ice was in the apoplast region where all ice was thawed at the same time. Conversely, ice nucleation happened at various times and in different tissues suggesting the location, movement, and freezing of water was influenced by cell structure. Following cold treatment, leaves and stems were all free from visible injury even though ice formation was observed.

Stems pulsed with carbohydrates experienced freeze events earlier than those pulsed with water and those left untreated. Stems of MJE that were not pulsed froze 30 to 40 min later than pulsed stems. This may explain the higher injury ratings in pulsed stems. Extended time frozen may have allowed larger ice crystal formation and cellular dehydration. Sepals and petals of SB froze at various time points and experienced multiple freezes throughout treatment. The greater surface area of SB buds that were more fully developed may have allowed sepals and petals to individually freeze due to the lower petal density and weakened ability to retain heat. It is not clear as to why sepals and petals did not freeze along with the stem and leaves. Freezing of leaf tissue was sometimes delayed compared to sepals and petals. An ice barrier may be present within the receptacle or the high density of petals within the flower bud reduced heat loss. Ice barriers have been reported in the reproductive structures of Calluna vulgaris L. (Kuprian et al., 2016) and also wheat (Triticum aestivum L.) (Livingston et al., 2018). Ice barriers were associated with certain cell types and smaller or fewer xylem. Peony, specifically Paeonia lactiflora Pall., has been reported to have an abundance of xylem located in the receptacle connecting to the ovules (Camp and Hubbard, 1962). Bud development may be complete in harvested cut peonies, but the number of xylem and their structure are uncharacterized throughout the bud development of peony flowers. Development of floral primordia occur during the fall and is completed prior to spring (Barzilay et al., 2002). Petals and reproductive organs,
are the only structures that continue to develop after harvest, which could explain why they experienced the most injury. Petal freezing points of tree peony varieties ‘Luoyanghong’ and ‘Fengdanbai’ were reported to increase as buds continued to develop (Wang et al., 2013). On the contrary, our study showed petals of further developed SB flowers did not have higher injury ratings than petals of less developed KR and MJE buds.

1.4 Conclusions

Minimizing of bud development during storage and shipping is vital to preserve vase life for consumers and avoid mechanical damage. The current study supports the industry standard storage temperature of 0.6 °C as a temperature, which maintains bud maturity. This study is one of the first to explore the potential use of sub-zero temperatures to store perishable peony cut flowers. Carbohydrates pulses were ineffective at improving vase life and preventing freeze injury. However, some cultivars opened more quickly in response to pulse or cold treatments without decreasing total vase life. Fast opening could help consumers appreciate peonies for a longer period of time. Lack of injury to dry stored flowers at sub-zero temperatures and confirmation of freezing at -4 °C through infrared video warrants more research on sub-zero temperatures. Temperatures just below 0 °C could avoid ice formation. The cut peony industry needs a long-term storage technique that preserves vase life and extends season availability. Other species such as tulips (Tulipa sp.) and Dutch iris (Iris × hollandica) could likely withstand sub-zero temperatures, allowing this research to be applied to those species.

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Conflicts of interest

The authors declare no conflict of interest
Literature Cited


Herman, E.M., K. Rotter, R. Premakumar, G. Elwinger, R. Bae, L. Ehler-King, S. Chen, and D.P. Livingston III. 2006. Additional freeze hardiness in wheat acquired by exposure to -


Table 1.1. Effect of pulse and cold treatments on petal freeze injury ratings for two peony cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Cold treatment (°C)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No cold</td>
<td>0</td>
</tr>
<tr>
<td>Karl Rosenfield</td>
<td>No pulse</td>
<td>0 c</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>0 c</td>
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<tr>
<td></td>
<td>Sucrose</td>
<td>0 c</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0 c</td>
</tr>
<tr>
<td>Monsieur Jules Elie</td>
<td>No pulse</td>
<td>0 c</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>0 c</td>
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<td>Sucrose</td>
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Petal freeze injury as water-soaked spotting was rated based on a scale of 0 to 5: 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 %.

For each cultivar, different letters indicate significant differences with P ≤ 0.05 (n = 16) according to Tukey’s HSD.
Figure 1. ‘Karl Rosenfield’ bud time (d) during post-storage evaluation as influenced by pulse and temperature interactions ($P \leq 0.0012$). Different letters indicate significant differences with $P \leq 0.05$ ($n = 16$) according to Tukey’s HSD.
Figure 1.2. ‘Monsieur Jules Elie’ bud time (d) during post-storage evaluation as influenced by pulse and temperature interactions ($P < 0.0001$). Different letters indicate significant differences with $P \leq 0.05$ ($n = 16$) according to Tukey’s HSD.
Figure 1.3. Total vase life (d) of ‘Karl Rosenfield’ flowers in response to carbohydrate pulses ($P < 0.0001$). Different letters indicate significant differences with $P \leq 0.05$ (n = 64).
**Figure 1.4.** Time lapse images of ice propagation through the two center stems of ‘Sarah Bernhardt’ held at -4 °C for 5 h. Lighter colors depict warmer temperatures. The light coloration in the lower portion of frame B indicate exothermic heat from the propagation of ice up the stem and the center leaf of the left stem. Ice continues to propagate up the stem and into sepal tissue in Frame C. Frame D and E depict ice nucleation occurring in the bud tissue and propagation downward. Frame F shows freeze events in the left bud approximately 45 min after the initial ice nucleation. The time does not refer to the exact time of the freeze test. It can be used to determine the time elapsed between each frame. Additional freeze events in petal and sepal tissue can be viewed in the supplemental video to gain a better understanding of the freezing process (https://go.ncsu.edu/jahnkepeonyfreeze).
CHAPTER 2:

Storage and vase life extension of cut *Paeonia lactiflora* Pall. using sub-zero temperatures

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(Written in the style of HortScience)
Abstract

Cut peonies (*Paeonia lactiflora* Pall.) have a relatively short vase life and limited availability due to seasonal production. Two sub-zero temperatures, -3.1 and -0.6 °C, were compared to more common storage temperatures of 0.6 °C, the industry standard, and 3.5 °C to determine whether storage temperatures could be reduced to extended the storage life of cut peonies. Cultivars Festiva Maxima, Monsieur Jules Elie, and Sarah Bernhardt stored at 0.6 °C had a longer flower open time at 12 weeks of storage compared to those held at -3.1 or 3.5 °C. Stems stored at a sub-zero temperature of -0.6 °C avoided freeze injury, had a reduced percentage of flowers that failed to open and fewer deformed flowers at 16 weeks of storage compared to 0.6 °C. Pre-treating stems before storage with pulses of a commercial hydrator solution or a 200 g·L⁻¹ sucrose solution for 2 h at 4 °C had little commercial significance compared to non-pulsed control. Total phenolic content, malondialdehyde, and superoxide dismutase were not effective indicators of vase life or quality loss. This study demonstrates the first use of non-freezing, sub-zero temperature storage for peony and represents the longest extension of peony vase life.

2.1 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 (Kamenetsky and Dole,
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 0.6 to 4.0 °C for up to 8 weeks, but vase life and cut flower quality of stored stems is reduced as storage duration increases (Gast, 1997; Walton et al., 2010). 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 (Gast, 1999; 2000; Loyola-López et al., 2012). These factors limit the storage life of cut peonies and leave a 1 to 3-month availability gap following August.

Cooling technology revolutionized postharvest handling of perishable crops like cut flowers. Holding flowers in cold storage preserves vase life and quality by reducing respiration (Celikel and Reid, 2002), carbohydrate loss (Walton et al., 2010), and flower development (Heuser and Evensen, 1986; Prisa et al., 2013), and limits ethylene production and sensitivity during storage (Celikel and Reid, 2002; Faragher et al., 1986). Cut flowers historically fall into two storage temperature classes; chilling sensitive flowers, consisting of many tropical and subtropical species that should be held no colder than 12 or 2 °C, respectively, and those that should be held no lower than 0 °C (Halevy and Mayak, 1981). The industry standard temperature for cut peony storage is between 0 and 2 °C (Gast, 1999; Heuser and Evensen, 1986; Post and Fischer, 1952; Walton et al., 2010; Xue et al., 2019) where over 25 cultivars were held for 4 to 12 weeks of storage. Vase life of stems stored for 4 weeks near 0 °C is reduced by 1 to 2 d when compared to freshly cut stems (Eason et al., 2002). 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 (Gast, 1999; Walton et al., 2010). 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 (Xue et al., 2019).

Near-freezing storage temperatures (< 0 °C) have gained recent interest in edible crops for improved storage length. Apricots (Fan et al., 2018; Liu et al., 2019), cherries (Zhao et al., 2019), and nectarines (Zhao et al., 2018) 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 (Fan et al., 2018; Zhao et al., 2018) and respiration rate (Fan et al., 2018; Zhao et al., 2019). 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 (1952) reported a higher vase life for cut Chrysanthemum, Narcissus, Iris × germanica, Convallaria majalis, and Tulip 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 (1972) 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 (Barzilay et al., 2002) 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 (Wang et al., 2013). Sepal tissue of tree peony (P. suffriticosa) ‘Luoyanhong’ and ‘Fengdanbai’ did not freeze until exposed to -3.8 and -4.0 °C, respectively (Wang et al., 2013).
Developing peony buds are high in starch (Walton et al., 2007), which could indicate low tolerance of sub-zero temperatures and freezing (Galindo et al., 2007; Jones et al., 1999; Patton et al., 2007). However, hydrolysis of starch to soluble carbohydrates like fructose, glucose, and later sucrose occurs within buds after 2 weeks of storage at 0 °C (Walton et al., 2010), which could improve sub-zero tolerance by increasing solute concentration (Ashworth et al., 1993; Yu et al., 2017). Floral preservatives containing soluble carbohydrates may also be used to improve sub-zero tolerance of cut peonies. Sucrose pulses of 200 g·L\(^{-1}\) decreased freezing points of cut carnation by 2.6 °C (Heins et al., 1981). 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.

Available literature describes the effects of above 0 °C storage on peony vase life, but not the impact of storage on quality. 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 (Heuser and Evensen, 1986; Walton et al., 2010). However, long storage durations inhibit the bud opening of other cut flower species like Dutch iris that are bud harvested (Goszczyńska and Rudnicki, 1982; van Doorn et al., 2014). Bud opening has been linked to water movement into petals causing cell expansion (van Meeteren et al., 2006) and 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 (Mayak et al., 1973), lilies (Doi and Reid, 1995), and sweet pea (Lathyrus)
(Mor et al., 1984). Carnations 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 (Heins et al., 1981).

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 (Chen et al., 2015; Droillard et al, 1989; Lee et al., 2017) in the form of reactive oxygenating species (ROS) that cause cellular damage. ROS and antioxidant concentration impact the of senescence of day lily (Chakrabarty et al., 2009), rose (Kumar et al., 2007) and carnation (Droillard et al, 1989; Mayak et al., 1983) flowers. Petals contain numerous phenolic compounds including antioxidants and anthocyanins that have antioxidant properties to reduce the impact of ROS (Bartoli et al., 1995; Li et al., 2008). Total phenolic content (TPC) is a general measurement of the bioactive compounds correlated to antioxidant activity (Chen et al., 2015). Superoxide dismutase (SOD) is one of the more prevalent antioxidant enzymes and has associated with the initial stages of flower senescence (Droillard et al, 1989; Kumar et al., 2007; Mayak et al., 1983) and potentially flower longevity. A common stage of senescence, also influenced by stressors like temperature and ROS, is cell membrane dysfunction and injury. Studies indicate lipid peroxidation, measured as malondialdehyde (MDA) (Hodges et al., 1999), increases as floral tissue senescence (Bartoli et al., 1995), following freeze tests (Wang et al., 2014) and periods of drought simulated on marigold (Tagetes) species (Tian et al., 2012). Peony flowers (Fan et al., 2012) and roots (Li et al., 2008) have been used medicinally for centuries due to the number of bioactive compounds (Wu et al., 2010). The high total phenolic content, >30 ascorbic acid equivalent (Fan et al., 2012) and 26.8 mg·g⁻¹ gallic acid equivalent (GAE) (Li et al., 2008), 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 i) determine the effects of 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.

2.2 Materials and Methods

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. All stems had one apical bud. Peony stems were processed by cutting to a length of 50 cm from 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. (2002), but did include some stems with buds at stages 1.5 and 2.5. Stems were assigned to each treatment ensuring all treatments had the same number of stems at each bud stage.

Expt. 1 - Broad temperature range storage

Treatment combinations of 3 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, 0.6 ± 0.7, or 3.5 ± 0.9 °C. Relative humidity (RH) for each temperature was 89, 94, and 93 %, respectively. 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.

**Expt. 2 - Near-freezing temperature storage**

Treatment combinations of 3 pre-storage pulses, 2 storage temperatures, 8 storage durations contained 13 replicates, 10 for postharvest evaluation (n=10) and 3 for destructive physiological indices (n=3), for a total of 48 groups for each cultivar. Groups were treated with pulses 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), 200 g·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 two storage temperatures: 0.7 ± 0.2 or -0.6 ± 0.2 °C. RH for each temperature was 93 and 96 %, respectively. Groups of 13 stems of every cultivar were removed from each storage temperature every 2 weeks for 16 weeks for a total of 8 storage durations.

**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 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 µmol·m$^{-2}$·s$^{-1}$. Fresh weight lost (FWL) was calculated as a percentage using the following equation:

$$\text{Equation 1. FWL} = \frac{\text{Initial}_{FW} - \text{Post-storage}_{FW}}{\text{Initial}_{FW}} \times 100$$

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 %. Vase life was calculated as the number of days as a bud (bud time) plus the number of days flowers were open (open time). 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). 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 (Eason et al., 2002).

Indices of physiological stress and antioxidant activity

Following storage in Expt. 2, 3 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 (1968) was used to determine 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). The puree was incubated on ice for 10 min and then centrifuged at 11500 x g for 15 min at 4 °C. 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 x 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). The equation used to calculate MDA concentration was:

\[
\text{Equation 2. MDA equivalents (nmol/ml) = } \frac{(A-B)}{157000*1000000}
\]

\[
A = [(\text{Abs}_{532} + \text{TBA}) - (\text{Abs}_{600} + \text{TBA})] - (\text{Abs}_{532} - \text{TBA}) - (\text{Abs}_{600} - \text{TBA})
\]

\[
B = [(\text{Abs}_{440} + \text{TBA}) - (\text{Abs}_{600} + \text{TBA}) \times 0.0571]
\]

Total phenolic content (TPC) was determined using the Folin-Ciocalteau (F-C) reagent based on the methods described by Singleton et al. (1999). 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 (0.25N) followed by 20 uL of sodium bicarbonate (1N), and 120 uL of H2O 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). 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) in MJE and SB 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 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) 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 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.

Experimental design and statistics

A completely randomized design was used for both Expt. 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). 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 honestly 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) where the model with the best $r^2$ value was reported in Appendix A.

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.

2.3 Results and Discussion

Vase life

Expt. 1 - Broad temperature range storage

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, open time declined for all cultivars (Table 2.1). 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 2.1). At 9 or more weeks of storage, open time of FM stored at -3.1 °C was significantly shorter than stems stored at 3.5 or 0.6 °C. In contrast, 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 open time was usually lowest for stems storage at -3.1 °C but no different than stems stored at 3.5 °C, which was no different than stems stored at 0.6 °C. Across cultivars, open time was longest for stems stored at 0.6 °C at 12 weeks of storage.

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, 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 (1986) 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 (Walton et al., 2010) induced by cold temperatures (Miller and Langhans, 1990). 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 2.1) 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 (Heins et al., 1981).

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 (van Doorn et al., 2014) and carnations (Goszczyńska and Rudnicki, 1982). The cause has been linked to collapsed or blocked vascular tissue including air embolisms, dehydrated cells, and bacterial growth (van Meeteren et al., 2006).

Total vase life (bud + open time) data for stems stored at 0.6 °C was similar to reports of peonies stored between 0 and 2 °C (Gast, 1999; Heuser and Evensen, 1986; Rehman et al.,
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 production temperature (Hall et al., 2007). This could explain a shorter vase life when cut stems were stored at 3.5 °C compared to 0.6 °C. Conversely, as storage duration at -3.1 °C increased, bud time increased yet 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 2.1). In all cultivars, 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.

Expt. 2 - 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. 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 2.2). There was also no significant decline in open time throughout the storage period when stored at -0.6 °C. On average, FM open time was 3.1 d on average and significantly longer by 0.2 d when stored at -0.6 compared to 0.7 °C. Stored SB open time was significantly ($P < 0.0001$) shortened from 4.0 to 2.9 d at 14 weeks of storage. Independent of storage duration, open time was significantly ($P < 0.0001$) 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.
Better storage life of cut peonies held at 0.6 °C compared to -0.6 °C was reported by Post and Fischer (1952) although cultivar used and vase life data were not reported. The current study suggests benefits of sub-zero storage may be cultivar specific, which would align with previous storage studies in other cut flower species (Eason et al., 2002; Gast, 1997; 2000). 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 vase life of SB stored for 8 weeks in the current study was 1 to 2 d shorter than vase life reported by Walton et al. (2010) who used 0 °C, but similar to that of stems stored at 2 to 3 °C for 8 and 10 weeks (Gast, 1999). 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, as SB stems were obtained 1 week later than FM and MJE.

Open time of all cultivars was influenced by pre-storage pulse treatment, but only FM vase life was influenced by the interaction of pulse and storage duration treatments. 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 2.3). 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 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 (van Doorn et al., 2011) and Expt. 2 results match previous reports on peonies (Gast, 1999; 2000; Loyola-López et al., 2012). In conjunction with storage, Loyola-López et al. (2012) reported a
lower vase life for ‘Karl Rosenfield’ when pulsing with 100 g·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 a 24 h pulses as sucrose concentration increased from 0 to 16 % (Bravdo et al., 1974). 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 (Xue et al., 2018). Cut flower species and peony cultivars may differ in their ability to transport and use sucrose, which could inhibit vase life improvement.

Bud time of non-stored SB was between 1.1 and 1.8 d; it was the only cultivar to have slightly longer bud time as storage duration reached 10 or more weeks. All MJE flowers opened within 1.0 to 1.4 d regardless of treatment and storage duration. Non-stored flowers of FM opened within 1.6 to 2.8 d. Following 2 or more weeks of storage, FM bud time was reduced to 1.1 to 1.9 d depending on pulse treatment and storage temperature, but no trend was seen following 2 or more weeks of storage. These data reflect bud time results of Expt. 1 when stems were stored at 0.6 °C. Additionally, Nichols and Wallis (1972) reported no difference in bud time when storing \(Narcissus\) ‘Carlton’ at either 0.6 or -0.6 °C.

Based on just vase life, data from both Expt. s 1 and 2 support the industry standard storage temperature of 0.6 °C. Data also indicate that 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 (1971) and Nichols and Wallis (1972) suggested 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 (Post and Fischer, 1952), there could be increased interest in this storage temperature. The slightly longer open time seen in stems held at -0.6 °C in Expt. 2 may provide enough buffer for shipping long-term stored stems or using shipment methods like sea freight.

*Flower quality*

Expt. 1 - Broad temperature range storage

Percent FWL increased linearly over the storage duration regardless of storage temperature (Figure 2.1), 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 where FWL slowed between 8 and 12 weeks of storage. Across cultivars, regression slopes for percent 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 (Cevallos and Reid, 2001; Xue et al., 2018). Storage at -3.1 °C also caused stems, leaves, and bud 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 storage temperature (Figure 2.1D-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 A3. At 4 weeks of storage, there was no significant difference in FTO for FM among storage temperatures. However, at 8 weeks and 12 weeks of storage, odds of FTO after storage at 0.6 °C were significantly lower than at -3.1 and 3.5 °C (Table A3). There was no significant duration and temperature interaction for MJE and SB meaning the odds of MJE and SB FTO were significantly lower when stored at 0.6 °C compared to -3.1 and 3.5 °C (Table A2).

The inability of peony flowers to open following storage has been missing from a majority of cut peony literature, but has been related to harvest bud maturity of stored stems (Eason et al., 2002). Water movement into petal tissue is considered a primary factor in petal expansion and flower opening (van Meeteren et al., 2006). 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 (1971) reported that 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 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, flower diameter either decreased as a linear or quadratic function (Figure 2.2). The industry standard of 0.6 °C preserved flower diameter longest in comparison to the other temperatures for both FM and SB. At this temperature, 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.

MJE had measurable flower diameters at all temperatures and storage durations (Figure 2.2). Statistically, diameter was not consistently higher at one storage temperature. Diameter was significantly lower for stems held at 3.5 °C compared to diameter of flowers held at either both or one of -3.1 and 0.6 °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 A4).

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, 0.6 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 0.6 °C (Figure 2.2). Logistic regressions of the deformity probability indicate highest probability of flowers being deformed when stored at -3.1 °C (Figure 2.2). Only SB had a significantly higher odds of deformity when comparing stems stored at 3.5 and 0.6 °C (Table A6). 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 like outer petals (Figure 2.3). 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.

Necrosis (Figure 2.4A-B) and water-soaked spotting of petals were observed (Figure 2.4C-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 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 (Pearce, 2001). Although plants have been shown to recover from such injury (Min et al., 2014), this was not observed in the current study.

Ice formation in peony petal tissue was reported to occur between -3.8 and -4.0 °C for *P. suffruticosa* (Wang et al., 2013) and -1.7 °C (Wright, 1942) for *P. lactiflora*. No freeze injury was reported on cut peony stems of FM, ‘Karl Rosenfield’ and SB after 5 h at -2 °C (Jahnke et al., 2020). 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.

**Expt. 2 - Near-freezing temperature storage**

FTO occurrence was low in both FM and MJE. Logistic regressions depict a slight increase over the storage duration for FM (Figure 2.5A), but no significant difference was seen in the odds ratio comparison (Table A8). 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 odds when stored at -0.6 compared to
0.7 °C (Table A8). Overall, the FTO occurrence was less in Expt. 2 when considering storage at 0.7 compared to -0.6 °C in Expt. 1. Raising the sub-zero temperature to -0.6 °C in Expt. 2 from -3.1 °C in Expt. 1 reduced the FTO percentage likely due to the avoidance of ice formation as none was observed throughout the 16 weeks of storage.

FM flower diameter significantly declined ($P < 0.0001$) 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 ($P < 0.0001$) larger by 0.4 cm. Storage duration and temperature had similar significant effects on 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. SB flower diameter also declined as storage duration increased, and a significantly smaller diameter was measured when stored for 4 or more weeks (Table 2.4). Following 6 or more weeks of storage, SB flower diameter was larger when stored at -0.6 °C, but was only significant at 10 or 14 weeks of storage (Table 2.4). 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 2.5D-F). The odds of FM, MJE and SB flowers being deformed were significantly lower when stored at -0.6 compared to 0.7 °C (Table A10). A higher percentage of SB flowers were deformed when pulsed with sucrose, but this was not statically significant. Deformity incidence in Expt. 2 was similar to Expt. 1. The extension of storage from 12 to 16 weeks in Expt. 2 increased the incidence of flower deformity in FM and MJE by 5 to 10%.

Overall, better quality flowers were obtained 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 (Fan et al., 2018), cherry (Zhao et al., 2019), and nectarine (Zhao et al., 2018) fruit by reducing respiration rate, retaining weight, preserving color, and maintaining 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 (Dole et al., 2017). 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 aesthetic of the cut stem. Leaf removal following harvest increased vase life of cut *Euphorbia pulcherrima* (Dole, 2005) and *Hydrangea* (Kitamura and Ueno, 2015), but had no effect on vase life of *Lilium* ‘Stargazer’ (Han, 2003). It is unknown how leaf removal prior to storing cut peonies would affect subsequent hydration of cut peony.

**Stress indices**

Total phenolic content (TPC) GAE ranged between 31.1 and 59.9 mg·g⁻¹ FW in peony petals and was generally higher at longer storage durations (Table 2.5). 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* < 0.0001) and SB (*P* < 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. (2017) 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 (Chen et al., 2015). They greatly exceeded that of
strawberry (2470 mg·kg⁻¹ FW) (Shin et al., 2007) and blackberry (6818.3 mg·kg⁻¹ DW) (Kim et al., 2015).

High TPC values may be explained by the numerous and concentration of bioactive compounds. The F-C reagent reacted with many compounds other than polyphenols (Everette et al., 2010) including carbohydrates, thiols, vitamins, phenolic acids like gallic acid contained in peony (Chen et al., 2015), and soluble protein. Anthocyanins may also attribute to higher TPC given their reducing properties. Total anthocyanins of *P. lactiflora* ranged from 2 to 180 mg·g⁻¹ (Bao et al., 2020) compared to 17.6 to 33.5 g·kg⁻¹ in blackberry (Kim et al., 2015). This may also explain lower TPC values in peony roots (*P. lactiflora* 26.75 mg·g⁻¹ DW Li et al., 2008 and *P. suffruticosa* 24.5 mg·g⁻¹ DW) Li et al., 2008, which lack pigmentation compared to petals. In the current study, TPC seemed to correlated with the 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. (2017), and anthocyanins were undetectable to near zero in white *P. lactiflora* petals (Bao et al., 2020; Jia et al., 2008).

Storage temperatures near 0 °C likely maintained TPC similar to that seen in strawberry (Shin et al., 2007), apricot Fan et al., 2018, nectarine (Zhao et al., 2018), and cherry fruit (Zhao et al., 2019). 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 (Kim et al., 2015) which were 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 (Pennycooke et al., 2005). Similar compounds may have increased while storing cut peonies as they contain gallic acid, phenolic acids, and tannins which are derivatives of flavanols (Bao et al., 2020; Wu et al., 2010).
MDA content remained relatively constant throughout storage with the exception of stems stored for 10 weeks (Table 2.6). 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 (Min et al., 2014). 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 (Wang et al., 2014). However, no phenotypic data was provided limiting the inference of stress indices used by Wang et al. (2014). 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.

Based on the values, it seems 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 (1968) compared to that of Hodges et al. (1999) 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 (Walton et al. 2010). In contrast, fresh blueberry fruit, one of the large anthocyanin containing fruit tested by
Hodges et al. (1999), can contain around 500 g·kg\(^{-1}\) total soluble sugars (Mikulic-Petkovsek et al., 2012).

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 SOD content of non-pulsed control stems. Values ranged from 8,000 to 10,573 U·g\(^{-1}\) FW for MJE and 7,838 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 Li et al. (2017) sometimes 100 times higher than values in peony roots (Wang et al., 2014). 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 (Droillard et al., 1989) All three forms plus isoforms were identified in carnation petals (Droillard, et al., 1989), 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 Expt. 1 where vase life and quality data declined when stems were stored at 3.5 and -3.1 °C.

2.4 Conclusions

While the vase life of cut peonies is still relatively short in comparison to many other cut flower species, storage of cut peonies can be extended to 12-16 weeks when held at -0.6. Data in this paper also supports the use of the industry standard of 0.6 °C; however, higher quality was achieved when stems were stored at -0.6 °C. Neither pre-storage pulses nor sub-zero temperatures improved open time of cut peonies. Although TPC, MDA, and SOD values did not
distinguish 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 vase life 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 by storing 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 like tulips and other spring flowering, seasonal crops would make this technology more attractive.

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Literature Cited


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Dole, J., B. Stamps, A. Carlson, I. Ahmad, L. Greer, and J. Laushman. 2017. Postharvest handling of cut flowers and greens. ASCFG Press, Oberlin, OH.


Table 2.1. 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 Expt. 1.

<table>
<thead>
<tr>
<th>Storage duration (weeks)</th>
<th>ST (°C)</th>
<th>FM Open time (d)</th>
<th>MJE Open time (d)</th>
<th>SB Open time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.5</td>
<td>0.6</td>
<td>-3.1</td>
<td>3.5</td>
</tr>
<tr>
<td>1</td>
<td>3.3*ns</td>
<td>2.6*ns</td>
<td>2.9* ns</td>
<td>5.3*b</td>
</tr>
<tr>
<td>2</td>
<td>2.3*ns</td>
<td>2.6*ns</td>
<td>2.0* ns</td>
<td>4.0*ns</td>
</tr>
<tr>
<td>3</td>
<td>2.5*ns</td>
<td>2.9*ns</td>
<td>2.0* ns</td>
<td>4.4*ns</td>
</tr>
<tr>
<td>4</td>
<td>2.6*a</td>
<td>2.6*a</td>
<td>1.5* b</td>
<td>4.2*ns</td>
</tr>
<tr>
<td>5</td>
<td>3.4*a</td>
<td>3.4*a</td>
<td>2.0* b</td>
<td>5.1 a</td>
</tr>
<tr>
<td>6</td>
<td>3.0*a</td>
<td>3.0*a</td>
<td>2.0* b</td>
<td>4.0*ns</td>
</tr>
<tr>
<td>7</td>
<td>2.2*a</td>
<td>2.2*a</td>
<td>0.9* b</td>
<td>4.0*a</td>
</tr>
<tr>
<td>8</td>
<td>3.8*a</td>
<td>3.3*a</td>
<td>1.2* b</td>
<td>3.9*ns</td>
</tr>
<tr>
<td>9</td>
<td>1.8*</td>
<td>3.0*</td>
<td>0.0*</td>
<td>3.7*a</td>
</tr>
<tr>
<td>10</td>
<td>1.0*</td>
<td>2.5*</td>
<td>0.0*</td>
<td>3.8*a</td>
</tr>
<tr>
<td>11</td>
<td>1.0*</td>
<td>2.4*</td>
<td>0.0*</td>
<td>3.0*b</td>
</tr>
<tr>
<td>12</td>
<td>0.0*</td>
<td>3.0*</td>
<td>0.0*</td>
<td>2.3*</td>
</tr>
</tbody>
</table>

*Non-stored* 4.6 5.5 7.0

*Indicates least squared means within cultivar are significantly different than the non-stored, control when *P < 0.05.*

1Least 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.

2A value of 0.0 d was reported when no flowers opened during post-storage evaluation.

3Values followed by a “.” had 3 or fewer observations and were not statistically compared in the partitioned analysis.
Table 2.2. Expt. 2 flower open time (d) for *Paeonia lactiflora* cultivar Monsieur Jules Elie (MJE) in response to storage temperature and storage duration.

<table>
<thead>
<tr>
<th>ST (°C)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>Non-stored</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>3.3* a&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.9 a</td>
<td>3.2* b</td>
<td>3.5 b</td>
<td>3.8*b</td>
<td>4.0* b</td>
<td>3.2*b</td>
<td>3.6 b</td>
<td>4.1</td>
</tr>
<tr>
<td>-0.6</td>
<td>3.3* a</td>
<td>4.2 a</td>
<td>3.9* a</td>
<td>4.1 a</td>
<td>4.4*a</td>
<td>5.4* a</td>
<td>4.5*a</td>
<td>4.3 a</td>
<td>4.1</td>
</tr>
</tbody>
</table>

*Indicates least squared means within cultivar are significantly different than the non-stored, control when \( P < 0.05 \).

<sup>1</sup>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.
Table 2.3. Mean flower open time (d) for *Paeonia lactiflora* cultivar Festiva Maxima (FM) in response to pre-storage pulse treatments and storage duration in Expt. 2 independent of storage temperature (0.7, -0.6 °C).

<table>
<thead>
<tr>
<th>Pre-storage pulse treatment¹</th>
<th>Non-pulsed</th>
<th>Hydrator</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open time (d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.1*a</td>
<td>2.8*a</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.5*a</td>
<td>3.4 a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.0*a</td>
<td>2.9 a</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.0*a</td>
<td>2.7*a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.2*ab</td>
<td>2.6*b</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.1 b</td>
<td>3.0 b</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.0*a</td>
<td>2.8*a</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>2.9*a</td>
<td>2.9 a</td>
</tr>
<tr>
<td>Non-stored</td>
<td>3.7*a</td>
<td>3.3 a</td>
<td>3.6 a</td>
</tr>
</tbody>
</table>

¹Pre-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 least squared means within cultivar are significantly different than the non-stored, control when $P < 0.05$.

²Least 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.
Table 2.4. Mean flower diameter (cm) for *Paeonia lactiflora* cultivar Sarah Bernhardt (SB) in response to storage temperature and storage duration in Expt. 2 independent of pre-storage pulses.

<table>
<thead>
<tr>
<th>Storage duration (weeks)</th>
<th>Storage temperature (°C)</th>
<th>Diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.7</td>
<td>-0.6</td>
</tr>
<tr>
<td>2</td>
<td>12.1 a(^1)</td>
<td>12.1 a</td>
</tr>
<tr>
<td>4</td>
<td>19.8*a</td>
<td>19.6*a</td>
</tr>
<tr>
<td>6</td>
<td>10.3*a</td>
<td>10.6*a</td>
</tr>
<tr>
<td>8</td>
<td>19.2*a</td>
<td>19.7*a</td>
</tr>
<tr>
<td>10</td>
<td>18.6*b</td>
<td>10.1*a</td>
</tr>
<tr>
<td>12</td>
<td>19.4*a</td>
<td>19.6*a</td>
</tr>
<tr>
<td>14</td>
<td>18.8*b</td>
<td>10.0*a</td>
</tr>
<tr>
<td>16</td>
<td>18.6*a</td>
<td>19.5*a</td>
</tr>
<tr>
<td>Non-stored</td>
<td>13.3 a</td>
<td>12.8 a</td>
</tr>
</tbody>
</table>

\(^1\)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 least squared means within cultivar are significantly different than the non-stored, control when \( P < 0.05 \).
Table 2.5. 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.

<table>
<thead>
<tr>
<th>Storage duration (weeks)</th>
<th>FM</th>
<th>MJE</th>
<th>SB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPC (µg·kg(^{-1}) FW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>31.1 e(^1)</td>
<td>38.9 e</td>
<td>38.0 cde</td>
</tr>
<tr>
<td>4</td>
<td>34.8 de</td>
<td>42.4 de</td>
<td>39.6 cd</td>
</tr>
<tr>
<td>6</td>
<td>38.1 abc</td>
<td>42.9 de</td>
<td>42.1 abc</td>
</tr>
<tr>
<td>8</td>
<td>37.2 bcd</td>
<td>46.3 cd</td>
<td>41.0 bcd</td>
</tr>
<tr>
<td>10</td>
<td>36.3 bcd</td>
<td>47.5 cd</td>
<td>41.4 bcd</td>
</tr>
<tr>
<td>12</td>
<td>35.1 cd</td>
<td>58.8 a</td>
<td>36.0 de</td>
</tr>
<tr>
<td>14</td>
<td>39.8 ab</td>
<td>59.9 a</td>
<td>47.4 a</td>
</tr>
<tr>
<td>16</td>
<td>41.3 ab</td>
<td>53.6 b</td>
<td>45.9 ab</td>
</tr>
<tr>
<td>Non-stored</td>
<td>34.3 cd</td>
<td>38.5 e</td>
<td>33.9 e</td>
</tr>
</tbody>
</table>

\(^1\)Least squared means followed by the same letter are not significantly different when adjusted with Tukey’s HSD test with \(P < 0.05\).
Table 2.6. 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.

<table>
<thead>
<tr>
<th>Storage duration (weeks)</th>
<th>FM</th>
<th>MJE</th>
<th>SB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA (nmol·g⁻¹ FW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.7 b</td>
<td>6.2 bc</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>1.6 b</td>
<td>6.9 bc</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>1.6 b</td>
<td>7.6 ab</td>
<td>3.9</td>
</tr>
<tr>
<td>8</td>
<td>1.6 b</td>
<td>7.6 ab</td>
<td>4.1</td>
</tr>
<tr>
<td>10</td>
<td>3.9 a</td>
<td>9.4 a</td>
<td>3.7</td>
</tr>
<tr>
<td>12</td>
<td>1.6 b</td>
<td>8.0 ab</td>
<td>3.3</td>
</tr>
<tr>
<td>14</td>
<td>1.3 b</td>
<td>4.8 cd</td>
<td>3.4</td>
</tr>
<tr>
<td>16</td>
<td>1.4 b</td>
<td>7.1 b</td>
<td>4.0</td>
</tr>
<tr>
<td>Non-stored</td>
<td>1.3 b</td>
<td>4.8 cd</td>
<td>3.1</td>
</tr>
</tbody>
</table>

\[ P \text{ value} \leq 0.0001 \leq 0.0001 \leq \text{NS} \]

\[ ^1 \text{Least squared means followed by the same letter are not significantly different when adjusted with Tukey's HSD test with} \ P < 0.05. \]
Figure 2.1. Expt. 1 fresh weight loss (%) (A, B, C) and probability of flowers that failed to open (FTO) (D, E, 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. 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 observed percentage that did occur as a decimal (n = 15). Regression models and fit statistics are located in the Appendix Tables A1-3.
Figure 2.2. Expt. 1 flower diameter (A, B, C) and percentage of deformed flowers (D, E, 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. Markers for diameter are the least squared means along with ± SD (n ≤ 15) with linear or quadratic regressions. Least squared means were used to account for missing samples when flowers did not open. Logistic regressions are the predicted deformity probability along with observed percentage as a decimal (n ≤ 15). Regression models and fit statistics are located in Appendix Tables A4-6.
Figure 2.3. 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 or those that did not fully expand, or as center petals not opening like outer petals.
Figure 2.4. 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.
Figure 2.5. Expt. 2 percentage of flowers that failed to open (FTO) (A, B, C) and percentage of deformed flowers (D, E, 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 or pre-storage pulses at nine storage durations. Logistic regressions show the predicted FTO or deformity probability along with the percentage that did occur as a decimal (n ≤ 10). Logistic models and associated statistics are located in Appendix Tables A7-10.
CHAPTER 3:

Sub-zero temperatures reduce impacts of Botrytis and shipping following long-term storage of cut Paeonia lactiflora Pall.

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(Written in the style of HortScience)
Abstract

Storage and shipping are vital components to the world-wide supply of cut peonies (*Paeonia lactiflora* Pall.). However, these stages of the supply chain provide adequate time and environments for *Botrytis* species to infect and disease to develop, reducing quality and causing losses for growers and suppliers. A sub-zero temperature of -1 °C was used to store cut stems of two peony cultivars, ‘Monsieur Jules Elie’ (MJE) and ‘Sarah Bernhardt’ (SB), in comparison to 1 °C for up to 8 weeks following inoculation with *Botrytis cinerea* conidia. Stems were then evaluated either directly after storage or following an overnight shipment via a commercial courier. Shipments experienced air temperatures between 15 and 25 °C over a 15 h period. Vase life was similar when comparing stems stored at 1 and -1 °C and when comparing shipped and non-shipped stems. The percentage of flowers that failed to open following storage increased as storage duration increased, but was lowest when stems were stored at -1 °C. Inoculation with *B. cinerea* conidia increased disease ratings on peony flower receptacle domes. Stems stored at -1 °C had lower disease ratings compared to stems held at 1 °C. SB was overall a less tolerant cultivar having higher ratings of disease and reduced vase life and quality following storage and shipping compared to MJE. While the sub-zero storage temperature did not significantly improve all responses for both cultivars, it may be a valuable tool when considering season extension of cut peony, quality preservation, and disease development mitigation.

3.1 Introduction

After centuries of cultivation, herbaceous peonies, primarily *Paeonia lactiflora* Pall. and its hybrids, continue to increase in popularity as cut flowers. Recently, cut peonies were added to the USDA floriculture survey with over $11.8M in wholesale value in 2018, which increased by over $3M in 4 years (USDA, 2019). Seasonality and high-value have also led to the development
of niche production in areas such as Alaska to fill production gaps during July and August (Auer and Holloway, 2008; Holloway, 2019; Kamenetsky and Dole, 2012). Currently, the United States is second in production value following The Netherlands (Kamenetsky and Dole, 2012). Distribution of cut peonies relies on cold storage and shipping due to meet global demand (Kamenetsky and Dole, 2012). Peonies are stored most commonly between 4 to 8 weeks at 0 to 2 °C (Gast, 1999, 2000; Heuser and Evensen, 1986; Walton et al., 2010). Shipping is the least controlled period between harvest and vase. Domestically, growers and suppliers use commercial couriers who often lack adequate cooling facilities and climate controlled transportation. International couriers may have cooling capacity (Reid, 2006), but flowers may be exposed to sub-optimal temperatures before or following air transport. Respiration increases at above optimal temperatures which decreases vase life resulting in early senescence (Celikel and Reid, 2002).

Storage and shipping introduce conducive environments for infection and disease development if a pathogen is present or cut stems are already infected. *Botrytis* spp., specifically *B. cinerea*, is one of the most common fungal pathogens causing production and postharvest losses of cut flower species (Trollinger and Strider, 1985; Wegulo and Vichez, 2007; Whetzel, 1930). Whetzel (1939) reported losses of peonies caused by Botrytis development following storage as early as 1929. In recent years, interest has grown to better understand the impact *Botrytis* has on the cut flower industry, which has led to the discovery of many new *Botrytis* species, specifically those infecting peonies (Garfinkel et al., 2017, 2019), and the characterization of *Botrytis* resistance and management of this pathogen (Muñoz, 2018). There are a large number of chemical and biological control options (Chastagner and Garfinkel, 2020:
Elad et al., 1993; Hammer et al., 1990; Muñoz, 2018), but no method provides 100% control leaving stems susceptible when conditions are favorable for disease development.

The storage environment includes temperature and relative humidity (RH) control. *Botrytis* can infect and develop over a wide temperature range -0.5 to 30°C (Coertz and Holz, 1999). Plus, the high relative humidity (≈90%) used at common storage temperatures (0 to 2°C) to prevent plant wilting also encourages *Botrytis* development (Carisse, 2016). Decreasing RH and storage temperature can slow the development of *Botrytis*. Temperatures currently used for cut peony storage allow for the development of *Botrytis* which has led to an increase in disease incidence and severity the longer peonies were stored (Gast, 2000; Heuser and Evensen, 1986).

*Botrytis* development during shipping is less understood than storage. Lack of control and variability in the environment during shipping could have the potential to increase plant stress and thereby infection and disease development.

Sub-zero temperatures are not used commercially to store cut flowers, but positive benefits have been noted in multiple cut flower (Nichols and Wallis, 1972; Post and Fischer, 1952), fruit species (Liu et al., 2019; Zhao et al., 2019) and conifer species (Camm et al, 1994, Hee, 1987), including increased storage life and preserved quality. There are also reports of reduced in vitro growth of *Botrytis allii* (Tian and Betolini, 1995) and a slower progression of *Botrytis* development at sub-zero temperatures (Carisse, 2016). Naturally, senescent tissue is more susceptible to the necrotrophic pathogen *Botrytis*. Any methods that delay senescence by slowing physiological processes such as respiration could increase the potential quality and vase life of stems following storage and shipping.

A number of postharvest methods, including fungicides (Gast, 2000; McCain and Welch, 1982), controlled atmospheres with high CO₂ (Hammer et al., 1990; Reyes, 1990) and biocontrol
agents (Elad, 1993), are effective at reducing the impact of *Botrytis* when applied prior to or during storage. However, many of these methods can be costly, labor intensive, or require precise control and technical skills. Also, many fungicides that are effective and used during production are not labeled for use during postharvest handling of cut flowers, specifically peonies. Storage facilities with temperature control are already in place making it a more viable option for disease mitigation.

The effect of sub-zero temperatures throughout the supply chain are not well understood. Additionally, sub-zero temperatures may minimize the impact of *Botrytis* on peonies providing an additional tool for integrated pest management during postharvest handling. The objectives of this study were (1) determine how sub-zero storage temperatures influence disease development following storage and shipping and (2) evaluate the resulting flower quality in comparison to the industry standard temperature.

### 3.2 Materials and Methods

*Plant material, inoculation, shipping.*

Cut stems of *Paeonia lactiflora* Pall. hybrids ‘Monsieur Jules Elie’ (MJE) and ‘Sarah Bernhardt’ (SB) were harvested and graded by separate local growers in Washington on 24 May 2019 and 5 June 2019, respectively. Stems were transported in bulk to Washington State University (WSU) Puyallup Research and Extension Center. All stems were processed to a length of 60 cm from bud tip to stem end. All stems had one apical bud between 1.9 and 2.3 cm in diameter. All but the three uppermost lobed leaves were removed. A total of 120 bunches of 3 stems were made for each cultivar. The buds on half of the bunches were sprayed to run off with a $3 \times 10^4$ *B. cinerea* conidia·ml$^{-1}$ suspension (inoculated) and half with tap water (non-
inoculated). The conidia used to inoculate cut stems were collected from 3 to 4 week-old cultures of WSU isolate MS05 grown on potato dextrose agar at 20 to 35 °C (Garfinkel et al., 2019).

Bunches were air dried at room temperature for 3 to 4 h following inoculation. Ventilated cardboard boxes were used to hold inoculated and non-inoculated stems at one of two temperatures (-1 or 1 °C). Ten bunches of each non-inoculated and inoculated stems were removed from each storage temperature at 1, 4, and 8 weeks. Following each storage duration, half of the non-inoculated and inoculated bunches from each storage temperature were shipped overnight via a commercial courier to North Carolina State University (NCSU). Air temperature and relative humidity were recorded every 5 to 15 min using data loggers, located in the middle of each package.

Postharvest evaluation.

Following storage at WSU and receiving stems at NCSU, stem ends were recut, removing the lower 2.5 cm. Each bunch of 3 stems were placed into a vase filled with 500 mL of tap water. The WSU Puyallup post-storage environment was maintained at 20.5 ± 0.5 °C, 40% relative humidity (RH), 12.8 µmol·m⁻²·s⁻¹ with a photoperiod of 24 h. The post-shipping environment at North Carolina State University was held at 20 ± 2 °C, 40 to 60 % RH, and a 12-h photoperiod at 15 µmol·m⁻²·s⁻¹. The vase life of each flower was recorded as the number of days from placing stems into water until termination. Reasons for termination included the petal drop, disease covering >10% of the bud, >50% non-botrytis related petal necrosis, >50% wilted petals, or stem collapse. Flowers failed to open (FTO) when guard petals did not separate by the time stems were terminated. Flower diameter (cm) of open flowers was measured on the fifth day after placing stems into water or the day of termination, whichever occurred first. After termination, petals were removed and the *Botrytis* decay on the receptacle dome was rated on a
scale of 0 to 4 where 0 = no damage, 1 = <25%, 2 = 25-50%, 3 = 51 to 75% and 4 = >75% of the edge of the dome decayed.

**Experimental design and statistics.**

Treatments were a 2 x 3 x 3 x 2 factorial arrangement consisting of inoculation with *Botrytis* spores (inoculated) or tap water (non-inoculated), two storage temperatures (-1 and 1 °C), three storage durations (1, 4, 8 weeks), and shipping (no or yes). The experimental design was a completely randomized block experiment with 5 blocks (n=5) consisting of 1 bunch from each treatment combination. Blocks were made to control for variation in the evaluation environment. Each bunch had 3 peony stems, which were averaged together as subsamples. Data from each cultivar were analyzed separately using analysis of variance through the GLIMMIX procedure (SAS 9.4 Cary, NC, USA). Tukey’s honest significant difference (HSD) was used when appropriate for mean separation with $P \leq 0.05$. The SLICE feature was used to implement a partitioned analysis or simple effect analysis when effects had a significant interaction with storage duration. Binary data measuring a yes or no response for FTO were analyzed and regressed using PROC LOGISTIC using a generalized linear model parameterization. Logistic models, odds ratios and comparisons are reported in the Appendix B. Odds ratio was used to explain the influence of either storage temperature and or storage duration had on flowers either FTO or being deformed while keeping other variables constant. Comparisons were adjusted with Tukey’s HSD. Partially open and fully open flowers were combined for FTO regression. Only significant terms ($P \leq 0.05$) were included in models.
3.3 Results and Discussion

Vase life

MJE vase life was influenced by the interaction of shipping and storage duration ($P < 0.0001$). Shipped stems lasted significantly longer than non-shipped stems at 1 (6.8 d) and 8 weeks (5.0 d) of storage, but not at 4 weeks (4.4 d). Non-shipped stems had a vase life of 6.0, 5.0 and 4.1 d at 1, 4, and 8 weeks of storage, respectively. Simple effects were compared to explain the four-way interaction among shipping, storage temperature, inoculation, and storage duration on the vase life of SB (Table 3.1). At 1 week of storage, vase life of shipped stems stored at 1 °C was significantly shorter than non-shipped stems that were not inoculated, and shorter than inoculated stems stored at -1 °C. A similar pattern was seen at 4 weeks of storage where the vase life of most shipped stems was significantly lower than non-shipped stems. At 8 weeks of storage, the effect of shipping was not as strong. Only shipped, inoculated stems stored at 1 °C had a significantly shorter vase life than non-shipped stems. This was largely due to disease development, which caused all flowers to not open resulting in a vase life of 0 d. While not significant, the vase life of inoculated stems (2.7 d) was also lower than non-inoculated stems (3.1 d) held at 1 °C for 8 weeks. Vase life decline of SB over the storage duration was consistent with results from MJE where vase life did not significantly decline until 8 weeks of storage. The exceptions were when shipped stems were not inoculated and stored at 1 °C or when both inoculated and non-inoculated stems were stored at -1 °C. This may indicate a benefit of using sub-zero temperatures when considering disease development.

Overall, there was a lack of significant evidence that the overnight shipping shortened vase life of cut peonies. This was a surprising result as temperature logs (Figure 3.1) show stems experienced temperatures between 15 and 25 °C over 15 h during shipping from WSU to NCSU.
Celikel and Reid (2002) have shown increases in temperature was positively correlated with respiration and a decline in vase life of snapdragon (*Antirrhinum majus* L.). In comparison to previous storage studies, the vase life of shipped MJE stored for 8 weeks was similar to Gast (2000) who stored stems at 1 °C. Vase life of non-shipped, and non-inoculated SB after 8 weeks was 4 d shorter than reports by Walton et al. (2010) and over 1 d shorter than Gast (2000). Pre- and postharvest factors can all attribute to differences in vase life. Vase life itself is a subjective measurement and there were some differences in the environmental conditions within the display room at WSU and NCSU. Stems were tapped while in the vase to ensure petal drop which provided a consistent method for vase life determination in the current study.

Results indicate vase life was similar if not slightly longer for stems stored at -1 °C compared to those stored at 1 °C. Post and Fischer (1952) and Halevy and Mayak (1981) suggested the small vase life improvement achieved when using a sub-zero storage temperature of -1 °C on multiple cut species does not outweigh the cost cooling and equipment needed to maintain precise temperatures and avoid freezing. No freezing was observed in the current study meaning ice nucleation may have been unable to occur at -1 °C. Cut flowers such as daffodil (*Narcissus*) (Nichols and Wallis, 1969), tulip (*Tulipa*) and rose (Post and Fischer, 1952) can tolerate -0.5 °C for multiple weeks with no injury. Peonies, specifically *P. suffruticosa*, petal tissue has been reported to have a freezing point of between -3.8 and -4.0 °C (Wang et al., 2013) and *P. lactiflora* petals froze at -1.7 °C (Wright, 1942). In controlled freeze tests, no injury was reported on cut peonies held at -2 °C for 5 h (Jahnke et al., 2020). Given the potential benefits of extending the availability of seasonal crops and improvement of cooling facilities and equipment, sub-zero temperatures may now be more applicable to the industry.
**Flower quality**

Peonies are harvested in a bud stage, which requires buds to open to be enjoyed by the consumer. Models developed from logistic regression depict lower FTO probability for both MJE and SB when stored at -1 °C compared to 1 °C (Figure 3.2). Shipping SB also increased the FTO probability regardless of storage temperature. Periods of cold storage often induce faster peony bud opening (Gast, 2000; Walton et al, 2010). However, the combination of long-term dry storage and warm temperatures during shipping may have increased the FTO percentage by reducing water uptake and therefore flower opening (van Meeteren, 2006).

MJE flower diameter was significantly \( P = <0.0001 \) smaller as each storage duration increased, decreasing from 14.3 cm at 1 week to 11 cm at 8 weeks. Flower diameter of stems stored at -1 °C was significantly \( P = 0.0038 \) smaller on average by 0.4 cm. The impact of storage and shipping on SB flower diameter was more complex and simple effects were compared in Table 3.2. In most cases, an increase in storage duration caused a significant decline in flower diameter. While not always significant, flower diameter of stems held at -1 °C was larger than those stored at 1 °C. Inoculated stems also had multiple cases of smaller diameters than non-inoculated stems. However, there is evidence to suggest that storage at -1 °C minimized disease development and allowed for larger flowers when comparing diameters of inoculated and non-inoculated stems stored at -1 to those stored at 1 °C.

The presence of *Botrytis* on inoculated stems, specifically the receptacle, may have reduced diameter by limiting water movement into petals. Similar to SB vase life, diameter was 0 cm when flowers were inoculated, stored for 8 weeks at 1 °C and shipped to NCSU (Table 3.2). This was again caused by disease, which prevented opening of all flowers in this treatment. Given that *Botrytis* development is common on peony flower buds (Garfinkel and Chastagner,
2018), it is not surprising that diameter would have been influenced by inoculation; any mitigation of disease or its progression would then allow full flower opening and larger flower diameters.

_Disease ratings_

The domed top of the flower receptacle, was rated for disease severity given its importance in connecting the petals and reproductive organs to the stem. Inoculated MJE stems had a significantly \( P = 0.0438 \) lower rating of 0.5 compared to 1.2 when stored at -1 °C and 1 °C, respectively. A rating of 0.5 for inoculated stems stored at -1 °C was also no different than non-inoculated stems stored at 1 °C further emphasizing the positive impact of the sub-zero storage temperature. Disease ratings were similar between shipped and non-shipped stems, with inoculated stems having a slightly higher disease rating as storage duration increased (Table 3.3). Inoculated MJE shipped to NCSU at 8 weeks did not increase in disease rating like inoculated stems evaluated at WSU. Botrytis decay on the outside of the peony bud likely caused stems to be terminated early, therefore limiting the disease development on the receptacle dome. Disease ratings were more severe on SB than MJE. However, storage temperature had a similar effect. Ratings were significantly lower at most storage durations when stems were stored at -1 °C compared to 1 °C (Table 3.3).

Currently there is no literature on _Botrytis_ development on cut flowers when stored at sub-zero temperatures. The reduction of disease development by lowering temperatures during postharvest handling has been well studied and reviewed by Romanazzi and Droby (2016). It is likely that a storage temperature of -1 °C slowed disease development (Van Den Berg and Lentz, 1968) compared to 1 °C resulting in a slightly longer vase life and larger flower diameter. However, disease still developed and caused some peonies to not open following storage.
Botrytis conidia have been reported to survive and be infectious after 30 months at -80 °C (Gindro and Pezet, 2001) and disease has continued to develop at -0.5 °C on grapes (Coertze and Holz, 1999). While the sub-zero storage does not provide complete control it can be coupled with current integrated pest management techniques to mitigate disease while improving post-shipping and storage quality.

Disease development on the peony dome may have restricted water movement and thereby decreased vase life, flower opening, and flower diameter. Lower disease ratings on MJE compared to SB mirror effects on vase life, FTO, and diameter responses indicating that MJE may be more tolerant to Botrytis than SB. Winters (1930) considered SB to be a resistant cultivar, but MJE was not evaluated. Cultivar differences may be linked to bud morphology and stage when inoculated. SB petals were more exposed, while in general MJE petals are covered by green sepals (Figure 3.3). Less susceptible cultivars of cut Eustoma grandiflorum (Raf.) Shinners were suggested to be used by Wegulo and Vilchez (2007) and similarly susceptibility differences could be exploited in peony production (Zhao et al., 2015). However, cultural practices, fungicides, biological control are a more practical control method due to the perennial nature and long-term production of the crop along with slow development and consumer acceptance of new cultivars.

3.4 Conclusion

Storing stems at -1 °C compared to 1 °C had a positive effect on SB, which was echoed in some cases with MJE. Overall, -1 °C resulted in a slightly longer vase life as storage duration increased, a lower number of buds FTO even when shipped, larger flower diameter, and lower disease ratings. While the impact of sub-zero temperatures was not large for any one parameter,
a sub-zero temperature has the potential to reduce postharvest losses by minimizing disease and maintaining a higher number of high quality stems. Acceptance and adoption of sub-zero temperatures will rely on future research on the what temperatures and duration will cause freeze injury on peonies and other cut flower species. Peony, bud stage, in particular may be a limiting factor in storage duration and botrytis susceptibility. Even if sub-zero storage temperatures are not used, this work confirms the importance storage temperature plays in the long-term storage of cut peonies

**Acknowledgments**

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Literature Cited


Chapter 22. PpIn:. 1,365p.


Coertz, S. and G. Holz. 1999. Surface colonization, penetration, and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of Botrytis cinerea. Plant Dis. 83:917-924.


doi:10.1016/j.postharvbio.2010.05.008.


Table 3.1. The mean vase life of *Paeonia lactiflora* ‘Sarah Bernhardt’ in response to a three-way interaction among shipping, storage temperature, inoculation *Botrytis cinerea* conidia.

<table>
<thead>
<tr>
<th>Shipping</th>
<th>Storage temperature (°C)</th>
<th>Inoculation</th>
<th>Storage duration (weeks)</th>
<th>Vase life (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>No</td>
<td>6.2</td>
<td>a&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>5.6</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>No</td>
<td>5.6</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>6.1</td>
<td>a</td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>No</td>
<td>4.9</td>
<td>bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>4.5</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>No</td>
<td>4.8</td>
<td>bc</td>
</tr>
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<td></td>
<td></td>
<td>Yes</td>
<td>4.8</td>
<td>bc</td>
</tr>
</tbody>
</table>

<sup>1</sup>Inoculations consisted of a $3 \times 10^4$ *B. cinerea* conidia·ml<sup>-1</sup> suspension (inoculated) with no inoculation stems sprayed with tap water.

<sup>2</sup>Least squared means followed by the same lowercase 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.

<sup>3</sup>Least squared means followed by the same uppercase letter within interaction level are not significantly different when adjusted with Tukey’s honest significant difference test with $P < 0.05$ in a partitioned analysis of simple effects.
Table 3.2. The mean flower diameter (cm) of *Paeonia lactiflora* ‘Sarah Bernhardt’ in response to a three-way interaction among shipping, storage temperature, inoculation *Botrytis cinerea* conidia.

<table>
<thead>
<tr>
<th>Shipping</th>
<th>Storage temperature (°C)</th>
<th>Inoculation(^1)</th>
<th>Flower diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>No</td>
<td>12.3 (^{a2}) A(^{3})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>11.6 b A</td>
</tr>
<tr>
<td>-1</td>
<td></td>
<td>No</td>
<td>12.9 ab A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>11.8 b A</td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>No</td>
<td>14.1 a A</td>
</tr>
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<td>14.1 a A</td>
</tr>
<tr>
<td>-1</td>
<td></td>
<td>No</td>
<td>14.1 a A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>13.4 ab A</td>
</tr>
</tbody>
</table>

\(^1\)Inoculation consisted of a \(3 \times 10^4\) *B. cinerea* conidia·ml\(^{-1}\) suspension (yes) and non-inoculated stems were sprayed with tap water (no).

\(^2\)Least squared means followed by the same lowercase letter within each storage temperature are not significantly different when adjusted with Tukey’s honest significant difference test with \(P < 0.05\) in a partitioned analysis of simple effects.

\(^3\)Least squared means followed by the same uppercase 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.
Table 3.3. Dome ratings measuring *Botrytis* decay for *Paeonia lactiflora* ‘Monsieur Jules Elie’ (MJE) and ‘Sarah Bernhardt’ (SB) in response to significant two-way interactions. Cultivars were analyzed separately.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Shipping</th>
<th>Inoculation(^1)</th>
<th>Storage duration (weeks)</th>
<th>Dome rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>MJE</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
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<td>d(^2)</td>
</tr>
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<td></td>
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<td>0.0</td>
<td>cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
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<td>SB</td>
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*Botrytis* decay on the receptacle dome was rated on a scale of 0 to 4: 0, 0 % outer edge receptacle colonized by *Botrytis*; 1, <25 %; 2, 26 to 50%; 3, 51 to 75%; 4, >75 % following flower termination.

\(^1\)Inoculation consisted of a $3 \times 10^4$ *B. cinerea* conidia·ml\(^{-1}\) suspension (yes) and non-inoculated stems were sprayed with tap water (no).

\(^2\)Least squared means for each separate cultivar followed by the same letter are not significantly different when adjusted with Tukey’s honest significant difference test with $P < 0.05$. 
Figure 3.1. Air temperatures of four overnight shipments of cut *Paeonia lactiflora* ‘Monsieur Jules Elie’ (MJE) and ‘Sarah Bernhardt’ (SB) peonies from Washington State University Puyallup, WA to North Carolina State University, Raleigh, NC between June 5 and August 8, 2019 via a commercial courier. Shaded areas are the time boxes were held at 4 °C in WA (blue), in transit (red), and held at room temperature of drop-off facility in NC (green). The first arrow at the overlapping region was the general pickup time by the courier. The second arrow and overlapping region is the general delivery time.
Figure 3.2. Logistic regressions showing the probability of *Paeonia lactiflora* flowers failing to open (FTO) over time for (A) ‘Monsieur Jules Elie’ (MJE) in response to storage temperature and (B) ‘Sarah Bernhardt’ (SB) in response to storage temperature and shipping treatments. Only significant effects were used to create logistic models. Markers are the actual percentage of flowers that failed to open.
Figure 3.3. Botrytis susceptibility of *Paeonia lactiflora* buds may be influenced by sepal coverage and petal exposure. (A) ‘Monsieur Jules Elie’ (MJE) has large green sepals that cover petals for a large period before bud opening. (B) ‘Sarah Bernhardt’ (SB) seem to have more exposed petals. (C) Botrytis infected SB bud showing necrosis on petals, sepals, and the peduncle.
CHAPTER 4:
Postharvest handling techniques for improved storage and vase life of cut tulip and Dutch iris

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(Written in the style of HortScience)
Abstract

Postharvest handling is a multifaceted stage of the cut flower supply chain intended to maintain or improve the quality of perishable cut flower material. During this stage, cold storage is used to maintain quality and extend availability. Three experiments were conducted using cut Tulipa sp. (tulip) and Iris × hollandica (Dutch iris) cultivars to evaluate the impacts of dry storage with the bulb attached to the stem, sub-zero temperatures, and pre- and post-storage preservative pulses on vase life. In Expt. 1, six tulip and two Dutch iris cultivars were stored for up to 6 or 2 weeks, respectively. The longest vase life at 6 weeks of storage was achieved for all tulip cultivars when stems were stored with the bulb still attached at -0.6 °C. Storing cut stems at 0.7 °C for 6 weeks resulted in the shortest vase life. Vase life of Dutch iris ‘Telstar’ and ‘River King’ was longest at 2 weeks of storage when stored at -0.6 °C. Additionally, 75 to 100 % of flowers fully opened when stems were stored with the bulb still attached. Expt. 2, utilized a storage temperature of -0.6 °C; cut tulips and ‘River King’ Dutch iris were pulsed prior to storage for 8 h at 4 °C with Bulb 100 and BVB containing BA and GA4,7 phytohormones. Cut stored stems maintained a vase life similar to that of non-stored, pulsed stems at 6 weeks of storage. The longest tulip vase life was achieved in a third experiment combining a sub-zero storage temperature of -0.6 °C, storing stems with the bulb attached and pulsing stems with Bulb 100 or BVB following storage. Vase life did not significantly decline over a 6-week storage duration. These three experiments demonstrate various strategies for unprecedented storage life and vase life of cut tulips and Dutch iris.

4.1 Introduction

Many plant species grown for cut flowers and foliage have specific postharvest handling guidelines. Implementation of these guidelines increases the chance of preserving or improving
quality throughout the supply chain. Holding cut stems in cold storage is a reliable method of preserving quality and can range from a matter of hours or days to weeks or even months. Multiple scenarios warrant cold storage of perishable cut material such as season extension, preparing for high demand, or long shipping durations. Optimization of storage practices primarily involves the traits and processing of the plant species being stored and controlling the air temperature of the storage environment.

*Tulipa* spp. (tulip) and *Iris × hollandica* (Dutch iris) are popular cut flower species commonly produced during cool spring seasons. These species have a relatively short storage and vase life (Dole et al., 2017). Both crops are unique in the cut flower industry because they are true bulb geophytes and are treated as annual crops even though both perennialize in the landscape. The time, labor, facilities, space, and lower quality flower of second year bulbs encourage growers to purchase new bulbs each year. Forcers can also achieve a longer stem length on tulips when by uprooting the bulb with the stems instead of cutting at ground level. Dutch iris have more fibrous root systems, naturally longer stems length, and are typically cut at ground level. For decades, multiple industry sources and published literature (DeHertogh, 1996) claimed that tulips can be stored for up to 2 to 3 weeks when the bulb is left attached to the stem compared 5 to 7 d when cut stems are stored dry, without water. However, there is little to no data provided to support these findings. Mayak and Halevy (1971) reported Dutch iris stored dry as a cut stem had a higher vase life that those stored with only the bulb basal plate still attached.

Dry storage, without stem ends in water, is a common practice to increase storage length of cut flowers. Vase life and quality of some species decline quickly when stored dry due to their inability to rehydrate i.e. *Ranunculus asiaticus* (Natarella and Kays, 1979), *Dahlia* and *Papaver* (Dole et al., 2009). However, successful dry storage is highly influenced by storage duration and
temperature. The vase life of rose (Rosa) ‘Ambiance’, daffodil (Narcissus) ‘King Alfred’ and ‘Paperwhite’, carnation (Dianthus caryophyllus) ‘Imperial White’, Dutch iris ‘Telstar’, and Tulipa gesneriana were no different when stored dry compared to those stored wet, cut stems in water, at 0 °C for 6 d (Cevallos and Reid, 2001). Failure to rehydrate can be caused by the drying out of vascular tissue and air embolisms (Mayak and Halevy, 1971; van Meeteren et al., 2006), microbial growth in stem ends (van Doorn and Perik, 1990; Zagory and Reid, 1986), and whole plant desiccation. Recutting stem ends following storage or shipping is a common industry practice to improve water uptake. If not recut, the 16% of dry-stored cut Dutch iris buds opened compared to 100% when recut after storage (Mayak and Halevy, 1971).

Cut flowers fall into temperature classes when considering cold storage (Goszczyńska and Rudnicki, 1982; Halevy and Mayak, 1981). Tropical flowers (Anthurium, orchids, and Euphorbia pulcherrima) are chilling sensitive and should be stored between 12 to 18 °C; subtropicals (Gladiolus, Anemone and protea) are stored between 2 to 8 °C; finally a majority of cut flowers like roses, carnations, Chrysanthemum, Dutch iris, and tulips are stored between 0 to 2 °C. A fourth class could be considered from results reported by Post and Fischer (1952), Nichols and Wallis (1972), and Hardenburg (1986), who concluded that some species like tulips, lily of the valley (Convallaria), Gardenia, sweet pea (Lathyrus), and chrysanthemums are best stored at a sub-zero temperature of -0.6 °C. The industry has not adopted this storage temperature likely due to the inability to precisely control air temperatures to avoid freezing, lack of knowledge on adaptable species, and the cost of the equipment. Freezing occurred in tulip petals and bulbs at -1.7 and -3.7 °C, respectively (Hardenburg, 1986). Sub-zero temperatures just below 0 °C with a small temperature fluctuation may keep plants in a supercooled state to avoid freezing. Research into these near-freezing temperatures is gaining popularity in fruit crop
Storage. Storage life of cherries (Zhao et al., 2019), nectarines (Zhao et al., 2018), and apricots (Fan et al., 2018) at temperatures between -1.2 and -3.0 °C exceeded that of fruit stored at temperatures above 0 °C. Authors reported lower respiration rates and ethylene production when stored at this “near-freezing temperature.

Floral preservatives are common products made up of one or a combination of the following: carbohydrates, acidifiers, antimicrobials, plant growth regulators, nano-particles (Dole et al., 2017). These products can be incorporated into water and used throughout postharvest handling. Pulse treatments are versatile short period treatments of floral preservatives lasting for less than 24 h. Vase life of peonies (Sang et al., 1998), *Limonium* (Doi and Reid, 1995), lisianthus (*Eustoma*) (Huang and Chen, 2002) and gladiolus (Mayak et al., 1973) have all been improved using pulse treatments. Tulips are commonly treated with a pulse combination of gibberellic acid (GA) and benzyladenine (BA) as studied by van Doorn et al. (2011).

Formulations combining BA and GA$_{4,7}$ have been commercialized and are readily available on the market and advertised for use on cut tulips (A. Ranwala, personal communication) and Dutch iris (R. Timmerman, personal communication). These formulations have not been tested in conjunction with various storage methods or storage temperatures.

The objective of this study was to evaluate the effects of long-term storage on tulip and Dutch iris cultivars’ vase life utilizing stems with or without bulbs still attached, sub-zero storage temperatures, and premixed commercial preservative pulses before and after storage. Through these data we demonstrate multiple postharvest handling procedures for both cut tulips and Dutch iris for optimized storage life and vase life.
4.2 Materials and Methods

Plant material

Non-cooled bulbs tulips were planted in bulbs crates filled with a peat based substrate mix (Old Castle, Fafard® 4P Mix; Sun Gro, Agawam, MA). Bulbs were rooted and vernalized through a temperature regime of 8.9 °C for 8 weeks, 5.0 °C for 7 weeks, and 0.6 °C for approximately 4 weeks. The total duration range was 16 and 19 weeks and dependent on variety. Plants were forced under natural day length in 2019 from 13 March to 8 April and in 2020 from 22 March to 25 April. Greenhouse temperatures were maintained at 18/15 °C (day/night). Plants were watered daily with non-amended water and once per week with 15N-0P-12.5K (Jack’s Professional Calcium Nitrate, JR Peters Inc., Allentown, PA) water-soluble fertilizer at 150 mg·L⁻¹. Tulips were harvested by uprooting the stem with the bulb attached when tepals were 25 to 50% colored. Dutch iris cultivars River King (RK) and Telstar (TS) were harvested with the bulb attached on 21 April 2019 by a local grower at a bud stage when little to no color was showing. The Dutch iris were transported within 3 h of harvest to North Carolina State University (NCSU) in ice water. Stems were stored at 4 °C for 12 h before processing stems for each experiment.

Expt. 1 - Bulb vs cut storage.

Treatments consisted of two storage temperatures, two storage methods, and two storage durations for a 2 x 2 x 2 factorial arrangement. A total of 9 groups of 5 to 10 stems were made of each cultivar using ‘Black Parrot’ (BP), Foxy Foxtrot (FF), ‘Golden Oxford’ (GO), ‘Lingerie’ (LG), ‘Menton’ (MN), and ‘Renown’ (RN) tulips and RK and TS Dutch iris. One group was used as a non-stored control (duration = 0) and taken directly to postharvest evaluation. Half of the remaining 8 groups were left with bulb attached and half were cut. Tulips were cut to either...
30 or 40 cm depending on the cultivar and all Dutch iris to 45 cm measuring from petal or sheath tip to stem end. Groups were wrapped in newspaper and placed in cardboard boxes lined with polyvinyl wrap. One box per cultivar and both bulb-attached and cut stems was held dry at -0.6 °C and one box at 0.7 °C. Relative humidity (RH) was maintained between 80 to 90 %. One bulb attached and cut group per cultivar was removed from each temperature after 3 and 6 weeks of storage.

**Expt. 2 - Pre-storage pulses.**

Stems of GO, MN, and ‘Piste’ tulips and RK Dutch iris were used to determine the effect of pre-storage pulses on stored cut stems. Stems of each cultivar were cut to a set length of either 35 or 45 cm before sorting stems into 9 groups of up to 10 stems. Groups were placed into treatments arranged in a 3 x 3 factorial consisting of three pre-storage pulses and three storage durations. Groups were pulsed by placing stem ends into one of three pulse solutions: tap water, Bulb 100 (2 ml·L⁻¹) (Floralife Inc., Waltersboro, SC), or BVB (2 ml·L⁻¹) (Chrysal Americas, Doral, FL) for 8 h at 4 °C. Groups were wrapped in newspaper and held at -0.6 °C in cardboard boxes lined with polyvinyl wrap. RH was maintained at 80 to 90 %. One group of each cultivar and pulse treatment was removed from storage at 3 and 6 weeks. A non-stored group of each pulse treatment was placed directly into postharvest evaluation (duration = 0).

**Expt. 3 - Post-storage pulses.**

Stems of MN tulip with the bulb still attached were sorted into 16 groups of 10 stems and assigned to treatments. Treatments consisted of 4 storage durations and 4 post-storage pulses arranged as a 4 x 4 factorial. Groups of bulb-attached stems were wrapped in newspaper and each cultivar was held at -0.6 °C in separate cardboard boxes. Four groups of each cultivar were removed from storage after 3, 6, and 9 weeks. Following storage, stems were
cut to 35 cm and one group was held dry while the other three were pulsed with either tap water, BVB (2 ml·L⁻¹) or Bulb 100 (2 ml·L⁻¹) for 8 h at 4 °C. A non-stored group of each pulse treatment including the dry control were placed directly into postharvest evaluation (duration = 0).

Pulse treatments.

Pulse treatments used in Expt. 2 and 3 consisted of tap water and two commonly used commercial preservatives, BVB and Bulb 100. Both commercial products contain gibberellic acid (GA₄₋₇) and benzyladenine (BA). Products were mixed per manufacturer instructions at a rate of 2 mL·L⁻¹ in 9.5 L containers. Each container held 1 L of solution per 25 stems. Stems were pulsed for 8 h at 4 °C with average RH of 86 %.

Post-storage evaluation.

Stems were recut, removing 2.5 cm from the stem end prior to evaluation. This follows industry practices to remove dried vascular tissue and improve water uptake. The length of tulips after recutting ranged from 32.5 to 38.5 cm and 42.5 cm for Dutch iris. Stems were individually placed into separate vases with filled with 400 mL of tap water. The evaluation environment was maintained at a 20 ± 2 °C, 40 to 60 % RH, and a 12-h photoperiod at 15 µmol·m⁻²·s⁻¹. Tulip vase life, the number of days a flower remained appreciable in tap water, was calculated as the number of days until tepals senesced. Tepal senescence was characterized when >50 % of tepals were slightly wilted, translucent, or any number of tepals abscised. Dutch iris vase life was calculated as the number of days until banners or tepals abscised, when >50 % in-rolled, or >50 % were discolored. Dutch iris flower opening was rated as either failed to open (FTO), partially open, or fully open. Tulip stem length gained during evaluation was calculated as the length at termination minus initial length measured from cut end to tepal tip. Percent fresh
weight (FW) lost following storage and recovered pulsing was determined on at least 5 stems of each cultivar.

Experimental design and statistics.

A completely randomized design was used for each experiment. Data from each cultivar were subjected to analysis of variance separately using the Generalized Linear Models (GLM) procedure (SAS 9.4, Cary, NC). Post hoc tests were implemented using Tukey’s honestly significant differences (HSD) with $P < 0.05$ for significant interactions and main effects. Reported values are the least squared means to account for missing samples, such as flowers that failed to rehydrate following storage or flowers that FTO, which were not used in vase life calculations.

4.3 Results

Expt. 1 - Bulb vs cut storage.

The percent FW lost during storage was between 9 and 25 % for all tulip cultivars. Weight lost was significantly higher when stems were stored for 6 weeks compared to 3 weeks and when stored with the bulb attached compared to without the bulb (cut) (Table 4.1) with the exception of MN. Percent FW change of ‘Telstar’ Dutch iris significantly increased from 9 to 15 % when stored for 2 weeks compared to 1 week.

Vase life of non-stored tulip BP, FF, GO, LG, MN, RN was 7.6, 8.1, 5.9, 7.9, 7.5, and 6.4 d, respectively. Vase life of all stored stems, independent of storage method and temperature, was significantly lower than non-stored stems with the exception of BP stored for 3 weeks, which had a vase life of 5.5 d. Among stored stems, the main effects were significant for most cultivars and the significant three-way interaction was ignored for FF and GO to simplify interpretation of the results. Vase life was significantly lower for stems stored for 6 weeks
compared to 3 weeks, 0.7 compared to -0.6 °C and all but two cultivars had a significantly longer vase life when stored with the bulb attached compared to being stored as a cut stem (Figure 4.1). On average, vase life was 1.4 d longer at 3 weeks and 0.6 d longer when stored with the bulb or at -0.6 °C.

Non-stored stems of RK and TS Dutch iris had a vase life of 6.8 and 6.7 d, respectively. Vase life did not significantly decline as storage duration increased and was not affected by storage temperature. On average, ‘River King’ had a significantly longer vase life ($P = 0.0204$) when stored with the bulb attached (6.0 d) compared to those stored as a cut stem (5.4 d). If buds were able to partially open, flowers of both cultivars stayed open for approximately half of the vase life. Non-stored stems fully opened over 90% of time. On average, ‘River King’ stored with the bulb attached fully opened 81% of time compared to 25% of cut stored stems. No trend was seen in ‘Telstar’ with 50 to 60% of buds full opening when stored.

Cut tulips continue elongation following harvest specifically in the last internode between the flower and uppermost leaf. The stem length gained was primarily influenced by storage duration, which caused a significant decrease as storage duration increased (Table 4.2). On average, cut stored stems did not increase in length as much as bulb stored stems. In most cultivars, storage at -0.6 °C increased stem length gained during post-storage evaluation compared to storage at 0.7 °C, but this was only significant for GO and MN.

**Expt. 2 - Pre-storage pulses.**

The main effects, storage duration and pre-storage pulses, significantly impacted the vase life of stored cut stems of tulip MN and ‘Piste’ and Dutch iris ‘River King’ independent of each other. Vase significantly declined for MN ($P <0.0001$) from 7.0 d for non-stored stems to 5.6 d
When stems were stored for 3 weeks at -0.6 °C, but not at 6 weeks of storage (4.9 d), ‘Piste’ vase life significantly \( P < 0.0001 \) declined at each duration from 6.3 to 5.1 d and finally 4.4 d at 6 weeks of storage. Bulb 100 and BVB pulsed stems had a significantly longer vase life than tap water pulsed stems. Vase life was on average 1.4 and 2.0 d longer for MN \( P = 0.0006 \) and ‘Piste’ \( P < 0.0001 \), respectively. Similarly, vase life was significantly shorter at 2 weeks of storage (7.0 d) compared to non-stored stems (7.5 d) for Dutch iris ‘River King’, and vase life was significantly longer (+1.1 d) when pulsed with Bulb 100 and BVB compared to tap water pulsed stems (6.5 d). While a significant interaction was found for treatment effects on vase life of GO (Table 4.3), vase life was longer for Bulb 100 and BVB-pulsed stems when stored for 3 or 6 weeks at -0.6 °C.

Stem length gained was not impacted by storage duration, but was significantly higher for both GO \( P = 0.0029 \) and ‘Piste’ \( P = 0.0001 \) when pulsed with Bulb 100 and BVB compared to tap water (10.1, 8.0 cm, respectively). GO pulsed with BVB or Bulb 100 gained on average 1.5 to 2.2 cm more in length and ‘Piste’ gained 0.9 to 1.5 cm in length compared to water pulsed stems. MN gained 11.5 cm during post-storage evaluation regardless of treatment.

**Expt. 3 - Post-storage pulses.**

Only bulb-attached stems were stored at -0.6 °C for Expt. 3. Percent FW lost following storage increased significantly as storage duration increased, but percent FW gained following 8-h pulses also significantly increased as storage duration increased (Figure 4.2). Eight-hour pulse treatments did not replace all of the FW lost following storage leaving stems with a deficit of 3, 4 and 8 % after 3, 6, and 9 weeks of storage respectively. There was no difference in the percent FW lost or gained among tap water or commercial preservatives Bulb 100 and BVB.
Vase life of MN tulips was significantly higher when pulsed with commercial preservatives Bulb 100 and BVB compared to the non-treated control and stems pulsed with tap water until 9 weeks of storage when vase life of BVB pulsed stems (5.9 d) was no different than tap water pulsed stems (4.2 d) (Table 4.4). There was no significant decline in vase life of Bulb 100 pulsed stems while vase life of BVB pulsed stems was significantly lower at 9 weeks. However, this value was no different than stems stored for 9 weeks and pulsed with Bulb 100. Vase life of tap water pulsed stems was statistically similar to that of the non-treated control throughout the storage duration. Over 9 weeks of storage, non-treated stems lost 4.8 d of vase life compared to 1.7 d when Bulb 100 was used following storage.

Compared to non-stored stems (11.6 cm), stem length gained was significantly shorter following 6 and 9 weeks of storage (9.8 and 9.1 cm, respectively). was lower as storage increased by 11.6 cm for non-stored stems. In comparison to the control and tap water stems, BVB and Bulb 100-pulsed stems were significantly longer by 3.4 cm. Independent of storage duration, stems pulsed with Bulb 100 and BVB gained significantly more length (P <0.0001) compared to tap water pulsed stems and the non-treated control. On average, length gained was 2.8 cm more for Bulb 100 and BVB-pulsed stems.

4.4 Discussion

Percent FW change and flower opening

Preservation of FW and turgidity is a key point of cut flower postharvest handling. At most, stored tulips in Expt. 1 lost 25% by 6 weeks of dry storage. Differences were observable statistically and visually primarily between stems stored with the bulb attached and those stored as a cut stem (Figure 4.3A). Stems with the bulb attached were visually less wilted than stored cut stems, which contradicts the higher mount of FW lost following storage compared to FW lost
by cut stems. Considering bulbs were not measured before and after storage, we cannot pinpoint which tissue, the stem or bulb, lost more FW. However, it is likely that FW was lost primarily from the bulb tissue by water moving into stem, leaf, and floral tissue where it was transpired. This would explain less severe wilting and higher percent FW lost following storage in bulb-attached stored stems.

The ability of bulb-attached stems to recover FW following storage was measured in Expt. 3 after placing cut stem ends into 8-h pulse treatments. Percent FW recovered increased as stems became more dehydrated with increasing storage duration. However, tulip stems likely have a limit in their ability to rehydrate based on the amount of dehydration because the difference between percent FW lost after storage and percent FW recovered after pulsing was larger at 9 weeks compared to 6 weeks. Throughout Expt. 3, no stems were lost due to an inability to rehydrate. Turgidity, meaning stiff stems and leaves, were observed 30 to 60 min after placement into tap water or commercial preservatives following storage.

Dutch iris reacted similarly to tulips where cut stored stems lost less FW and were visually more wilted compared to stems stored with the bulb still attached. In comparison to tulips, Dutch iris were visually less wilted. *Iris siberica*, a related species, has lignified hydrocytes, which likely increase stem strength (Tikhomirova et al., 2018) which could mask dehydration. The difference in ability of Dutch iris and tulips to rehydrate and develop floral structures could be explained by differences in cell makeup and vascular structure. *Tulipa gumusanica* Terzioglu and *Tulipa armena* Boiss. var. *armena* were reported to have a large amount of succulent parenchyma in and around the vascular bundles (Coskuncelebi et al., 2008), which could explain its propensity to rehydrate quickly.
In Expt. 1, 0 to 50% of Dutch iris ‘Telstar’ failed to open after 2 weeks of storage, regardless of storage temperature. Dutch iris have been reportedly difficult to rehydrate compared to tulips following dry storage (van Doorn et al., 2014) even with a portion of the basal plate still attached (Mayak and Halevy, 1971). Water movement (van Meeteren, 2006) and elongation of the pedicel and ovary are responsible for petal expansion and Dutch iris flower opening (van Doorn et al., 2014). In most cases a higher percentage of ‘River King’ and ‘Telstar’ flowers fully and partially opened when stored with the bulb attached compared to cut stems in which a higher percentage failed to open when stored for 2 weeks (Figure 4.3). By retaining the bulb scales, the vascular system may have avoided desiccation, which could have improved water uptake following recutting. Both BVB (A. Ranwala, personal communication) and Bulb 100 (R. Timmerman, personal communication) contain GA$_{4,7}$, which improved flower opening of ‘Discovery’ Dutch iris (Macnish et al. 2010). The pulse treatment duration was 16 h longer in Macnish et al. (2010), which may explain why there was no improvement of flower opening when stems were treated with BVB and Bulb 100, compared to tap water.

Vase life

Storing tulips with the bulb attached resulted in the longest vase life at the longest storage duration without the use of a preservative pulse treatment. These results validate decades of use and claims from the industry and literature (DeHertogh, 1996), which lacked supporting data. Retaining bulbs may allow for the translocation of carbohydrates, starch, water, and other nutrients, which would have been used to complete flowering and daughter bulb formation (DeHertogh, 1996; Miller and Langhans, 1990). Bulb scales likely reduce vascular desiccation by providing water, thereby preserving vascular tissue for future water uptake when cut and placed into a vase.
In conjunction with a sub-zero storage temperature of -0.6 °C, storage life of stems stored with the bulb attached was extended to 6 weeks with all cultivars having a vase life of over 4 to 6 d in Expt. 1 with the exception of GO (3.6 d). The vase life of cut tulips at 6 weeks using this combination was similar to tulips stored for 6 d at 0 °C (Cevallos and Reid, 2002). The closest report to the results of Expt. 1 was a vase life of 6.7 d after 31 d of storage when held in modified atmospheric packaging (MAP) at 0 °C (Aros et al., 2017). However, combination of sub-zero storage and bulb attached stems is likely a more cost effective method. MAP can be expensive and require technical skills to achieve accurate atmosphere concentrations, while cooling systems currently used by cut flower growers may already have the ability to maintain a sub-zero temperature between 0 and -1 °C.

Preservative pulses improved vase life of stored cut stems when used prior to storage on cut stems (Expt. 2) and when applied following storage of bulb attached stems (Expt. 3). Both, Bulb 100 and BVB contain GA₄,₇ and BA phytohormones; impacts of these phytohormones align with past reports on cut tulip vase life and quality (Kim and Miller, 2008; van Doorn et al., 2011). Vase life improvement was less than results reported by van Doorn et al. (2011); however, both the current study and van Doorn et al. (2011) indicate cultivars differ in their responses to pulses and the concentrations of BA and GA may differ between the commercial preservatives and that used by van Doorn et al. (2011). Flower quality consisting of color retention, petal enlargement, and delay of abscission was also observed to be better when Bulb 100 and BVB were used (Figure 4.3). The BA component likely alleviated petal discoloration (van Doorn et al., 2011).

The difference in vase life of pulsed stems compared to non-pulsed stems was greater when pulses were used before storage on cut stems than when pulses were used after storage on
stems stored with the bulb-attached. However, the vase life of bulb attached stems pulsed following storage was longer than cut stems pulsed before storage. Specifically, vase life of ‘Menton’ in Expt. 2 at 6 weeks of storage was between 6.1 and 6.4 d and between 8.0 and 8.1 d in Expt. 3. This comparison matches with results of Expt. 1 where bulb attached stems had a longer vase life than cut stored stems. While there was no statistical comparison between experiments, Expt. 3 resulted in the longest vase life at 6 weeks of storage. Sub-zero storage of cut tulips was first reported by Post and Fischer (1952) who claimed cut tulips could be stored dry for 8 weeks at -0.5 °C. Expt. 3 results support the first use of sub-zero temperatures for cut tulip storage by Post and Fischer (1952) and provide evidence extending storage to 9 weeks when storing stems with the bulb attached and pulsing stems with a preservative containing BA and GA.

Dutch iris responded similarly to cut tulip varieties, but did not benefit as much from being stored with the bulb attached. Vase life was slightly longer when stems were stored for 2 weeks at -0.6 °C. There were no negative effects of storing stems with the bulb attached nor when storing stems at -0.6 °C and there was a positive impact on the number of flowers that fully opened, specifically for TS. Water conductivity and flower opening was lower on ‘Wedgwood’ and ‘Professor Blaauw’ Dutch iris when stored for 4 d at 4 °C with the basal plate still attached (Mayak and Halevy, 1971). Potentially removing more stem from bulb attached stems may have further reduced vascular blockage, which may explain the ability of flowers to open. However, a preliminary test looking at stem length removal above the attached bulb indicated that there was no difference in flower opening in ‘Telstar’ when 2.5 cm was removed above the attached bulb compared to 15 cm following storage (data not presented).
van Doorn et al. (2014) also reported ‘Blue Magic’ to be chilling sensitive when stored at 0.5 °C, but there was no injury observed on either Dutch iris cultivar tested. Many of the differences between the current studies results compared to the literature could have been due to the cultivar. For example, TS and RK were used for these experiments and are grown by the NC grower due to the reliability to open fully compared to other varieties (M. Hommes, personal communication). As stated during the fresh weight loss section, the combination of storing stems with the bulb attached and at -0.6 °C may have minimized the development of a vascular occlusion, which improved flower opening and vase life.

Pulse treatments were not as effective on RK Dutch iris as they were on tulips. However, a positive benefit was still measurable and may be applicable to other cultivars like ‘Telstar’. A 24 h pre-storage pulse of a combination of thidiazuron (TDZ) and GA₃ improved vase life of ‘Discovery’ Dutch iris after 2 weeks of storage at 0 °C (Macnish et al., 2010). Dutch iris were not tested in Expt. 3, but it could be beneficial to store stems with the bulb attached and pulse stems with a commercial preservative following storage to further improve vase life.

Length gain

In-vase stem elongation can be an undesirable characteristic of tulips. Increasing storage duration decreased stem length gained during postharvest evaluation as did storing at 0.7 °C compared to stems stored at -0.6 °C. Cold temperatures are often used to increase tulip stem length during the forcing phase of production (DeHertogh, 1996; DeHertogh and Le Nard, 1993). It is suggested that cold temperatures induce invertase and water-channel proteins for increased water potential which causes internodal stretching (Balk and Douwe de Boer, 1999). Preservative pulses of BVB and Bulb 100 increased length gained during both Expt. 2 and 3. van Doorn (2011) reported increased stem elongation and bending in stems treated with GA₃, while no
effect was seen when treating with BA. van Doorn (2011) also found that the addition of ethephon, a precursor to ethylene, reduced stem elongation and is now included with BA and GA in commercial floral preservatives. The use of this product may yield positive results if used as a post-storage pulse similar to Expt. 3 methods.

4.5 Conclusion

The cut flower industry is constantly looking for ways to improve postharvest handling practices to preserve or improve their products. These three experiments illustrate three methods of improving storage and vase life of cut tulips and Dutch iris. The longest vase life was achieved when storing stems with the bulb attached at -0.6 °C. Leaving the bulb attached is also a simple and effective method of preserving vase life when sub-zero temperatures are not available. Pre-storage pulses in Expt. 2 were not as beneficial as the combination of post-storage pulses on bulb-attached stored stems; however, this may be an effective method when keeping the bulb is undesirable or cut stems need to held for an extended period. Finally, the best method for long-term storage of cut tulips was storing stems with the bulb attached at -0.6 °C followed by an 8-h pulse of either BVB or Bulb 100. It is likely that this method would also have a positive impact on the vase life of Dutch iris when stored with the bulb attached.

The methods studied for tulips and Dutch iris, such as holding stems with a storage organ or using sub-zero temperatures, could be extended to other cut flower species. Sub-zero storage temperatures are not currently used in the industry and adoption of -0.6 °C would increase if more species were tolerant of this temperature. While not all species require long-term storage capabilities, having the option to store multiple crops at the same temperature for long periods of time gives growers and suppliers flexibility by allowing them to store product for periods of high demand, when warm production temperatures results in early harvest or when other issues arise.
The rehydration ability of tulips following storage is uncharacteristic in cut flowers. A better understanding of the vascular structure and water transport may provide insight to improving hydration of many other cut flower species.

**Funding**

This research was supported by funding from the Association of Specialty Cut Flower Growers, the Gloeckner Foundation, and the North Carolina Department of Agriculture & Consumer Services through the North Carolina Specialty Crop Block Grant Program.

**Acknowledgments**

The authors would like to thank Castle Hayne Farms and Ednie Flower Bulbs for their donation of plant material to conduct this research.

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Literature Cited


Dole, J., B. Stamps, A. Carlson, I. Ahmad, L. Greer, and J. Laushman. 2017. Postharvest handling of cut flowers and greens. ASCFG Press, Oberlin, OH.


Table 4.1. Expt. 1 stem fresh weight (FW) loss (%) following storage at both 0.7 and -0.6 °C as affected by storage treatments: duration and method main effects for tulip ‘Black Parrot’ (BP), ‘Foxy Foxtrot’ (FF), ‘Golden Oxford’ (GO), ‘Lingerie’ (LG), Menton’ (MN), and ‘Renown’ (RN).

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>Stem FW loss (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (weeks)</td>
<td>BP</td>
<td>FF</td>
<td>GO</td>
<td>LG</td>
<td>MN</td>
<td>RN</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>8</td>
<td>11</td>
<td>9</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>12</td>
<td>16</td>
<td>13</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>P value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulb</td>
<td>18</td>
<td>11</td>
<td>13</td>
<td>13</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Cut</td>
<td>14</td>
<td>9</td>
<td>13</td>
<td>9</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>P value&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt;0.007</td>
<td>&lt;0.0009</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>1</sup>P < 0.05 indicate a significant difference between least squared means using mean separation adjusted with Tukey’s HSD within each cultivar and treatment; NS = nonsignificant.

<sup>2</sup>Storage method included stems stored with the bulb still attached (bulb) and stems without the bulb (cut).
Table 4.2. Expt. 1 stem length gained during post-storage evaluation as affected by storage treatments: duration, method, and temperature main effects for tulip ‘Black Parrot’ (BP), ‘Foxy Foxtrot’ (FF), ‘Golden Oxford’ (GO), ‘Lingerie’ (LG), ‘Menton’ (MN), and ‘Renown’ (RN).

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>Stem length gained (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP</td>
</tr>
<tr>
<td>Duration (weeks)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>6</td>
<td>8.5</td>
</tr>
<tr>
<td>P value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>NS</td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Bulb</td>
<td>9.0</td>
</tr>
<tr>
<td>Cut</td>
<td>8.4</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>8.2</td>
</tr>
<tr>
<td>-0.6</td>
<td>9.2</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup><sup>P < 0.05</sup> indicate a significant difference between least squared means using mean separation adjusted with Tukey’s HSD within each cultivar and treatment; NS = nonsignificant.

<sup>2</sup>Storage method included stems stored with the bulb still attached (bulb) and stems without the bulb (cut).
Table 4.3. Expt. 2 vase life of tulip ‘Golden Oxford’ as affected by a significant ($P < 0.05$) two-way interaction between pre-storage pulse treatment and storage duration at -0.6 °C when stored as cut stems.

<table>
<thead>
<tr>
<th>Pre-storage pulse</th>
<th>Storage duration (weeks)</th>
<th>Vase life (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Tap water</td>
<td>6.2</td>
<td>ab$^2$</td>
</tr>
<tr>
<td>Bulb 100</td>
<td>6.6</td>
<td>a</td>
</tr>
<tr>
<td>BVB</td>
<td>6.6</td>
<td>a</td>
</tr>
</tbody>
</table>

$^1$Pre-storage pulses were applied by placing cut ends of stems into pulse solutions for 8 h at 4 °C.

Commercial preservatives, Bulb 100 and BVB, contain benzyladenine and GA$_{4,7}$ and were mixed per manufacturer instructions with tap water at a rate of 2 ml·L$^{-1}$.

$^2$Least squared means followed by the same letter are not significantly different when adjusted with Tukey’s HSD test with $P < 0.05$. 

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Table 4.4. Expt. 3 vase life of tulip ‘Menton’ as affected by a significant \((P < 0.05)\) two-way interaction between pre-storage pulses and storage duration -0.6 °C when stored with the bulb attached.

<table>
<thead>
<tr>
<th>Pre-storage pulse</th>
<th>Storage duration (weeks)</th>
<th>Vase life (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Non-treated, control</td>
<td>7.6</td>
<td>bcd</td>
</tr>
<tr>
<td>Tap water</td>
<td>7.5</td>
<td>cd</td>
</tr>
<tr>
<td>Bulb 100</td>
<td>8.8</td>
<td>a</td>
</tr>
<tr>
<td>BVB</td>
<td>8.9</td>
<td>ab</td>
</tr>
</tbody>
</table>

\(^1\)Pre-storage pulses were applied by placing cut ends of stems into pulse solutions for 8 h at 4 °C.

Commercial preservatives, Bulb 100 and BVB, contain benzyladenine and GA\(_{4,7}\) and were mixed per manufacturer instructions with tap water at a rate of 2 ml·L\(^{-1}\).

\(^2\)Least squared means followed by the same letter are not significantly different when adjusted with Tukey’s HSD test with \(P < 0.05\).
Figure 4.1. Expt. 1 vase life (d) of *Tulipa* ‘Black Parrot’ (BP), ‘Foxy Foxtrot’ (FF), ‘Golden Oxford’ (GO), ‘Lingerie’ (LG), ‘Menton’ (MN), and ‘Renown’ (RN) flowers as affected by the significant main effects of storage method (A), storage temperature (B) and storage duration (C). Treatment analyses were separated by cultivar given different entry into the experiment and flower type. The same lowercase letters within each cultivar are not significantly different according to Tukey’s HSD at $P < 0.05$. 

129
Expt. 3 percent FW change for tulip ‘Menton’ as affected by storage duration. The same lowercase letters are not significantly different according to Tukey’s HSD at $P < 0.05$ for percent FW lost following storage ($P < 0.0001$). The same uppercase letters are not significantly different according to Tukey’s HSD at $P < 0.05$ for percent FW recovered after pulsing ($P < 0.0001$) stems for 8 h at 4 °C with either tap water or commercial preservatives Bulb 100 and BVB at 2 ml·L$^{-1}$. 

Figure 4.2.
Figure 4.3. (A) Expt. 1 visual wilting symptoms of tulip ‘Renown’ following storage with the bulb still attached to stems or as cut stems at 0.7 or -0.6 °C for 6 weeks. (B) Expt. 1 Dutch iris ‘River King’ generalized flower opening following storage with the bulb still attached to stems or as cut stems at 0.7 or -0.6 °C for 2 weeks. (C) Expt. 3 flower quality of 6 d old tulip ‘Menton’ which were stored for 6 weeks with the bulb still attached at -0.6 °C. Pulses consisted of 8 h at 4 °C in either tap water or commercial preservatives, Bulb 100 and BVB, containing benzyladenine and GA4,7.
CHAPTER 5:

Simulated spring freezes Cause Bud abortion and receptacle necrosis in *Paeonia lactiflora* Pall. ‘Festiva Maxima’

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ᵃ Corresponding author

(Written in the style of HortScience)
Abstract

Sub-zero temperatures during spring freeze events are suspect of causing losses in North Carolina (NC) cut peony (*Paeonia lactiflora* Pall.) production fields. Damaged buds fail to open and internal necrosis is visible in the receptacle. Two protocols were developed to simulate spring freezes using data from three weather stations surrounding two of the largest NC cut peony producers. The cultivar Festiva Maxima was subjected to simulated spring freezes as potted plants at two growth stages prior to flowering where the minimum temperatures were either -3 or -6 °C. Bud abortion was only observed on peonies at the second growth stage and the highest percentage of buds aborted when simulated freezes had a minimum temperature of -6 °C. Peony shoots always froze from the root system upwards if freezing occurred and was confirmed and visualized using an infrared camera. Some shoots at both growth stages 2 and 3 remained supercooled throughout both simulated freezes of -3 and -6 °C. Histological sections of a necrotic receptacle depicted air pockets in the receptacle tissue characteristic of ice damage. This research represents the first steps to understanding the freezing temperatures responsible for peony bud damage and cellular injury behind this damage.

5.1 Introduction

Peonies, primarily *Paeonia lactiflora* Pall. and its hybrids, are a spring flowering herbaceous perennial grown for cut flowers. North Carolina (NC) is the largest cut peony producer in the United States based on the 2018 Floriculture Survey (USDA, 2019) and one of the earliest producers in the Northern Hemisphere’s spring season (Kamenetsky and Dole, 2012). The majority of peony growth occurs before the average frost-free date of April 13 in the region of two large NC peony growers. Cut flowers are harvested generally between mid-April to early May leaving high-value peony buds exposed to sub-zero temperatures. Peony growers in NC
suspect freezes to be major component of peony losses within the past ten years (C. van Staaldruinen, personal communication), sometimes occurring as late as 2 to 3 weeks before harvest.

Peony flower buds develop in renewal buds called eyes on the underground crown during the fall prior to winter dormancy (Barzilay et al., 2002). Following the completion of vernalization, which is 8 to 10 weeks of 4 to 10 °C in greenhouse forcing experiments (Fulton et al., 2001; Kamenetsky et al., 2000, 2003;), flowering occurs 30 to 60 d after shoot emergence depending on the cultivar (Hall et al., 2007). Growth and development is highly responsive to soil and air temperatures following vernalization. Increasing air temperature causes earlier shoot emergence, faster development, and earlier flowering (Hall et al., 2007). Stems are harvested when the apical bud is at a specific stage to ensure flower opening and maximize vase life (Heuser and Evensen, 1986). Each stem has one apical bud. Overall flower bud size, the number of stems with viable flower buds, and stem length are reliant on many factors including the day and night temperature (Hall et al., 2007), age of the crown, size of the eye (Barzilay, 2002), nutrition, and vernalization (Fulton et al., 2001). Shoots are monocarpic and plants flower once each year following winter dormancy which limits availability of cut peonies. Propagules are also expensive and take three to four years to reach profitable yield. However, cut peony stems are high-value, making world-wide production important for the cut flower industry.

The occurrence and severity of spring freeze events are often unpredictable. A major spring freeze hit the Southeastern United States during April 2007 damaging and causing crop losses in grape (Vitis spp.), strawberry (Fragaria ananassa Duch.), blueberry (Vaccinium spp.), and blackberry (Rubus subgenus Rubus Watson) (Warmund et al., 2008). Peony growers in NC also saw injury (Figure 5.1) and crop losses (C. van Staalduinen, personal communication).
During this spring freeze, temperatures were recorded as low as -5.7 °C in NC and the temperature was below 0 °C for 16 to 47 h in two NC locations. Injury was sustained even when protection methods were used.

Plant species differ in their ability to tolerate low temperatures. Cut flowers species in particular are split into different temperature classes. Chilling sensitive flowers, consisting of many tropical and sub-tropical species which experience injury below 12 and 2 °C, respectively (Halevy and Mayak, 1981). A majority of cut flowers including peonies, roses (Rosa) and Chrysanthemums do not experience chilling injury and can handle temperatures as low as 0 °C (Halevy and Mayak, 1981). Cut flowers such as daffodil (Narcissus) (Nichols and Wallis, 1969), tulip (Tulipa) and rose (Post and Fischer, 1952) can tolerate -0.5 °C for multiple weeks with no injury. P. suffruticoso petal tissue has been reported to have a freezing point of between -3.8 and -4.0 °C (Wang et al., 2013) and P. lactiflora petals froze at -1.7 °C (Wright, 1942). In controlled freeze tests, no injury was reported on cut peonies held at -2 °C for 5 h (Jahnke et al., 2020).

Natural, spring freezes are composed of many uncontrollable factors including the air movement, soil moisture and temperature, and cloud cover which can influence minimum temperature, duration below 0 °C, and the rate of cooling and warming which influence ice nucleation and the duration a plant is frozen (Livingston et al., 2018). Each freeze event is therefore unique. The plant species in question, plant growth stage, and the amount of deacclimation or acclimation to freezing temperatures also influence plant tolerance to freeze events (Ashworth et al., 1989; Li, 1984; Min et al., 2014; Warmund et al., 2007). Simulation of freeze events in controlled chambers followed by survival observations have been the primary method of determine temperature tolerance. Histological studies (Kuprian et al., 2016; Livingston et al., 2018) have provided additional information about freeze avoidance.
mechanisms and susceptible cell tissue. The use of infrared cameras further allows researchers to confirm and visualize freezing of plant tissue (Livingston et al., 2018; Takeda and Glenn 2016). Infrared imaging can help explain how fast and where ice developed and determine if plants remained supercooled. Supercooling, the ability of water to remain below 0 °C without ice nucleation, is an ice avoidance mechanism commonly seen in woody perennials during winter dormancy when temperatures fluctuate below 0 °C (Ashworth, 1989; Pearce and Fuller, 2001; Kuprian et al., 2016).

The potential for freeze injury, high-value, world-wide production, and lack of remontency make peonies a valid candidate for freezing temperature research. The objectives of this study were (1) determine if the freezing temperatures could cause flower bud abortion and similar injury reported on field peonies and (2) confirm freezing and evaluate the cellular injury following freezing using infrared thermography and histology.

5.2 Materials and Methods

Plant culture

Bareroot propagules of *Paeonia lactiflora* Pall. ‘Festiva Maxima’ containing 4 to 5 eyes were received from a local producer and planted in 24.5 cm diameter pots with a peat-based commercial substrate (Fafard® 4P Mix; Sun Gro, Agawam, MA) on Dec. 2018. Plants were then held at 4.4 °C for 10 weeks to allow rooting and vernalization based on the recommendations of Fulton et al. (2001) and Hall et al. (2007). Peonies were forced in a poly-covered greenhouse at North Carolina State University under natural light conditions from March 1 through flowering, which occurred between April 10th and 30th during 2019 and 2020. Temperatures were held at 18.3/15.6 °C day/night. Plants were irrigated every other day using unamended water.
Simulated spring freeze tests

Two-year old peonies at growth stages 2 (Figure 5.2B) and 3 (Figure 5.2C) were subjected to simulated spring freeze events with minimum temperatures of either -3 or -6 °C during 2020. Preliminary tests on stage 1 (Figure 5.2A) were inconclusive due to the number of buds that aborted. The freeze protocol was designed based on temperature data from three weather stations (CRONOS, 2020), surrounding the two largest peony growers on the Eastern coast of NC. Freeze events, when air temperatures dropped below 0 °C for 1 h or more, occurring between February, March, and April from 2016 to 2018 were used to calculate the averages for the maximum air temperature prior to freeze event, the duration between the maximum air temperature prior to freeze events and the minimum air temperature, the cool down rate to the minimum air temperature, the duration of the minimum air temperature, the warmup rate to the maximum air temperature following a freeze event, and the maximum air temperature following a freeze event. These averages were used to create the freeze protocol detailed below.

One potted peony was placed into a modified upright freezer (Model 253.9260410, 20.3 cu. ft., Kenmore, Chicago, IL) and held at 8 °C for 2 h. Freezers cooled at a rate of 1 °C·h⁻¹ until reaching either -3 or -6 °C. The minimum temperatures were held for 1 h. Temperatures rose at a rate of 1.5 °C·h⁻¹ to 10 °C at which plants were held for at least 2 h before removal. One thermocouple was placed in close proximity to peony buds and one thermocouple into the top 2.5 cm of soil during the freeze test. Thermocouples were calibrated at 0 °C with an ice-water slurry. Actual air temperatures were recorded every 10 s (CR10X, Campbell Scientific, Utah, USA). Following freeze tests, the peonies were brought back to the greenhouse and observed to determine if buds developed and opened or aborted. Each simulated spring freeze temperature was replicated four times on each growth stage using 1 to 2 plants.
Infrared thermography

Two stage 2 and stage 3 peonies were monitored via infrared (IR) camera (FLIR T620, FLIR Systems, Wilsonville, OR) (640 × 480 pixels) during each simulated freeze with minimum temperatures of -3 and -6 °C. One thermocouple was placed in close proximity to each peony bud and one 2.5 cm into the peat-based substrate. Actual air temperatures were recorded every 10 s. The simulated spring freeze event was recorded with a computer and IR software (FLIR Systems, Wilsonville, OR) at 1 frame·s⁻¹ and a continuous video recording with BandiCam software at 30 frames·s⁻¹.

Histology

The methods used for preparing bud tissue for histological investigation were adapted from Kuprian et al. (2016). Buds of stage 3 peonies were harvested by cutting 2.5 cm below the receptacle 3 d after simulated spring freezes. Buds were cut in half to increase the surface area of tissue exposed for 24 h to a fixative solution composed of methanol:formaldehyde:glacial acetic acid:distilled water (45:10:5:40). Following fixation, buds were dehydrated using an ethanol series and a micro-wave assisted protocol (Livingston et al., 2009) was used to embedded buds in paraffin. A rotary microtome (Leica,Model RM2255,Wetzlar, Germany) was used to cut 12 μm thick longitudinal sections from paraffin blocks. Sections were stained with a triple stain of Safranin, Fast Green, and Orange G to visualize receptacle cell structure.

5.3 Results and Discussion

Ice formation and propagation

Ice nucleation was consistently observed in the peat-based substrate between -0.3 and -1.4 °C. All shoots of both stage 2 and 3 supercooled during -3 and -6 °C simulated freezes and in some shoots ice nucleation was never observed. All shoots of stage 1 peonies froze when the
minimum temperature was -6 °C. Ice propagation always started from the base of the shoot and moved rapidly upwards to the bud. However, not all leaves or sides of the shoot froze before bud tissues. In some cases, ice propagated up one side of the shoot, into sepals and petals, and then back down the shoot and into leaves. The freezing process of a stage 3 peony bud during a simulated spring freeze with a minimum temperature of -6 °C was recorded using infrared video (https://go.ncsu.edu/jahnkech5peonybudst3freeze). Freezing is depicted as a whitening of the shoot and bud as latent heat is released during freezing. Ice propagation into this stage 3 peony shoot was observed between a chamber temperature of -4.9 and -6.0 °C. The initial rapid freezing ostensibly within the vascular system has been termed as stage 1 freezing (Livingston et al., 2018; Pearce and Fuller, 2001) and is generally not considered lethal to non-chilling sensitive species.

The second stage of freezing is slower and brighter. It can be seen throughout the bud tissue in the supplemental video within second to minutes following the initial freezing. The second stage is reported coupled with cellular dehydration as cells export water to the apoplast (Livingston et al., 2018; Pearce and Fuller, 2001). This may delay or help cells avoid intracellular ice formation by reducing the available water for ice formation. Shoots and buds that froze during the freeze tests reached equilibrium with the sub-zero temperature of the chamber before air temperatures were warm enough to induce thawing. This means a third and more damaging stage of freezing may have occurred (Livingston et al., 2018). All above-ground peony tissue thawed at the same time and was most evident when plants regained turgidity around -0.4 °C. Visually plants showed signs of wilting, loss of turgidity and potential thawing, at -2.0 °C.
Plant injury post-freezing

Peony leaves and stems of each growth stage were visually uninjured following freeze tests. Bud abortion primarily occurred in peonies at growth stage 2 when the minimum temperature reached -6 °C. The percentage of buds that aborted for peonies frozen at stage 2 was 12 and 86 % when the minimum temperature was -3 and -6 °C, respectively. Stage three flower buds were more tolerant with 0 % of buds aborting regardless of the minimum freeze test temperature. This was surprising as increasing plant development increased floral organ susceptibility in fruit trees (Rodrigo, 2000). Also, older wheat leaves (Livingston et al., 2018) and larger, more developed strawberry flowers (Hummel and Moore, 1997) experienced injury at warmer freezing temperatures compared to younger tissues. The tolerance of stage 3 peony buds to freezing temperatures may be related to the large number of petals, which could help retain intrinsic heat. This is in contrast to leaf tip injury on wheat (Livingston et al., 2018) and floral injury of strawberries (Ariza et al., 2015); both lack a large mass and overlapping tissues for protection.

Aborted flower buds of plants at growth stage 2 desiccated and turned necrotic over a 14 d period following the simulated freeze test (Figure 5.2D). Seventeen percent of non-frozen control buds also aborted. Bud abortion has been a common issue when developing forcing programs for potted peonies (Byrne and Halevy, 1986; Fulton et al., 2001). Most bud abortion seen on frozen plants in this experiment was likely caused by the imposed freeze tests. Shoots with a well-developed bud that will likely flower often have longer stems compared to stems that will abort the buds (personal observation). Plants used for growth stage 2 and stage 3 had well developed buds on longer stems compared to non-frozen controls in which buds aborted. It is possible that some bud abortion was caused by other factors including insufficient nutrition,
disease, and insect damage. Stage 1 plants were not used for this study due to the inability to determine if bud abortion was caused by freezing or other factors.

_Bud necrosis and bud histology_

Following a spring freeze in April of 2016 in NC, buds failed to open and necrosis was visible in the receptacle region of buds (Figure 5.3A) (C. van Staalduinen personal communication). No necrosis was found in stage 3 non-frozen controls (Figure 5.3B). A longitudinal section of a stage 3 bud that experienced a simulated freeze to -6 °C developed receptacle necrosis (Figure 5.3D), similar to that of Figure 5.3A. Receptacle necrosis also developed in whole plant freeze tests of blackberry ‘Triple Crown’ (_Rubus_ spp.) when flowers were exposed to temperatures between -3 and -4 °C (Takeda and Glenn, 2016) and apple (_Prunus_ spp.) flower buds (Salazar-Gutiérrez, 2016). Histological sections of this necrotic region of the stage 3 peony bud revealed voids within the receptacle region following freezing and fixation (Figure 5.3E), not present in non-frozen controls (Figure 5.3C). This is consistent with voids seen in forsythia buds (Ashworth, 1990) and wheat leaves (Livingston et al., 2018) following ice formation induced by a freeze test. The supplemental video captured and confirmed the freezing of this stage 3 bud (Figure 5.3D). Freezing was rapid and there was no delay in ice propagation. Blackberry flowers also seem to have no barrier to ice formation (Takeda and Glenn, 2016), but _Calluna vulgaris_ (L.) Hull, an alpine species that commonly experience freeze events, was reported to have an ice barrier (Kuprian et al., 2016).

Peony flowers are quite large and require structural support and water for floral development. Melville (1982) reported a large amount of water-filled vascular tissue in the region of _P. veitchii_ Lynch. While the receptacle xylem size and cell composition was not thoroughly studied in this species of peony, a large amount of vascular tissue could lead to an
accumulation of ice and therefore injury to the receptacle. In *Calluna vulgaris*, the barrier to ice propagation was related to small xylem size and densely packed cells (Kuprian et al., 2016). Damage in the receptacle region of peonies could cause obstruction to water movement leading to the inability of petals expansion and flower opening (van Doorn and van Meeteren, 2003). Stage 3 buds that froze during freeze tests and were not destructively harvested were able to open. There are likely a number of factors that differed between this potted freeze test and natural spring freezes in field conditions which may explain why the lack of injury and bud death in controlled freezes.

A large amount of literature is available on the freezing of plant material using excised plant shoots, flowers, leaves, and stems (Ashworth, 1989; Kuprian et al., 2016; Pagter and Williams, 2011). However, using excised tissue exposes vascular tissue and its contents to the open air and nucleating factors, which could influence the temperature at which freezing occurs. Whole plant freeze tests, as in the current study, are less common presenting a challenge in terms of the amount of substrate, its composition and moisture, and the presence of intact roots, crown, and multiple shoots. This complication and mixed results limit the translation to field situations experienced by peony growers.

5.4 Conclusion

Ice formation in buds demonstrated via IR video, development of necrosis in a stage 3 buds that was similar to field results, and cellular damage observed in histological sections could all lead to bud abortioin at the second growth stage when frozen at -6 °C. While controlled freeze tests did replicate necrotic injury, stage 3 buds did not lose the ability to open following the freeze tests. Conditions seen in the NC field such as cultivar, soil moisture, soil type, minimum temperature, and duration below 0 °C may be factors worth exploring to study bud
injury. The protocol used for the current study averaged characteristics of many spring freezes over multiple years and months, instead of focusing on a specific growth stage and the types of spring freezes that occur during that stage of growth. A more severe simulated freeze including a lower temperature, longer duration, or faster cool- and warm-up rates based on spring freezes where damage was confirmed may result in consistent bud injury.

Two other factors that could influence future results in future studies are the use of a reliable flowering cultivar and well-established plants over 3 to 4 years in age. At this age, plants will likely produce multiple large viable buds that are less susceptible to bud abortion.

Acknowledgments

The authors would like to thank Terra Ceia Farms for their donation of plant material and the vital photographs and corresponding information needed to start the exploration of this issue.
Literature Cited


doi:10.2135/cropsci2009.02.0077


Figure 5.1. Necrosis in the receptacle of peony buds from Terra Ceia, NC between 9 and 11 d following a spring freeze at the beginning of April in 2007. Temperatures were below 0 °C for approximately 16 h with a minimum temperature of -5.7 °C (Warmund et al., 2008). Photo courtesy of C. van Staaldruinen of Terra Ceia Farms.
Figure 5.2. Growth stages of potted peonies (A) growth stage 1: red foliage, small bud size, leaves not fully expanded; (B) growth stage 2: transitioning to green foliage, medium bud size, leaves may not all be fully expanded; (C) growth stage 3: all leaves are green and expanded, petals may be visible between sepals; (D) Stage 2 desiccated flower bud 14 d after experiencing a simulated freeze with a minimum temperature to -6 °C.
Figure 5.3. (A) Injured peony bud following spring freeze April 2016 from a field in Terra Ceia, NC (courtesy of C. van Staalduinen of Terra Ceia Farms). Picture was taken approximately 15 d after plants experienced a 6 h freeze event below 0 °C; (B) Non-frozen stage 3 bud with no necrosis or injury; (C) Triple-stained, longitudinal-section of the bud from (C); (D) Stage 3 bud 10 d after a simulated spring freeze below 0 °C for 9 h with a low of -6 °C held for 1 h; (E) Triple-stained, longitudinal-section of the bud from (d) with arrows point to large pockets in the lower portion of the figure in the receptacle region could have formed due to ice formation.
APPENDICES
Appendix A. Models, fit statistics, and temperature comparisons for Chapter 2 figures.

Table A1. Percent weight loss linear regression models and fit statistics for Figure 2.1A-C.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Storage temperature (°C)</th>
<th>Model $x = storage\ duration$</th>
<th>Adjusted $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM (a)</td>
<td>-3.1</td>
<td>$y = 0.7544 + 0.9767x$</td>
<td>0.6470</td>
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<tr>
<td></td>
<td>-0.6</td>
<td>$y = 1.121 + 1.046x$</td>
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</tr>
<tr>
<td></td>
<td>-3.5</td>
<td>$y = -0.7941 + 1.491x$</td>
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<tr>
<td>MJE (b)</td>
<td>-3.1</td>
<td>$y = 0.4634 + 0.5826x$</td>
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</tr>
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<td>$y = 0.9683 + 0.7475x$</td>
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<tr>
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<td>$y = 0.3491 + 1.110x$</td>
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<tr>
<td>SB (c)</td>
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<td>$y = 2.236 + 2.197x - 0.01728x^2$</td>
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<tr>
<td></td>
<td>-0.6</td>
<td>$y = -0.7738 + 4.382x - 0.1834x^2$</td>
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<td>-3.5</td>
<td>$y = 0.5541 + 3.540x$</td>
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Table A2. Failure to open (FTO) logistic regression models for Figure 2.1D-F.

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<th>Pr &gt; ChiSq</th>
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Table A3. Failure to open (FTO) storage temperature treatment comparisons using least squares means and Tukey HSD

Figure 2.1D-F.

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<th>Storage duration (weeks)</th>
<th>Storage temperature (°C)</th>
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<th>Adj P value</th>
<th>Adjusted lower CI</th>
<th>Adjusted upper CI</th>
<th>Odds ratio</th>
<th>Adj lower CI for odds ratio</th>
<th>Adj upper CI for odds ratio</th>
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<td>FM (d)</td>
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<td>0.10</td>
<td>0.9868</td>
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<td>6.988</td>
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<td>-0.80</td>
<td>0.0151</td>
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<td>0.449</td>
<td>0.228</td>
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Table A4. Flower diameter linear regression models and fit statistics Figure 2.2A-C.

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<th>Model (x = \text{storage duration})</th>
<th>Adjusted (r^2)</th>
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</thead>
<tbody>
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<td>FM (a)</td>
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<td>(y = 11.36 + 0.2393x - 0.1075x^2)</td>
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<td>-0.6</td>
<td>(y = 11.69 - 0.2462x)</td>
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<tr>
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<td>-3.5</td>
<td>(y = 11.65 + 0.3188x - 0.1170x^2)</td>
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</tr>
<tr>
<td>MJE (b)</td>
<td>-3.1</td>
<td>(y = 11.69 - 0.2736x)</td>
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<td>-0.6</td>
<td>(y = 11.21 - 0.2064x)</td>
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</tr>
<tr>
<td></td>
<td>-3.5</td>
<td>(y = 11.81 - 0.3954x)</td>
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<tr>
<td>SB (c)</td>
<td>-3.1</td>
<td>(y = 10.68 + 0.0053x - 0.0729x^2)</td>
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</tr>
<tr>
<td></td>
<td>-0.6</td>
<td>(y = 11.60 - 0.3266x)</td>
<td>0.4050</td>
</tr>
<tr>
<td></td>
<td>-3.5</td>
<td>(y = 11.66 - 0.2269x - 0.0579x^2)</td>
<td>0.6056</td>
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Table A5. Flower deformity logistic regression models for Figure 2.2D-F.

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<th>Explanatory variable</th>
<th>Estimate</th>
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<th>Pr &gt; ChiSq</th>
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<td>FM (d)</td>
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<tr>
<td>Intercept</td>
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Table A6. Flower deformity storage temperature treatment comparisons using least squares means and Tukey HSD

Figure 2.2D-F.

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<th>Comparison</th>
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<th>Adj $P$ value</th>
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<th>Odds ratio</th>
<th>Adj lower CI for odds ratio</th>
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Table A7. Failure to open (FTO) logistic regression models for Figure 2.5A-C.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Estimate</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
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<td><strong>FM (a)</strong></td>
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<td></td>
</tr>
<tr>
<td>Intercept</td>
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<td>Storage duration (D)</td>
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</tr>
<tr>
<td>Storage temperature (T)</td>
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<tr>
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<td>0.3394</td>
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<tr>
<td><strong>MJE (b)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
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<td>10.666</td>
<td>0.0011</td>
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<tr>
<td>Storage duration (D)</td>
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<td>0.0047</td>
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<tr>
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<tr>
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<td>0.0011</td>
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<tr>
<td><strong>SB (c)</strong></td>
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<td></td>
<td></td>
</tr>
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<td>Intercept</td>
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<td>82.744</td>
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</tbody>
</table>
Table A8. Failure to open (FTO) storage temperature treatment comparisons using least squares means and Tukey HSD

Figure 2.5A-C.

<table>
<thead>
<tr>
<th>Storage temperature (°C)</th>
<th>Comparison</th>
<th>Estimate</th>
<th>Adj P value</th>
<th>Adjusted lower CI</th>
<th>Adjusted upper CI</th>
<th>Odds ratio</th>
<th>Adj lower CI for odds ratio</th>
<th>Adj upper CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM (a)</td>
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<td>0.7</td>
<td>1.368</td>
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<td>MJE (b)</td>
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<td>0.7</td>
<td>&lt;0.001</td>
<td>ns</td>
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<tr>
<td>SB (c)</td>
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<td>0.7</td>
<td>-0.7405</td>
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<td>-1.287</td>
<td>-0.193</td>
<td>0.477</td>
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Table A9. Flower deformity logistic regression models for Figure 2.5D-F.

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<th></th>
<th>Explanatory variable</th>
<th>Estimate</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FM (d)</strong></td>
<td>Intercept</td>
<td>-2.96</td>
<td>95.709</td>
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<tr>
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<td>Storage duration (D)</td>
<td>0.25</td>
<td>90.808</td>
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<td>Storage temperature (T)</td>
<td>-0.6</td>
<td>6.648</td>
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<td></td>
<td></td>
<td>0.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>MJE (e)</strong></td>
<td>Intercept</td>
<td>-7.34</td>
<td>53.153</td>
<td>&lt;.0001</td>
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<td>Storage duration (D)</td>
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<td>&lt;.0001</td>
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<td>Storage temperature (T)</td>
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<td>12.930</td>
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<td></td>
<td>0.7</td>
<td>0</td>
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</tr>
<tr>
<td><strong>SB (f)</strong></td>
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<td>-3.96</td>
<td>61.087</td>
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<td>Storage duration (D)</td>
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Table A10. Flower deformity storage temperature treatment comparisons using least squares means and Tukey HSD

Figure 2.5D-F.

<table>
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<th>Storage temperature (°C)</th>
<th>Comparison</th>
<th>Estimate</th>
<th>Adj P value</th>
<th>Adjusted lower CI</th>
<th>Adjusted upper CI</th>
<th>Odds ratio</th>
<th>Adj lower CI for odds ratio</th>
<th>Adj upper CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM (d)</td>
<td>-0.6</td>
<td>0.7</td>
<td>-0.60</td>
<td>0.0099</td>
<td>-1.066</td>
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<td>MJE (e)</td>
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<td>0.7</td>
<td>-1.42</td>
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<td>-0.0566</td>
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Appendix B. Models, fit statistics, and temperature comparisons for Chapter 3 figures.

Table B1. Failure to open (FTO) logistic regression model for Monsieur Jules Elie (MJE) and Sarah Bernhardt (SB) for Figure 3.2A-B.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Explanatory variable</th>
<th>Estimate</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; ChiSq</th>
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<tbody>
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<td>Storage duration</td>
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<td>Shipping</td>
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<td>Storage duration</td>
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</table>
Table B2. Failure to open (FTO) storage temperature and shipping treatment comparisons using least squares means and Tukey HSD for Monsieur Jules Elie (MJE) and Sarah Bernhardt (SB) in Figure 3.2A-B.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Storage temperature (°C)</th>
<th>Comparison</th>
<th>Storage duration (weeks)</th>
<th>Estimate</th>
<th>Adj P value</th>
<th>Adjusted lower CI</th>
<th>Adjusted upper CI</th>
<th>Odds ratio</th>
<th>Adj lower CI for odds ratio</th>
<th>Adj upper CI for odds ratio</th>
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<tr>
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</tr>
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<td>0.236</td>
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</tr>
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<td>0.0171</td>
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</tr>
<tr>
<td>SB</td>
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<td>-1.29</td>
<td>0.001</td>
<td>-1.96</td>
<td>-0.63</td>
<td>0.273</td>
<td>0.141</td>
<td>0.532</td>
</tr>
<tr>
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<td>-1.96</td>
<td>-0.63</td>
<td>0.273</td>
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<td>0.001</td>
<td>-1.96</td>
<td>-0.63</td>
<td>0.273</td>
<td>0.141</td>
<td>0.532</td>
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