HortScience 59(4):520–528. 2024. https://doi.org/10.21273/HORTSCI17651-23

Low Temperature Tolerance of Peach Flower Buds and Shoot Tissues Is Differentially Influenced by Freezing and High Temperature Exposure

Renae E. Moran
School of Food and Agriculture, University of Maine, PO Box 179, Monmouth, ME 04259, USA

Peyton Ginakes
Cooperative Extension, University of Maine, PO Box 179, Monmouth, ME 04259, USA

Keywords. acclimation, cambium, phloem, *Prunus persica*, winter hardiness, xylem

Abstract. **In northern temperate zones, peach trees are vulnerable to cold temperature injury in the fall, particularly as climate change prolongs warm weather in the fall and potentially delays the onset of cold acclimation. Four experiments evaluated how cold acclimation of flower buds and shoot phloem, cambium, and xylem is affected by exposure to varying temperatures in the fall. One-year-old peach shoots from trees grown in Maine, USA, were collected from October through November, exposed to 1, 3, or 6 days of low, high, and freezing temperatures, and subjected to stepwise controlled freezing to about −30 °C. Injury was visually quantified as oxidative browning of flower buds and shoot tissues. High temperature exposure, even of a single day, decreased cold tolerance of flower buds and shoot tissues until late November, when high temperatures only minimally decreased cold hardiness. In mid-November, increasing the duration of high temperature exposure from 1 to 3 days decreased cambium and phloem hardiness, but hardiness in flower buds was not further decreased by the longer duration of 3 days. By late November, hardiness in flower buds, cambium, and phloem was less responsive to high temperature, and was increased by prior exposure to 6 days of freezing. After high temperature, xylem lost hardiness to a small degree in mid-October and late November, but in mid-November this occurred in only one experiment. In this study, deacclimation during high temperature in the fall was greater in cambium and phloem than in flower buds and at times greater than in xylem.**

Winter injury to peach trees, which can occur from late fall through early spring, is a frequent cause for reduced fruit yield and tree death in temperate regions (Quamme et al. 2010). In North America, winter injury to peaches has been documented in regions as disparate as British Columbia, Canada (Caprio and Quamme 2006), Oklahoma, USA (Smith et al. 1994), and Tennessee, USA (Deyton et al. 1996). In late fall, trees remain vulnerable to injurious freezing temperatures when high temperature interferes with the development of cold hardiness. In many fruit tree species, the onset of cold tolerance begins with the environmental signals of shortening daylength and cold temperature (Howell and Weiser 1970). Additional hardiness develops after exposure to freezing temperatures (Andrews and Proebsting 1987; Howell and Weiser 1970; Minas and Sterle 2020; Ouyang et al. 2022; Szalay et al. 2010) without which the hardiness needed to tolerate sudden or severe decreases in temperature may not occur. Changing climates have increased the frequency of prolonged periods of high temperature in the fall and are predicted to delay the timing of the first frost by as much as 16 d by 2040 (Rochette et al. 2004). This delayed cold acclimation can leave fruit trees vulnerable to sudden or severe decreases in temperature.

The impact of both natural and simulated warming on peach hardiness in winter and spring is well-documented (Chaplin 1948; Durner 1995; Edgerton 1954; Layne and Ward 1978; Layne et al. 1977; Liu et al. 2019; Minas and Sterle 2020; Moran 2021; Shin et al. 2015; Szalay et al. 2010; Szymajda and Zurawicz 2016), but how it affects cold acclimation in the fall is not as well understood. Minimum temperatures in the 4 preceding days strongly influence peach flower bud hardiness during endodormancy (Minas and Sterle 2022). In fall, daytime highs above 20 °C or continuous storage at 15 °C limits tissue hardiness in flower buds, trunks, and limbs to −15 to −16 °C (Buchanan et al. 1976; Szalay et al. 2010). Differing hardiness among shoot xylem, cambium, and phloem is not commonly considered, but rates of acclimation can vary among these tissues. Arora et al. (1992) found that the bark in peach shoots was less hardy than the xylem in October but became hardier in November, whereas Yu et al. (2017) found that trunk and shoot bark both were as hardy as xylem from September to November. To address how climate change will affect peach tree hardiness, studies must consider how high temperature affects hardiness of individual shoot tissues and flower buds. Cambial hardiness, in particular, is not frequently measured, but injury in this tissue can lead to tree mortality (Deyton et al. 1996).

The goal of this research was to measure the difference in cold temperature tolerance of peach shoots and flower buds following a short duration of exposure to warm, cool, or freezing temperatures.

Materials and Methods

Plant material and growing conditions. One-year-old shoots of peach (*Prunus persica* L.) were collected from an orchard in Livermore Falls, ME, USA (GPS coordinates 44.469972, −74.22545) that was planted in 2014 with three cultivars grafted to 'Lovell' rootstocks. At each collection date, shoots were cut, placed into buckets with tap water to a depth of 2.5 cm, and transported to a laboratory where they were recut under water and returned to buckets before acclimation treatments. We used 'Redhaven' for Expts. 1 and 2, 'Gloria' and 'Madison' for Expt. 3, and 'Redhaven' and Gloria' for Expt. 4. We used three different cultivars, Redhaven, Gloria, and Madison, to obtain enough whole shoots for both experiments in 2021 as we avoided pruning more than five shoots from each tree because pruning in fall can be detrimental to cold hardiness (Durner 1995). In Maine, orchards with large plantings of single cultivars do not yet exist. Relative flower bud hardiness is greater in 'Madison' than 'Redhaven' based on survival after a natural freeze (Young 1988), but less is known about comparative shoot hardiness or about hardiness in 'Gloria'.

Daily minimum and maximum temperatures for the 7 d before each sampling were collected from the nearest National Oceanic and Atmospheric Administration weather station in Livermore Falls, ME, USA (GPS coordinates 44.4716, −70.1594).

Experimental set up. Four experiments, summarized in Table 1, were conducted to

Received for publication 6 Dec 2023. Accepted for publication 23 Jan 2024.
Published online 8 Mar 2024.
We thank The Berry Fruit Farm for their collaboration. This project was supported by the Maine Department of Agriculture, Conservation and Forestry Specialty Crops Block Grant and by the US Department of Agriculture National Institute of Food and Agriculture, Hatch Project Number ME0-31912 through the Maine Agricultural & Forest Experiment Station. Maine Agricultural and Forest Experiment Station Publication Number 7852. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product, nor does it imply approval or disapproval to the exclusion of other products or vendors that may also be suitable.
R.E.M. is the corresponding author. E-mail: rmoran@maine.edu.

Table 1. Sampling dates, peach cultivars, and durations and temperatures of acclimation treatments in four experiments.

Expt.	Sampling date	Cultivars	Duration (d)	Temperature (°C)	Name
1	14 Oct 2020	Redhaven	1	−4.0	F1[1]
			1	3.5	L1
			1	18.0	H1
2	6 Nov 2020	Redhaven	3	3.5	L3
	8 Nov 2021		2 + 1	3.5 + 20.0	H1
			3	20.0	H3
3	1 Nov 2021	Gloria	3	−3.0	F3
		Madison	1 + 2	−3.0 + 3.5	F1-L2
			3	3.5	L3
			3	20.0	H3
4	28 Nov 2022	Gloria	6	−5.0	F6
		Redhaven	3 + 3	−5.0 + 3.5	F3-L3
			6	3.5	L6
			6	18.0	H6

[1] F, L, H, and F-L indicate freezing, low nonfreezing, high, and freezing-low acclimation temperature, respectively, and the number indicates the duration in days.

compare various acclimation temperatures and durations. In all experiments, freezing, low nonfreezing, and high temperature acclimation treatments were applied using a programmable chest freezer, walk-in cold room, and closet, respectively. The low nonfreezing treatment was used as a control, to approximate cool outdoor ambient temperatures. Shoots were kept in the dark during acclimation treatment. Shoots subjected to freezing temperatures for acclimation were briefly thawed before they were exposed to stepwise controlled freezing. In the first three experiments, a set of four shoots was retained before acclimation treatments to measure prior injury that may have occurred in the orchard, and this served as a proxy for injury at 0 °C. Because the basal ends of shoots were typically hardier than shoot tips, in agreement with previous findings (Layne et al. 1977), we used entire shoots but eliminated 6 cm of the shoot tip, which varied too much in hardiness from the shoot base.

Expt. 1. To compare 1 d of freezing, low, or high temperatures, 'Redhaven' shoots were collected 14 Oct 2020. A total of 95 shoots were collected divided into 30 shoots for each acclimation treatment and five for measuring any prior injury. Shoots were subjected to 1 d of freezing (F1), low (L1), or high acclimation temperatures (H1), at −4.0, 3.5, or 18.0 °C, respectively. To assess cold hardiness, shoots from all acclimation treatments were subjected to controlled stepwise freezing for 1 h to −3.8, −11.7, −15.7, −20.0, −25.5, or −31.2 °C with five shoots per treatment at each temperature.

Expt. 2. To compare 1 and 3 d of high temperature exposure, 'Redhaven' shoots were collected on 6 Nov 2020 and 8 Nov 2021. A total of 120 shoots were collected and divided into 40 shoots for each acclimation treatment and an additional four for measuring any prior injury. Acclimation treatments included exposure to 3.5 °C for 3 d (L3), 3.5 °C for 2 d followed by 20 °C for 1 d (H1), and 20 °C for 3 d (H3). To assess cold hardiness, shoots from all acclimation treatments were subjected to 1 h at −2.7, −10.4, −15.2, −19.4, −25.5, and −30.0 °C in 2020. In 2021, shoots were subjected to 1 h at −4.0, −9.4, −12.8, −17.2, −18.8, −22.2, −25.0, and −28.6 °C with five shoots per treatment at each temperature.

Expt. 3. To compare freezing-thawing with constant freezing or low nonfreezing temperature, 'Gloria' and 'Madison' shoots were collected 1 Nov 2021. A total of 120 shoots were collected from each cultivar and were divided into 30 shoots for each acclimation treatment and an additional four for measuring any prior injury. Acclimation treatments were 3 d at either −3.0 (F3), 3.5 (L3) or 20 °C (H3), or 1 d of freezing at −3.0 °C followed by 2 d above freezing at 3.5 °C (F1-L2). To assess cold hardiness, shoots from all acclimation treatments were subjected for 1 h to −4.8, −9.8, −13.8, −17.8, −19.5, −22.0, −25.8, and −29.5 °C with four shoots per treatment at each temperature.

Expt. 4. To test longer durations of freezing or high temperature, as well as freezing followed by thawing, shoots from 'Redhaven' and 'Gloria' trees were collected 28 Nov 2022. A total of 140 shoots were collected from each cultivar and were divided into 35 shoots for each acclimation treatment and an additional four for measuring any prior injury. Acclimation treatments included 6 d at −5.0 °C (F6), 6 d at 3.5 °C (L6), 6 d at 18.0 °C (H6), or 3 d at −5.0 °C followed by 3 d at 3.5 °C (F3-L3). To assess cold hardiness, shoots from all acclimation treatments were subjected for 1 d to −4.8, −13.7, −17.6, −19.9, −23.5, −27.2, and −29.3 °C with five shoots per treatment at each temperature.

Controlled freezing. Following acclimation treatments, whole shoots were divided into buckets filled with tap water to a depth of 2.5 to 5.0 cm. In Oct 2020, temperature during controlled freezing was measured with an ISO 17025 Traceable® digital thermometer. In subsequent experiments, one to three Type T thermocouples (2 m in length) were placed in contact with shoots and connected to a Hobo data logger (model UX120; Bourne, MA, USA) to measure shoot temperature at 1-min intervals during controlled freezing. A Traceable® digital thermometer (Traceable Products, Galveston, TX, USA) was used to confirm functioning of the thermocouples. Buckets were then placed in a controlled, programmable freezer (SuperCold Freezer, Scientemp, Adrien, MI, USA) held at −3 to −4 °C for 17 h, followed by a decrease in temperature at a target rate of 3 to 5 °C per hour until −30 °C. Shoots were held at each set temperature for 1 h in Expts. 1, 2, and 3, and for 24 h in Expt. 4, at which time a bucket of shoots was removed, brought to a cold room, and held at 3.5 °C until analysis of injury. Actual temperature plateaus at each step were recorded for data analysis. Before analysis, shoots were held at ambient temperature (20 °C) for at least 1 d to allow for full oxidative browning of injured tissue.

Injury analysis. Flower buds were counted as dead or alive based on visual browning in cross section. Xylem tissue browning was visually rated using a scale from 0 to 10, where 0 indicated no browning and 10 indicated 100% of the xylem in cross section was discolored when cut just before observation. To assess browning of phloem and cambium, shoots were cut lengthwise to the depth of the outer xylem on two sides of each shoot and rated on the same scale as xylem. Phloem browning was measured as the length and cross-sectional depth of phloem tissue that was discolored. Cambial browning was measured as the relative length and circumference of cambium that was discolored. In addition, tissues were rated according to the intensity of browning using a scale of 0 to 5, where 0 indicated no browning and 5 indicated dark browning to blackening of the tissues (Supplemental Figs. 1 and 2; Moran et al. 2021). Both ratings were used to calculate an index of injury according to the equation:

$$index\ of\ injury = \frac{(discolored\ area + (discolored\ intensity * 2))}{2}$$

Reading glasses with +3.00 strength and light-emitting diode lighting were used to enhance visual observation of tissue browning.

Statistical analysis. Data were analyzed using SAS Version 9.4 (SAS Institute, Cary, NC, USA). Each shoot was considered an experimental unit with four to five replicate shoots for each temperature during controlled freezing. Prior injury was measured in four to five shoots as a measure of injury present on shoots that were not subjected to controlled acclimation or freezing, and was included in the model at a temperature of 0 °C. PROC NLIN was used to estimate the four parameters of an adjusted logistic sigmoid function:

$$y = \frac{Bmax}{(1 + e^{b(T-x)})} + d$$

where y is the survival or browning index, B_{max} the maximum injury or upper asymptote, b the slope at the inflection point, T the temperature at the inflection point, x as the controlled freezing temperature, and d the lower asymptote or injury that occurred before testing (Repo and Lappi 1989). The temperature at the inflection point was used as an estimate of the lethal temperature for flower buds (LT_{50}). For shoot tissues, the temperature at the inflection point is an estimate of temperature of injury (TI) rather than lethal

temperature, as shoots would occasionally have an inflection point associated with partial injury of the tissue that was not clearly lethal. The inflection point for flower buds was consistently very close to 50% mortality. Acclimation temperature, duration, and genotypic differences were evaluated based on the LT_{50s} or TIs and their 95% confidence intervals determined using PROC NLIN. Significant differences in injury among treatments but within a genotype and temperature were based on means separation using PROC GLM single-degree of freedom T-tests (contrasts) at $P \leq 0.05$.

Results

Air temperature in the week before sampling was highest before Expt. 1 in Oct 2020, the earliest sampling date, and lowest before Expt. 4 in late November, the latest sampling date (Fig. 1). Minimum daily temperatures fell below freezing during the week before each experiment, but for a greater number of days before Expts. 2 and 4 than before Expts. 1 and 3. The number of days that had at or below freezing daily temperature minimums varied from three in the week before Expt. 3 to every day in the week before Expt. 4. In Expt. 2, both years had the same number of days with freezing minimums, but maximum temperatures were higher on average in 2021.

Expt. 1. In October, flower bud survival was close to 100% at temperatures above −20.0 °C and was nearly 0% at temperatures of −25.5 and −31.2 °C (Fig. 2). At −20.0 °C, H1 resulted in lower survival than other treatments ($P = 0.001$). The F1 treatment did not increase flower bud hardiness relative to L1. Because of the wide interval of test temperatures in the critical range of −15 to −25 °C, the LT_{50} of the H1 treatment could not be accurately estimated, so LT_{50} was not statistically different among treatments (Table 2).

Cambial injury was minimal at temperatures above −11.7 °C and was severe below −20.0 °C in all acclimation treatments (Fig. 2). Shoots exposed to H1 had greater cambial injury at −15.7 °C and lost 4.5 °C of hardiness (Table 2) compared with F1 ($P = 0.018$), but not L1. Injury at other temperatures did not differ among acclimation treatments.

Phloem tissues were uninjured at temperatures of −11.7 °C and higher and were severely injured at temperatures colder than −25.5 °C in all acclimation treatments (Fig. 2). Injury at −15.7 °C was highly variable among shoots, so the loss of hardiness with H1 did not differ from other treatments ($P = 0.07$). Based on the TI, H1 reduced hardiness in phloem by 4.1 °C compared with L1, but not F1 (Table 2).

Variability in the level of injury among treatments was present in xylem at −31.2 °C (Fig. 2), when F1 was less severely injured than H1 ($P = 0.035$) or L1 ($P = 0.005$). A 7.4 °C loss of hardiness occurred in the TI for H1 compared with L1, but TI did not differ compared with F1 (Table 2).

Expt. 2. Flower bud survival was nearly 100% at temperatures of −15.2 °C and higher,

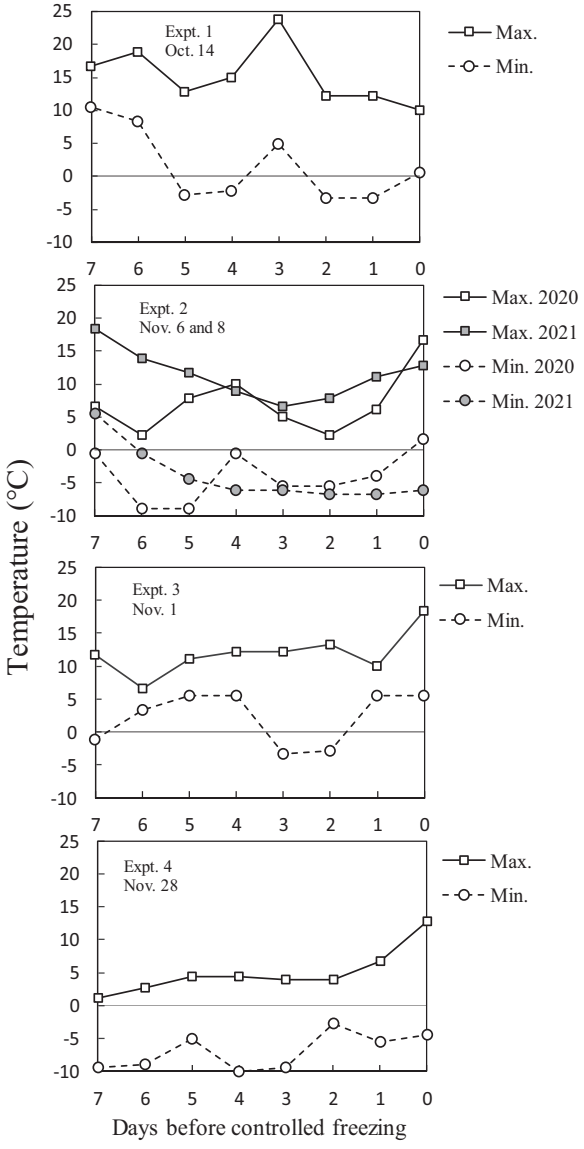

Fig. 1. Minimum and maximum air temperature in the 7 d before peach acclimation treatments with day 0 as the sampling date. Data obtained from the National Oceanic and Atmospheric Administration weather station in Livermore Falls, ME, USA.

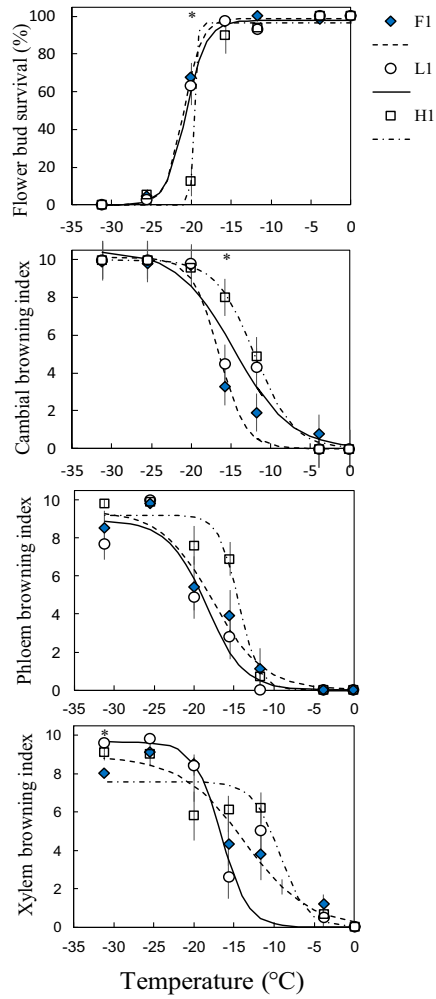

Fig. 2. 'Redhaven' peach flower bud survival and oxidative browning index in cambium, phloem, and xylem of 1-year-old shoots following 1 d at −4.0 °C (F1), 3.5 °C (L1), or 18.0 °C (H1) in Expt. 1, mid-Oct 2020. Shoots were subjected to controlled freezing from 0 to −31.2 °C. Bars indicate standard errors (n = 5). * indicates a significant treatment effect within a temperature.

Table 2. The lethal temperature (LT_{50}, °C[i]) of flower buds and temperature of injury (TI, °C) in 1-year-old shoot tissues of 'Redhaven' peach after a 1-d exposure to −4.0 (F1), 3.5 (L1), or 18.0 °C (H1) in Expt. 1, mid-October (14 Oct 2020).

Treatment	Flower buds	Cambium	Phloem	Xylem
L1	−20.6 a[ii]	−14.6 ab	−18.5 a	−16.7 a
F1	−20.9 a	−16.5 a	−17.7 ab	−13.7 ab
H1	−19.6	−12.0 b	−14.4 b	−9.3 b
		95% confidence interval (−°C)		
L1	19.9–21.4	12.4–16.7	16.8–20.2	14.8–18.4
F1	20.5–21.2	15.3–17.7	14.8–20.5	11.6–15.8
H1	ne[iii]	11.2–12.7	13.6–15.3	6.4–12.2

[i] Based on the inflection point estimated from nonlinear regression.
[ii] Means separation within a column based on 95% confidence interval of the regression estimate.
[iii] Not statistically estimable.

and was 0% at −25.0 °C and colder in all three treatments in mid-November of both 2020 and 2021 (Fig. 3). Greater bud survival occurred in L3 compared with H1 or H3 treatments at −19.4 °C in 2020 ($P = 0.001$) and −22.2 °C in 2021 ($P = 0.001$). At these temperatures, H1 resulted in similar bud survival as H3. In both years, the LT_{50} was 1.9 to 3.1 °C greater following H3 compared with L3, and compared with H1 in 2020 (Table 3). Flower buds were slightly hardier in 2021 than in 2020.

Cambial tissues were severely injured at −19.4 °C and below in both years (Fig. 3). In 2020, H3 increased injury at −19.4 °C relative to L3 ($P = 0.006$), although the difference in the amount of browning was minor. No difference occurred between H1 and other treatments. In 2021, H3 increased injury compared with L3 at −12.8 to −22.2 °C ($P \leq 0.004$) and compared with H1 at −12.8 °C ($P = 0.039$). Treatment H1 increased injury at −18.8 and −22.2 °C compared with L3 ($P \leq 0.008$). Compared with L3, loss of hardiness after H3 was 1.5 °C in 2020 and 5.2 °C in 2021. Compared with H3, the TI for H1 was 1.0 °C lower in 2020 and 3.4 °C lower in 2021 (Table 3). Similar to flower buds, cambial tissues were hardier in 2021 compared with 2020 in two of the treatments.

Phloem tissues remained uninjured or were slightly injured at −15.2 °C and were severely injured at −25.5 °C and colder after H1 and H3 in both years (Fig. 3). Injury after L3 was only partial at the coldest freezer temperatures. In 2020, H3 increased injury at −25.5 and −30.0 °C compared with L3 ($P \leq 0.010$). Injury was also increased by H1 at −25.5 °C ($P = 0.002$), but injury plateaued and was not different from L3 at −30.0 °C. In 2021, H3 substantially increased injury at all temperatures ranging from −18.8 to −28.6 °C compared with L3 ($P = 0.001$) and H1 ($P \leq 0.026$), whereas H1 had greater injury than L3 at −25.5 and −28.6 °C ($P \leq 0.002$). After L3, phloem injury at the coldest freezer temperature was only minor, so the TI could not be estimated for this treatment (Table 3). In 2020, H1 phloem had a similar TI as H3. In 2021, phloem was 6.2 °C higher after H3 than H1. The TI was similar in the 2 years for L3 and H3, but was 5 °C lower in H1 in 2021 compared with 2020.

Xylem injury was minimal at temperatures higher than −19.4 °C in 2020 (Fig. 3). For reasons unknown, injury at −25.5 °C was greater with L3 than H3 ($P = 0.001$) in 2020, although no difference between treatments were observed at −30.0 °C. Xylem injury in 2021 was partial at temperatures as cold as −28.6 °C and was unaffected by acclimation treatments. Xylem TI could not be estimated in H1 and H3 in Nov 2020, but was −24.7 °C after L3 (data not shown). In 2021, the TI for the partial level of injury that occurred was −3 to −9 °C and was not different among the treatments (data not shown).

Expt. 3. Flower bud survival was similar in the four treatments at temperatures above −17.8 °C and below −19.5 °C (Fig. 4). Treatment differences occurred at −17.8 and −19.5 °C, but not consistently in both cultivars. In 'Gloria', lower bud survival occurred with H3 at −19.5 °C compared with F3 and F1-H2 ($P \leq 0.037$), but not L3. Survival did not vary between F3 and F1-L2. In 'Madison', bud survival at −17.8 and −19.5 °C was lower following H3 than other treatments ($P \leq 0.010$). In both cultivars, H3 increased the LT_{50} compared with other treatments (Table 4). The LT_{50} for H3 was increased by 2.3 °C compared with F3, but by less than this compared with F1-L2 or L3. The F3 treatment lowered the LT_{50} compared with L3 in both cultivars and compared with F1-L2 in 'Madison'. Flower bud LT_{50} was similar in both cultivars, except with L3, which accrued 1 °C greater hardiness in 'Madison' compared with 'Gloria'.

Cambial tissue was severely injured at temperatures below −17.8 °C in both cultivars and across all treatments (Fig. 4). Injury was greater at −13.8 and −17.8 °C following H3 compared with other treatments in both cultivars ($P \leq 0.002$). In 'Gloria', H3 also lowered survival compared with F1-L2 and L3 at −9.8 ($P \leq 0.033$). Cambium TI was similar following F3, F1-L2, and L3 (Table 4). The TI was increased by 4.1 to 5.0 °C by H3 compared with all other treatments.

In both cultivars, H3 increased phloem injury at −17.8 to −25.8 °C compared with other acclimation treatments and at −29.5 °C in 'Madison' ($P \leq 0.039$; Fig. 4). In 'Gloria', other acclimation treatments had similar phloem injury at −17.8 to −22.0 °C, but at −25.8 °C, F3 and F1-L2 had less injury than L3 ($P \leq 0.02$). In 'Madison', L3 had less injury than F3 and F1-L2 at −22.0 °C ($P < 0.016$), but similar injury at other temperatures excluding −25.8 °C for which there were no shoots measured as it was not removed from the freezer at the right temperature. No difference occurred between F3 and F1-L2 in 'Gloria', but F3 had less injury at −22.0 °C than F1-L2 in 'Madison' ($P = 0.001$). The TI for phloem injury was higher by 4.8 to 7.2 °C after H3 compared with all other acclimation treatments in 'Gloria' and was unaffected by acclimation treatment in 'Madison' despite being several degrees higher with H3 (Table 4). Phloem TI was similar in the two cultivars, except following F1-L2, which occurred at a higher temperature in 'Madison' than in 'Gloria'.

Partial xylem injury in F3 occurred in the range of −9.8 to −16.0 °C with a plateau from −16.0 to −29.5 °C (Fig. 4). This plateau in injury also occurred in L3 and F1-L2 in 'Madison', but in 'Gloria', additional injury occurred at temperatures below −22.0 °C. In contrast, xylem injury continued to increase as temperature decreased with H3. Treatment differences in the amount of injury occurred within several test temperatures. In 'Gloria', xylem injury at −25.8 °C was greater in H3 than in L3 and F1-L2 ($P \leq 0.050$), but not F3. At −29.5 °C, injury was greater in H3 than in F3 ($P = 0.002$), but not L3 or F1-L2 °C. In 'Madison', xylem injury at −19.5 °C was greater after H3 and L3 than after F3 and F1-L2 ($P = 0.001$). Xylem injury at −29.5 °C was increased by H3 compared with other treatments ($P \leq 0.044$). The TI was unaffected by cold acclimation treatment (data not shown).

Expt. 4. In late November, flower bud survival was close to 100% at temperatures above −19.9 °C and was 0% below −23.5 °C (Fig. 5). At −17.6 °C, L6 had lower survival than other treatments in 'Gloria' ($P \leq 0.016$), but not 'Redhaven'. At −19.9 and −23.5 °C, bud survival was increased by F6 compared with other treatments in both cultivars ($P \leq 0.038$). Also at −19.9 °C, but only in 'Redhaven', H6 decreased survival compared with L6 and F3-L3 ($P \leq 0.025$). Flower bud LT_{50} was 3.6 to 4.6 °C hardier after F6 than after other treatments in both cultivars (Table 5). No difference in LT_{50} occurred between H6, L6, and F3-L3. In 'Redhaven', the LT_{50} occurred at a lower temperature than in 'Gloria' following F6, but this cultivar difference did not occur with other treatments.

Cambial injury was similar among treatments at temperatures of −17.6 °C and above (Fig. 5). Treatment differences occurred at other temperatures, albeit with inconsistent cultivar responses. In 'Gloria', H6 increased injury at −19.9 °C compared with other treatments ($P \leq 0.023$), and at −27.2 °C compared with F6 only ($P = 0.001$). The F6 treatment had less injury at −27.2 °C compared with other treatments ($P \leq 0.012$). In 'Redhaven', H6 increased injury compared with all other treatments at −23.5 and −29.3 °C ($P \leq 0.049$), and at −27.2 °C, F3-L3 had less injury than other treatments ($P \leq 0.022$). Differences among other treatments were not significant. In 'Gloria', the TI was lowered by F3-L3 compared with L6 and F6, but not H6, and was similar among treatments in 'Redhaven' (Table 5). The TI was similar in both cultivars.

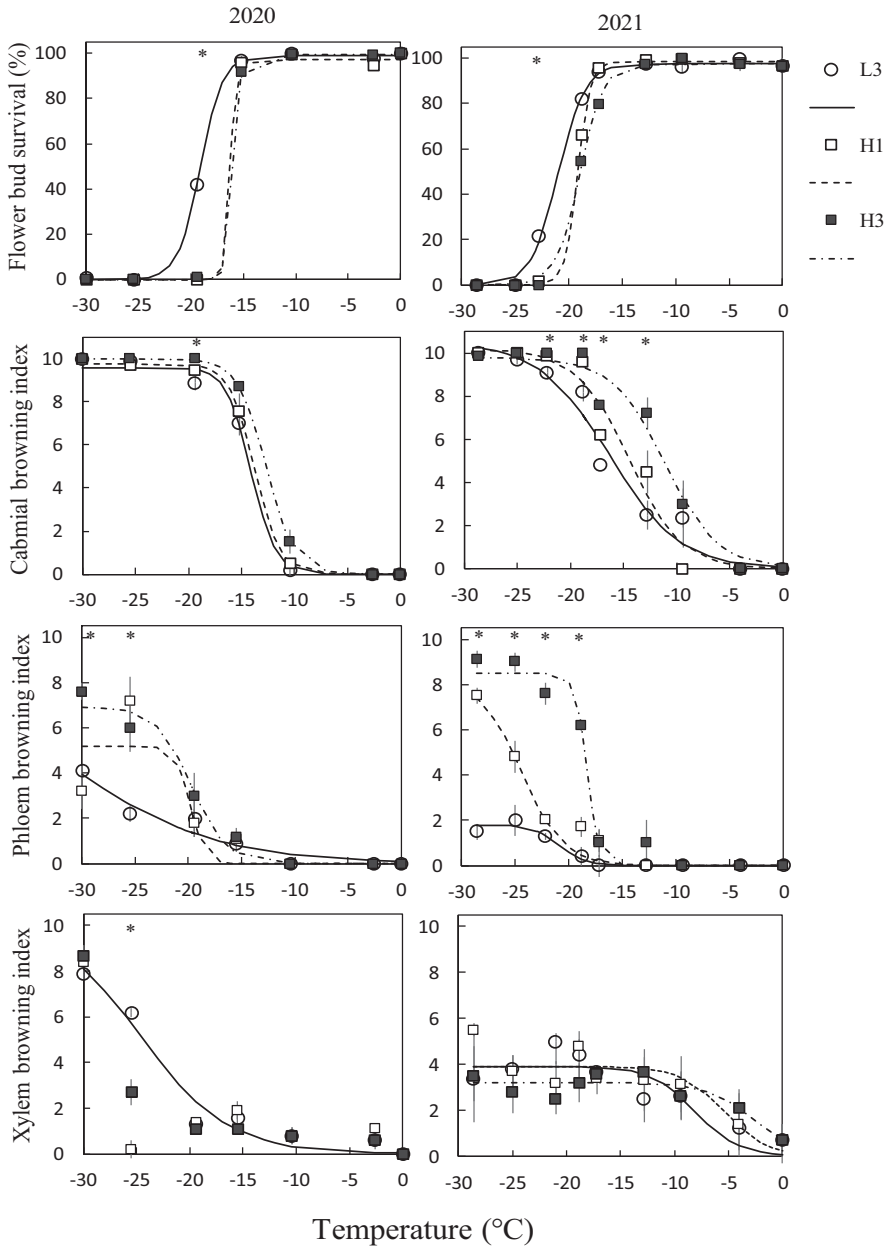

Fig. 3. 'Redhaven' peach flower bud survival and oxidative browning index in cambium, phloem, and xylem of 1-year-old shoots following 3 d at 3.5 °C (L3), 1 d at 20.0 °C (H1), or 3 d at 20.0 °C (H3) in Expt. 2, mid-Nov 2020 and 2021. Shoots and buds were subjected to controlled freezing from 0 to −30.0 and −28.6 °C, respectively. Bars indicate standard errors (n = 4). * indicates a significant treatment effect within a temperature.

Phloem injury was minor at 19.9 °C and above and was severe at −29.3 °C (Fig. 5). Injury varied among treatments at −27.2 °C in 'Gloria' and at −27.2 and −29.3 °C in 'Redhaven'. In both cultivars, H6 and L6 had greater injury at −27.2 °C than the two freezing treatments ($P \leq 0.037$). Greater phloem injury also occurred in H6 than L6 in 'Gloria' ($P = 0.001$), whereas the two treatments had similar levels of injury in 'Redhaven'. No difference occurred between the two freezing treatments at −27.2 °C. At −29.3 °C, injury in 'Redhaven' was greater in H6 than other treatments ($P \leq 0.026$), but similar in all treatments in 'Gloria'. The TI for phloem injury did not differ among treatments or cultivars (Table 5).

Xylem injury was minor at −17.6 °C and above in 'Gloria' at −19.9 °C and above in 'Redhaven' (Fig. 5) and was similar among treatments at temperatures above −19.9 and below −23.5 °C. In 'Gloria', xylem hardiness at −19.9 °C was decreased in H6 and L6 compared with F6 ($P \leq 0.015$), but injury for F3-L3 was similar to other treatments. In 'Redhaven', injury at −23.5 °C was increased by H6 compared with other treatments ($P = 0.001$) and was greater in L6 compared with both freezing treatments ($P = 0.001$). The TI was lowered by 2.3 to 6.6 °C after F6 compared with other treatments in both cultivars (Table 5). In 'Gloria', the TI occurred at a higher temperature following L6 than F3-L3, but not H6. In 'Redhaven', the TI occurred at a higher temperature following H6 compared with L6 and F3-L3. Xylem hardiness was 2.6 to 5.0 °C greater in 'Redhaven' than in 'Gloria' with all treatments.

Discussion

Flower bud and shoot tissue hardiness. Flower bud hardiness was relatively unchanged among the experiments and ranged from −19.0 to −21.1 °C after low temperature acclimation, which served as a control in this study. Cambial hardiness with low, nonfreezing temperature ranged from −14.6 °C in mid-October to −17.9 °C in late November, indicating a small increase with seasonal progression. Generally, cambial tissues were less hardy than flower buds and phloem tissues, and at times, less hardy than xylem. The level of hardiness in phloem after low temperature acclimation was highly variable among the experiments and ranged from −18.5 °C to below −30.0 °C. In the fall, the phloem of apple and peach shoots is more tender than xylem and cambium, but phloem develops greater hardiness than these tissues by midwinter (Nesmith and Dowler 1976; Quamme et al. 2010; Yu et al. 2017). In this study, phloem was as hardy as, or hardier than, cambium after low temperature acclimation. Hardiness of the xylem was also variable among experiments and ranged from a high of −16.7 °C in October to below −30.0 °C when only partial injury occurred at the coldest test temperature in Nov 2021.

High temperature acclimation. High temperature reduced hardiness compared with low temperature, although the degree of loss varied by shoot tissue and with seasonal progression. The reduction in hardiness was greater in phloem than in cambium and flower buds in mid-October through mid-November. However, in late November, flower buds did not respond to high temperature, and cambium and phloem responded to a lesser degree than at earlier sampling times. After high temperature, xylem lost hardiness to a small degree in mid-October and late November, and in mid-November in only one experiment. This inconsistency may have been because of the differing temperatures before sampling, but cultivars in these experiments also differed.

In this study, loss of hardiness in flower bud, cambium, and phloem after high temperature acclimation appeared to be greater with higher maximum temperatures before sampling and with fewer days with freezing temperatures, which occurred in Oct 2020 and Nov 2021. With 'Redhaven', the largest decrease in flower bud hardiness occurred in Nov 2020, when 3 d of high temperature reduced hardiness by 3.1 °C. Peach flower buds acclimate to −10 to −16 °C in fall despite exposure to daytime high temperatures above 15 °C (Buchanan et al. 1976; Szalay et al. 2010). This is comparable to the warmest level of flower bud hardiness in our study, −16.0 °C. A loss of 4 to 10 °C occurred in phloem following high temperature acclimation. The differing mechanisms of hardiness between xylem and flower buds compared

Table 3. The lethal temperature (LT_{50}, °C[i]) of flower buds and temperature of injury (TI, °C) in 1-year-old shoot tissues of 'Redhaven' peach after a 3-d exposure to 3.5 °C (L3) or 20.0 °C (H3), or a 1-d exposure to 20.0 °C (H1) in Expt. 2, mid-November (6 Nov 2020 and 8 Nov 2021).

	Flower buds		Cambium		Phloem	
Treatment	2020	2021	2020	2021	2020	2021
L3	−19.1 a[ii]	−21.1 a*[iii]	−14.2 a	−16.3 a*	<−30.0	<−28.6
H1	−16.2	−19.2 b	−13.7 a	−14.5 a	−19.5 a	−24.5 a*
H3	−16.0 b	−19.0 b*	−12.7 b	−11.1 b*	−19.8 a	−18.3 b
			95% confidence interval (−°C)			
L3	18.9–19.3	20.6–21.5	13.5–14.7	14.8–17.7	ne	ne
H1	ne[iv]	18.6–19.9	13.2–14.3	13.6–15.4	19.0–20.7	23.5–25.5
H3	15.9–16.2	18.5–19.5	12.4–13.1	10.2–12.0	18.3–21.2	17.9–18.6

[i] Based on the inflection point estimated from nonlinear regression.
[ii] Means separation within a column based on 95% confidence interval of the regression estimate.
[iii] The asterisk indicates a significant difference between years.
[iv] ne = not statistically estimable.

with phloem is a likely contributing factor for the larger and more consistent phloem response to high temperature. Xylem parenchyma and flower buds develop the ability to supercool due to anatomical properties and an increase in compatible solutes, whereas phloem must develop the capacity to rapidly lose cellular water to the apoplast and tolerate subsequent cellular dehydration, the process known as extraorgan freezing (Vitasse et al. 2014; Wisniewski et al. 2018). The cellular mechanism of hardiness development in cambium is not as well studied as in xylem and phloem. If the cambium supercools similarly to xylem parenchyma, differential thermal analysis with a high-resolution infrared camera could be used to detect a low temperature exotherm (Livingston et al. 2018) associated with injury in the cambium if it can be detected. However, because cambium is such a thin layer relative to xylem, the xylem would likely mask this exotherm, particularly when they occur near the same temperature.

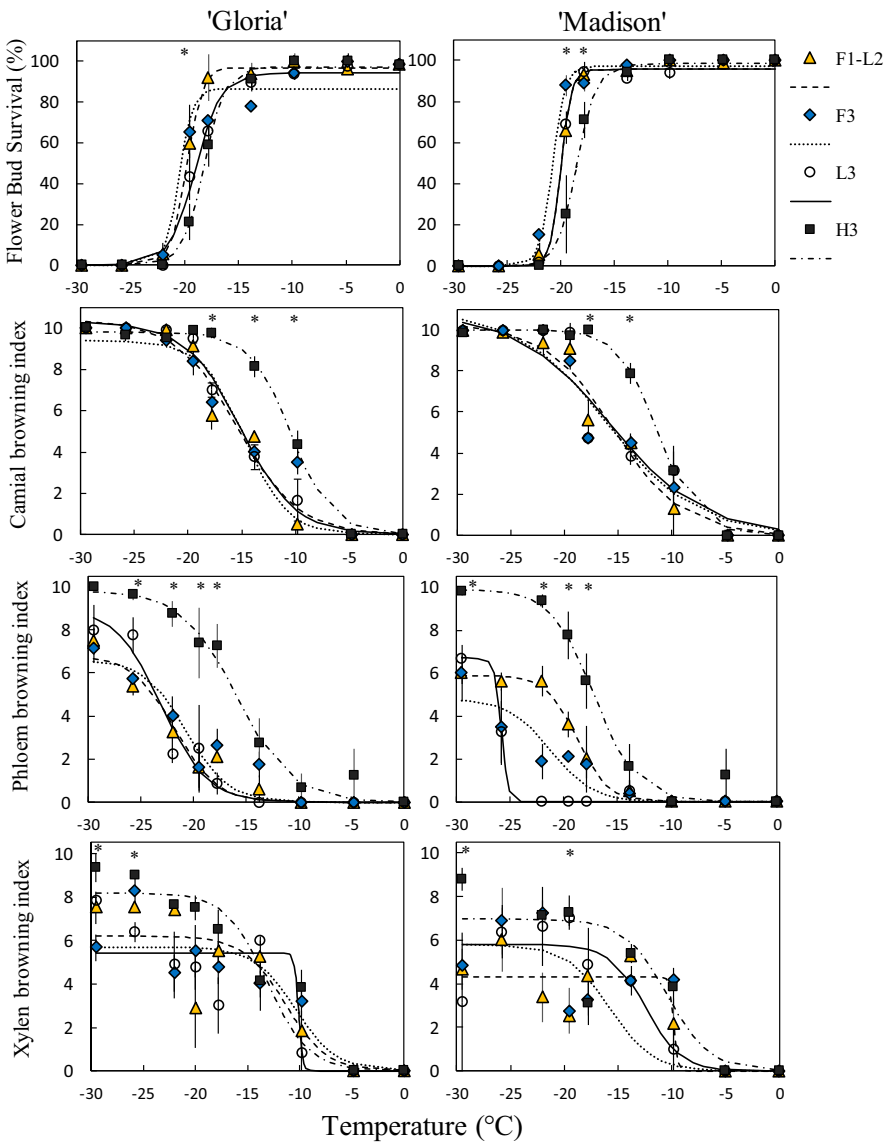

Fig. 4. 'Gloria' and 'Madison' peach flower bud survival and oxidative browning index in cambium, phloem, and xylem of 1-year-old shoots following 1 d at −3.0 °C followed by 2 d at 3.5 °C (F1-L2), 3 d at −3.0 °C (F3), 3 d at 3.5 °C (L3), or 3 d at 20.0 °C (H3) in Expt. 3, early Nov 2021. Shoots and buds were subjected to controlled freezing from 0 to −29.5 °C. Bars indicate standard errors (n = 4). * indicates a significant treatment effect within a temperature.

Lower temperatures predominated before the Nov 2020 and 2022 experiments, and hardiness responses to high temperature acclimation were smaller at these sampling times. In woody ornamentals and tree fruit species, flower buds are relatively resistant to high temperature deacclimation during endodormancy (Litzow and Pellet 1980), or they require a duration longer than 7 d for measurable response (Andrews and Proebsting 1987; Edgerton 1954). Similarly, during the first part of endodormancy, a duration of 2 weeks is needed to change hardiness in grape buds (Rubio et al. 2016), or longer in shoot tissues of red-osier dogwood (Litzow and Pellet 1980). Therefore, the 6-d duration of high acclimation temperatures in Nov 2022 may not have been long enough to elicit a measurable loss of hardiness in flower buds. However, periods of high temperature longer than 6 d do not frequently occur in Maine in late fall or early winter. The lack of response in flower buds in the late November experiment may have also been due to controlled freezing occurring over a longer duration (24 h) than in earlier experiments (1 h), but a small response to high temperature persisted in other shoot tissues. In the closely related species of plum and cherry, the onset of bud dormancy is induced primarily by cold temperature but is also favored by short days (Heide 2008). The loss of hardiness after the short durations of 1 and 3 d of high temperature in October or mid-November indicates that dormancy may not have fully developed by this time. Additional research is needed to confirm that the lack of bud response to high temperature is an indication of dormancy onset.

Freezing temperature acclimation. Freezing temperatures had variable effects on hardiness depending on shoot tissue and sampling date. In October, bud hardiness was unaffected by 1 d of freezing, but in early November this was sufficient to increase hardiness in 'Gloria'. In 'Madison', 3 d of freezing temperature were needed to increase hardiness compared with low nonfreezing temperature. Xylem, cambium, and phloem hardiness was unaffected by freezing treatment in October and early November, but durations longer than 3 d were not tested in those experiments, and increasing freezing temperature duration has been shown to increase the level of hardiness in other species (Andrews and Proebsting 1987; Hong and Sucoff 1982; Wu et al. 2019). Freezing in late November increased hardiness of flower buds and xylem, which have similar hardiness

Table 4. The lethal temperature (LT_{50}, °C[i]) of flower buds and temperature of injury (TI, °C) in 1-year-old shoot tissues of 'Gloria' and 'Madison' peach after a 3-d exposure to −3.0 °C (F3), 3.5 °C (L3), or 20.0 °C (H3), or a 1-d exposure to −3.0 °C followed by a 2-d exposure to 3.5 °C (F1-L2) in Expt. 3, early November (1 Nov 2021).

	Flower buds		Cambium		Phloem	
Treatment	Gloria	Madison	Gloria	Madison	Gloria	Madison
L3	−19.0 b[ii]	−20.0 b*[iii]	−15.1 a	−15.7 a	−23.1 a	−25.8 a
F1-L2	−19.8 a	−20.0 b	−15.4 a	−15.4 a	−21.8 a	−18.7 a*
F3	−20.5 a	−20.8 a	−14.7 a	−16.0 a	−20.7 a	−21.2 a
H3	−18.2 c	−18.5 c	−10.4 b	−11.3 b	−15.9 b	−17.1 a
			95% confidence interval (−°C)			
L3	18.6–19.5	19.6–20.3	14.4–15.9	13.8–17.6	20.9–25.2	15.9–30.0
F1-L2	19.5–20.2	19.8–20.3	14.4–16.4	13.9–16.8	20.2–23.4	17.5–20.0
F3	19.8–21.2	20.5–21.1	13.2–16.1	14.4–17.6	18.8–22.5	17.0–25.5
H3	17.8–18.6	18.0–19.0	9.8–11.0	10.8–11.8	14.3–17.6	16.1–18.2

[i] Based on the inflection point estimated from nonlinear regression.
[ii] Means separation within a column based on 95% confidence interval of the regression estimate.
[iii] The asterisk indicates a significant difference between cultivars.

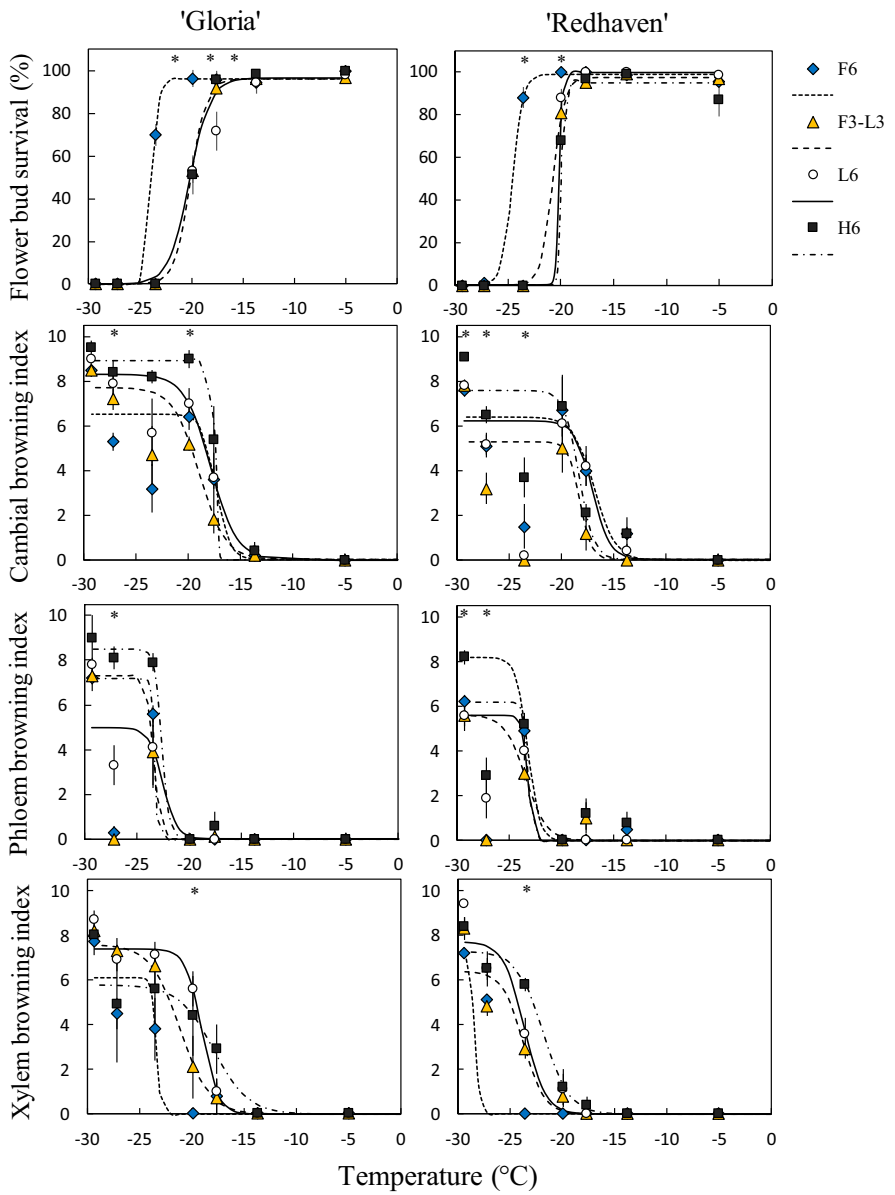

Fig. 5. 'Redhaven' and 'Gloria' peach flower bud survival and oxidative browning index in cambium, phloem and xylem of 1-year-old shoots following 3 d at −5.0 °C followed by 3 d at 3.5 °C (F3-L3), 6 d at −5.0 °C (F6), 6 d at 3.5 °C (L6), or 6 d at 18.0 °C (H6) in Expt. 4, late Nov 2022. Shoots and buds were subjected to controlled freezing from −4.8 to −29.3 °C. Bars indicate standard errors (n = 5). * indicates a significant treatment effect within a temperature.

mechanisms. Freezing did not affect cambium hardiness and minimally increased hardiness in phloem. Maximum hardiness may have developed in these tissues by late November.

Freeze-thaw cycles, more typical of ambient temperature patterns in the fall, resulted in similar hardiness as constant low acclimation temperature, but the impact of constant freezing was not large in October or mid-November. Moreover, the effect of freezing-thawing was not consistent in all cultivars or in both experiments. The freeze-thaw cycle in late November increased xylem hardiness in one of two cultivars and to a lesser extent than the constant freezing, but this may have been because of its shorter duration. In our study, the duration of thawing was longer than a diurnal cycle, but comparable to freeze-thaw cycles of a longer duration that are typical in late fall.

The level of hardiness at the time of sampling was not directly measured in this study, so it is not known if acclimation treatments altered ambient hardiness levels. Differences in hardiness were based on a comparison with low nonfreezing temperature, but this treatment itself may have altered hardiness during the experiment compared with hardiness at the time of shoot collection, 3 or 6 d prior depending on the experiment (Hong and Sucoff 1982). Freezing temperatures had occurred in the orchard by the time of sampling in all experiments. The prevailing cold weather in Maine's short growing season complicates studying the impact of low or freezing temperatures on acclimation. Future research might consider measuring hardiness changes from the time of sampling through a period of controlled temperature acclimation.

One-year-old shoots were used in this study, but they may not represent hardiness in the older portions of trees. Older branch sections (up to 4 years) of peach have greater xylem and cambial hardiness than 1-year-old sections, but phloem hardiness is unaffected by branch age in December (Moran and Ginakes 2022). Additional research is needed to assess the acclimation responses of variously aged branches.

This study explored the vulnerability of peach flower buds and 1-year-old shoot tissues to cold temperature injury following simulated warm spells in fall. Cold tolerance of these tissues has varied implications for peach production. For instance, flower bud loss has an impact on fruit yield, but minimal impact on overall tree health. Damage to the cambium causes rapid shoot or tree decline, and damage to the xylem leads to long-term decline and possible fungal infection (Chang et al. 1989; Deyton et al. 1996). Less is known about the impact of phloem injury on tree health and performance. In addition, xylem and phloem are regrown annually, provided cambial tissues are adequately healthy to function in new tissue production, but excessive damage of these tissues impairs tree vigor, favors disease, and reduces orchard lifespan. In addition, the ability to rapidly re-acclimate would determine the potential for injury after warm spells and should be considered

Table 5. The lethal temperature (LT_{50}, °C[i]) of flower buds and temperature of injury (TI, °C) in 1-year-old shoot tissues of 'Redhaven' and 'Gloria' peach after 6-d exposure to −5.0 °C (F6), 3.5 °C (L6), or 18.0 °C (H6) or a 3-d exposure to −5.0 °C following a 3-d exposure to 3.5 °C (F3-L3) in Expt. 4, late November (28 Nov 2022).

	Flower buds		Cambium		Phloem		Xylem	
Treatment	Gloria	Redhaven	Gloria	Redhaven	Gloria	Redhaven	Gloria	Redhaven
L6	−20.2 b[ii]	−20.2 b	−17.9 b	−17.0 a	−22.5	−23.2	−19.0 c	−23.6 b*
F3-L3	−20.1 b	−20.8 b	−19.0 a	−18.2 a	−23.4	−23.4 a	−21.1 b	−23.7 b*
F6	−23.8 a	−24.6 a*[iii]	−17.4 b	−16.8 a	−23.3	−23.2	−23.4 a	−28.4 a*
H6	−19.9 b	−20.0 b	−17.5 ab	−18.3 a	−22.6	−23.2 a	−17.9 bc	−21.8 c*
			95% confidence interval (−°C)					
L6	19.4–20.8	20.1–20.2	17.3–18.4	15.7–18.3	18–27	ne	18.5–19.6	22.9–24.3
F3-L3	19.9–20.2	20.0–21.5	18.5–19.6	16.7–19.8	ne[iv]	22.5–24.2	19.8–22.4	22.8–24.5
F6	23.7–23.9	24.3–24.8	16.6–18.3	15.2–18.4	ne	ne	23.1–23.7	28.3–28.5
H6	19.8–20.1	19.9–20.1	5–30	17.5–19.1	ne	22.8–23.6	14.8–20.9	21.0–22.6

[i] Based on the inflection point estimated from nonlinear regression.
[ii] Means separation within a column based on 95% confidence interval of the regression estimate. ne is not estimable.
[iii] The asterisk indicates a significant difference between cultivars.
[iv] ne = not statistically estimable.

in future work. In this study, shoot cambium was the least hardy tissue and highly susceptible to high temperature–induced loss of hardiness, making it especially vulnerable to potential fall freezes. Although it had greater hardiness than cambium, phloem was also highly susceptible to deacclimation and would be vulnerable to sudden, severe freezes. Warmer temperatures in fall will likely expose this vulnerability, threatening peach production in the temperate regions despite general warming and northward shifts in growing zones.

References Cited

Andrews PK, Proebsting EL. 1987. Effects of temperature on the deep supercooling characteristics of dormant and deacclimating sweet cherry flower buds. J Am Soc Hortic Sci. 112:334–340. https://doi.org/10.21273/JASHS.112.2.334.

Arora R, Wisniewski ME, Scorza R. 1992. Cold acclimation in genetically related (sibling) deciduous and evergreen peach (*Prunus persica* [L.] Batsch). Plant Physiol. 99:1562–1568.

Buchanan WW, Biggs RH, Bartholic JF. 1976. Cold acclimation of Florida peaches and nectarine cultivars. HortScience. 11:398–400.

Caprio JM, Quamme HA. 2006. Influence of weather on apricot, peach and sweet cherry production in the Okanagan Valley of British Columbia. Can J Plant Sci. 86:259–267. https://doi.org/10.4141/P98-028.

Chang LS, Iezzoni A, Adams G, Howell GS. 1989. *Leucostoma persoonii* tolerance and cold hardiness among diverse peach genotypes. J Am Soc Hortic Sci. 114:482–485. https://doi.org/10.21273/JASHS.114.3.482.

Chaplin CE. 1948. Some artificial freezing tests of peach fruit buds. Proc Am Soc Hortic Sci. 52:121–129.

Deyton D, Sams CE, Cummins JC, Lockwood DW. 1996. Cambial browning of cold injured peach nursery trees. Fruit Var J. 50:226–229.

Durner EF. 1995. Dormant pruning and fall ethephon application influence peach pistil hardiness. J Am Soc Hortic Sci. 120:823–829. https://doi.org/10.21273/JASHS.120.5.823.

Edgerton LJ. 1954. Fluctuation in the cold hardiness of peach flower buds during rest period and dormancy. Proc Am Soc Hortic Sci. 64:175–180.

Heide O. 2008. Interaction of photoperiod and temperature in the control of growth and dormancy of *Prunus* species. Scientia Hortic. 115:309–314. https://doi.org/10.1016/j.scienta.2007.10.005.

Hong S, Sucoff E. 1982. Rapid increase in deep supercooling of xylem parenchyma. Plant Physiol. 69:697–700.

Howell GS, Weiser CJ. 1970. The environmental control of cold acclimation in apple. Plant Physiol. 45:390–394.

Layne REC, Ward JM. 1978. Rootstock and seasonal influences on carbohydrate levels and cold hardiness of 'Redhaven' peach. J Am Soc Hortic Sci. 103:408–413. https://doi.org/10.21273/JASHS.103.3.408.

Layne R, Jackson HO, Stroud FD. 1977. Influence of peach seedling rootstocks on defoliation and cold hardiness of peach cultivars. J Am Soc Hortic Sci. 102:89–92. https://doi.org/10.21273/JASHS.102.1.89.

Litzow M, Pellet H. 1980. Relationship of rest to dehardening in red-osier dogwood. HortScience. 15:92–93.

Liu J, Lindstrom OM, Chavez DJ. 2019. Differential thermal analysis of 'Elberta' and 'Flavorich' peach flower buds to predict cold hardiness in Georgia. HortScience. 54:676–683. https://doi.org/10.21273/HORTSCI13518-18.

Livingston DP, Tuong TD, Murphy JP, Gusta LV, Willick I, Wisniewski ME. 2018. High-definition infrared thermography of ice nucleation and propagation in wheat under natural frost conditions and controlled freezing. Planta. 247:791–806. https://doi.org/10.1007/s00425-017-2823-4.

Minas IS, Sterle D. 2020. Differential thermal analysis sheds light on the effect of environmental and cultivar in peach floral bud cold hardiness. Acta Hortic. 1281:385–391. https://www.actahort.org/books/1281/1281_51.htm.

Minas IS, Sterle D. 2022. Exploration of environmental variables for peach floral bud cold hardiness prediction in western Colorado. Acta Hort. 1352:285–290. https://www.actahort.org/books/1352/1352_38.htm.

Moran R, Ginakes P. 2022. Cold hardiness in 1- through 4-year-old shoots of peach, cherry and apple (abstr). HortScience. 57:9S. https://ashs.confex.com/ashs/2022/meetingapp.cgi/Paper/37715.

Moran RE, Peterson BJ, Fazio G, Cline JA. 2021. Low temperature tolerance of apple shoots following exposure to warm temperatures in late winter. HortScience. 56(6):642–649. https://doi.org/10.21273/HORTSCI15665-20.

Moran R. 2021. Loss of cold temperature tolerance and reacclimation following warm temperatures in peach. Acta Hortic. 1346:15. https://doi.org/10.17660/ActaHortic.2022.1346.15.

Nesmith WC, Dowler WM. 1976. Cultural practices affect cold hardiness and peach tree short life. J Am Soc Hortic Sci. 101:116–119. https://doi.org/10.21273/JASHS.101.2.116.

Ouyang L, Leus L, De Keyser E, Van Labeke M. 2022. Temperature is an important driver for cold acclimation in garden roses. Scientia Hortic. 296:110905. https://doi.org/10.1016/j.scienta.2022.110905.

Quamme HA, Cannon AJ, Neilsen D, Caprio JM, Taylor WG. 2010. The potential impact of climate change on the occurrence of winter freeze events in six fruit crops grown in the Okanagan Valley. Can J Plant Sci. 90:85–93.

Repo T, Lappi J. 1989. Estimation of standard error of impedance-estimated frost resistance. Scand J For Res. 4:67–74. https://doi.org/10.1080/02827588909382547.

Rochette P, Belanger G, Castonguay Y, Bootsma A, Mongrain D. 2004. Climate change and winter damage to fruit trees in eastern Canada. Can J Plant Sci. 84:1113–1125.

Rubio S, Dantas D, Bressan-Smith R, Pérez FJ. 2016. Relationship between endodormancy and cold hardiness in grapevine buds. J Plant Growth Regul. 35:266–275. https://link.springer.com/article/10.1007/s00344-015-9531-8.

Shin H, Oh Y, Kim D. 2015. Differences in cold hardiness, carbohydrates, dehydrins and related gene expressions under an experimental deacclimation and reacclimation in *Prunus persica.* Physiol Plant. 154:485–499.

Smith MW, Carroll BL, Taylor GG. 1994. Cold injury of peach and nectarine cultivars after a fall freeze. HortScience. 29:821. https://doi.org/10.21273/HORTSCI.29.7.821.

Szalay L, Timon B, Németh S, Papp J, Tóth M. 2010. Hardening and dehardening of peach flower buds. HortScience. 45:761–765.

Szymajda M, Zurawicz E. 2016. Tolerance of peach flower buds to low sub-zero temperatures in winter. Hortic Sci (Prague). 42:126–133. https://doi.org/10.17221/206/2015-HORTSCI.

Vitasse Y, Lenz A, Körner C. 2014. The interaction between freezing tolerance and phenology in temperate deciduous trees. Front Plant Sci. 5:541. https://doi.org/10.3389/fpls.2014.00541.

Wisniewski ME, Nassuth A, Arora R. 2018. Cold hardiness in trees: A mini-review. Front Plant Sci. 9:1394. https://doi.org/10.3389/fpls.2018.01394.

Wu D, Kukkonen S, Luoranen J, Pulkkinen P, Heinonen J, Pappinen A, Repo T. 2019. Influence of late autumn preconditioning temperature on frost hardiness of apple, blueberry and blackcurrant saplings. Scientia Hortic. 258:108755. https://doi.org/10.1016/j.scienta.2019.108755.

Young RS. 1988. West Virgina peach and nectarine fruit and vegetative bud injury and crop rating resulting from −28 °C January temperature, p 362–365. In: Childers NF, Sherman S (eds). The peach. Somerset Press, Somerville, NJ, USA.

Yu DJ, Hwang JY, Chung SW, Oh HD, Yun SK, Lee HJ. 2017. Changes in cold hardiness and carbohydrate content in peach (*Prunus persica*) trunk bark and wood tissues during cold acclimation and deacclimation. Scientia Hortic. 219:45–52. https://doi.org/10.1016/j.scienta.2017.02.038.