Dormancy Effects on Supercooling in Deacclimated 'Meteor' Tart Cherry Flower Buds

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Abstract. Exposure of 'Meteor' tart cherry (*Prunus cerasus* L.) flower buds to deacclimating conditions resulted in an increase in the temperature of the low-temperature exotherms (LTEs) produced by the flower primordia during controlled freezing. Primordium supercooling temperature was related to chill unit accumulation, an indicator of depth of flower bud endodormancy. LTEs ceased to be detected after deacclimation earlier in 1986-87, a season of more rapid chill unit accumulation, than in 1987-88. Before deacclimation, the range of primordium LTE temperatures within a flower bud was normally $\leq 1^\circ\text{C}$, but in deacclimated buds considerable variability in LTE temperatures was observed. However, primordia within a flower bud lost the ability to supercool simultaneously. This change was generally concurrent with the appearance of mature xylem vessel elements (XVE) in the upper bud axis and in the flower primordium but did not entirely depend on vessel element maturation.

In western Montana, as in many other tree fruit growing regions, mild winter and spring temperatures often lead to flower bud deacclimation, resulting in increased susceptibility to freeze damage. Low-temperature survival of the flower primordia of many stone fruits, including 'Meteor' tart cherry, depends on freeze avoidance by supercooling (Quamme, 1974). At natural cooling rates, water migrates from the flower primordia to freeze in specific locations in the bud axis and scales, while the primordia remain supercooled until their critical temperature is reached (Andrews and Proebsting, 1987; Ashworth et al., 1989; Quamme, 1978; Quamme and Gusta, 1987).

The association between flower bud dormancy and cold hardiness was recognized by Proebsting (1963), who developed the concept of the minimum hardiness level (MHL) for peach flower buds. Peach flower bud hardiness fluctuated during endodormancy, defined by Lang et al. (1987) as an internally regulated growth control. Primordia did not dehardening above the MHL until termination of endodormancy, after which the MHL increased slowly with bud development. The MHL (Proebsting et al., 1989) and chill unit accumulation, an indicator of bud endodormancy, were used in sweet cherry bud hardiness modeling and forecasting (Andrews et al., 1987; Proebsting et al., 1989). Primordium supercooling temperature was also influenced by air temperature (Andrews and Proebsting, 1987; Andrews et al., 1987; Proebsting, 1963; Proebsting et al., 1989; Quamme, 1983), the presence of ice nucleators (Andrews et al., 1983b), and tissue hydration (Andrews and Proebsting, 1987; Quamme, 1983).

Supercooling in *Prunus* flower primordia can be studied using differential thermal analysis (DTA) (Quamme, 1974). Freezing of overwintering stone fruit flower buds is characterized by the release of heat of fusion in two or more exothermic events. Water freezing nonlethally within the bud axis and scales at -2 to -6°C produces a high-temperature exotherm. At the primordium killing temperature, the freezing of deep-supercooled water gives rise to a LTE for each individual primordium.

Deacclimation plays an important role in *Prunus* flower bud injury in the field (Andrews et al., 1983b; Ashworth, 1984). The current study was designed to characterize the influence of

controlled deacclimation on the extent of 'Meteor' tart cherry primordium supercooling during winter and spring and to clarify the relationship between bud endodormancy and deacclimation. The consequence of anatomical development on supercooling in individual flower buds was also examined.

Methods and Materials

'Meteor' tart cherries on Mahaleb rootstock were grown in the research orchards of the Western Agricultural Research Center, Montana Agricultural Experiment Station, Corvallis. 'Meteor' bears unmixed flower buds on spurs, with three to four primordia per bud. Plant samples for DTA and dormancy evaluation were collected from the field at 2-week intervals from Dec. to Mar. 1986-87 and 1987-88.

Differential thermal analysis and hardiness determination. 'Meteor' floral branches were placed in a growth chamber at $21/15^\circ\text{C}$ (day/night), 16-hr photoperiod, and 80% to 90% RH for deacclimation. In 1986-87, DTA was performed immediately and after 2, 4, and 10 days of deacclimation. In 1987-88, 6 and 8 days of deacclimation were added.

Plant material was prepared for DTA at ambient temperature. DTA used a 16-channel computerized data acquisition system with two Data Translation (Marlboro, Mass.) DT2805/5716 16-bit analog to digital (A/D) output conversion boards (Callan et al., 1986). Thermoelectric modules (9×23 mm, 132 junctions; Melcor Corp., Trenton, N.J.) were employed as heat sensors (Andrews et al., 1983a; Callan et al., 1986). Seven spur flower buds were affixed to each of seven thermoelectric modules with thermal conducting paste (Omegatherm 101, Omega, Inc., Stamford, Conn.) for a total of 49 buds per sampling date.

The cut surface of each bud was inoculated with a drop of ice water in 1986-87 and with an aqueous suspension of an ice nucleation-active strain of *Pseudomonas syringae* (Cit7, courtesy S. Lindow) in 1987-88. Prior testing had shown that the LTE temperature was not affected by nucleation method. Thermoelectric modules were suspended in a foam box within an ultralow freezer (Scientemp 5112, Adrian, Mich.) controlled by a cam programmer (Partlow, New Hartford, N.Y.). The temperature was lowered 2°C/hr in 1986-87 and 1°C/hr to -10°C followed by 2.5°C/hr in 1987-88. Temperature was monitored

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Abbreviations: BBI, budbreak index; DTA, differential thermal analysis; LTE, low-temperature exotherm; MHL, minimum hardiness level; XVE, xylem vessel element.

with a 36-gauge (0.127-mm diam.) copper-constantan thermocouple connected to the reference channel of the A/D board.

Supplemental DTA data were obtained on each date with a two-pen recorder (Linear 885, Hackensack, N.J.). Recorder data were used when tissue dehydration due to prolonged subfreezing temperatures reduced LTE peak height to below consistent detection limits of the computer system (0.05 mV). This occurred on the day of sampling in mid-January and early February of both years, but, as primordia rehydrated in the growth chamber, they produced detectable LTEs on freezing.

To monitor the behavior of individual deacclimating flower buds and to determine the primary source of LTE variability (among primordia within a bud vs. among buds), a single bud each was placed on eight thermoelectric modules and computerized DTA performed concurrently with the plant material described above. Of 184 buds thus examined during the season, 113 produced LTEs on freezing. To quantify variability among primordia within individual buds, the standard deviation (σ) of the LTEs of the primordia within each bud was calculated and the relationship of each σ to the mean LTE temperature for that flower bud determined by regression. The variability among flower buds was examined by calculating the σ of the mean bud LTE temperatures in each sample of eight buds and by determining the relationship of this σ to the overall mean LTE temperature for that sample. As some buds did not produce LTEs, n varied from two to eight.

The primordium LT_{50} (lethal temperature that kills 50% of flower primordia) was determined using flower buds prepared as for DTA. About 20 buds per test temperature were affixed with thermal paste to the bottom of an inverted plastic foam cup that was placed inside a wide-mouth vacuum bottle. Sample temperature was monitored with a 36-gauge copper-constantan thermocouple. Bottles were removed from the freezer at 4 to 5°C increments in the anticipated lethal temperature range. LT_{50} was calculated by regression.

Chill unit accumulation and floral budbreak index. Four 'Meteor' floral branches were placed at weekly intervals in the growth chamber described above. In 1986–87, endodormancy was considered complete when vegetative budbreak occurred within 2 weeks (Hatch and Walker, 1969). In 1987–88, a budbreak index (BBI) was used to characterize stage of flower bud development more precisely. After 2 weeks, all flower buds were classified as to numerical stage of development (1 = first swelling; 2 = side green; 3 = green tip; 4 = tight cluster; 5 = open cluster; 6 = first white) (Ballard et al., 1971). The BBI was then calculated by multiplying the mean bud developmental stage by 100. Chill units were calculated from daily minimum–maximum standard weather shelter temperatures using the Utah model (Richardson et al., 1974).

Anatomical development. Flower buds similar to those prepared for DTA in 1987–88 were fixed in formalin–acetic acid for microscopic examination. Three to 10 buds were observed for each DTA sampling date. XVE maturation in the bud axis and primordia, characterized by the development of annular thickenings, was observed in serial sections of paraffin-embedded material stained with periodic acid–Schiff's reagent and counterstained with aniline blue (Jensen, 1962).

To correlate the LTE capacity of individual buds with their anatomical development, individually frozen deacclimating buds, as described above, were fixed after DTA between mid-Jan. and the end of Feb. 1988. Only samples in which some buds did not produce detectable LTEs were selected for microscopy, for a total of 74 buds.

Results

Flower primordium supercooling. The pattern of 'Meteor' flower primordium supercooling temperature was similar in both years. Supercooling temperature on the day of field collection fluctuated with outdoor temperatures but remained below -20°C through most of the winter (Fig. 1). Primordium LTE temperature was consistently lowest on the day of collection and increased more rapidly with deacclimation as winter progressed. On most field collection dates, primordium LTE temperatures were distributed normally before deacclimation. After deacclimation, the LTE distribution widened and often appeared bimodal, with an increase in the number of LTEs in the -6 to -15°C range.

Detection of LTEs was also consistent between the two years. Nearly all primordia supercooled (3.0 to 3.5 LTEs per bud) on the day of sampling until field collection terminated in early Mar. 1987 and mid-Mar. 1988. The number of LTEs that were detected diminished with deacclimation as winter advanced (Fig. 1).

The LT_{50} approximated the modal LTE temperatures on the day of field collection during Winter and Spring 1988 (Table 1). After deacclimation, the modal LTE temperature remained near the LT_{50} but was often 1 to 2°C lower.

Individually frozen flower buds. Observations of supercooling in individual flower buds revealed that the primordia within a bud ceased to supercool simultaneously as deacclimation occurred (Table 2). The majority of deep supercooling buds produced either three or four LTEs at -6°C or lower, in agreement with the average number of primordia per bud. The infrequency of buds producing one or two LTEs indicates that primordia within a bud seldom supercool independently.

Primordia within acclimated buds exhibited considerable homogeneity of supercooling temperature, as evidenced by a low primordium LTE σ in buds supercooling to -25 to -30°C . (Fig. 2). The temperature range of LTEs within an acclimated bud was usually $\leq 1^{\circ}\text{C}$. Variability of LTEs of primordia within a bud increased as deacclimation progressed and mean bud supercooling temperatures rose above -15°C . Variability of mean LTE temperatures of buds frozen on a given date also increased with deacclimation (Fig. 3). LTE temperatures were generally more variable within a population of eight buds than among the primordia of an individual bud.

Chill unit accumulation and budbreak index. Chill unit accumulation generally occurs primarily in spring and fall in western Montana because of midwinter temperatures that are too low to be effective (Fig. 4). Chill unit accumulation was more rapid in 1986–87 than in 1987–88 due to earlier initiation of chilling, according to the Utah model. BBI in 1987–88 was lowest from the time measurement began in mid-December until early January. A rapid increase in BBI through late January was followed by a more gradual increase during February and March.

Both vegetative and flower bud endodormancy were terminated earlier in 1987 than in 1988 (Table 3). In 1987, vegetative bud endodormancy was complete by 23 Jan., since, in plant material collected on this date, budbreak occurred after 2 weeks in the growth chamber. This stage was reached 18 days later in 1988. The field collection date on which all flower buds reached a minimum of stage 3 (green tip) after 2 weeks in the growth chamber was 10 days later in 1988 than in 1987.

Supercooling behavior paralleled endodormancy during the two seasons (Table 3). The period during which primordia supercooled was longer and the LTE temperature was lower on a given date in 1988 than in 1987.

Fig. 1. Frequency distribution of 'Meteor' tart cherry flower bud exotherms after deacclimation in plant material collected (A) 1 Jan. 1988; (B) 18 Jan.; (C) 1 Feb.; (D) 14 Feb.; (E) 29 Feb.; (F) 14 Mar.

Anatomical development. Mature XVEs were not observed in the upper bud axis or primordia on the day of field collection at any time during 1987–88. Essentially all primordia in these samples produced LTEs. Mature XVEs were not observed after up to 10 days of deacclimation in Dec. 1987, but were first apparent after 8 days in buds collected in mid-Jan. 1988, after 6 days in early February, after 4 days in mid-February, and after 2 days in late February.

More extensive differentiation and maturation of XVEs was observed with successive field collection dates as deacclimation in the growth chamber progressed. In the majority of buds examined, those that exhibited LTEs lacked mature XVEs in the upper bud axis and primordium pedicels, while loss of a detectable LTE was accompanied by XVE maturation. However, some exceptions to this condition were observed. While 35, i.e., 80%, of the 44 flower buds in which LTEs were not observed had XVEs in the upper bud axis and primordium pedicels, no mature XVEs were seen in the remaining nine. In addition, 18

of 30 deacclimating buds with deep supercooling primordia contained at least some XVEs, although it could not be ascertained whether a functional vascular connection had been established between the stem and flower primordia.

Discussion

Deacclimation in 'Meteor' tart cherry flower buds resulted in a moderate increase in the LTE temperature when sampled in December and January but a progressively greater increase as the season advanced. Observations reported here are consistent with those of Andrews and Proebsting (1987), who found that sweet cherry flower primordia sampled in December retained the ability to supercool to below -15°C after 10 days of deacclimation at 20°C . As with 'Meteor' tart cherry, sweet cherry primordia lost supercooling ability in subsequent monthly samples. An increase in the LTE temperature and a widening of the LTE distribution comparable to that observed in this study have

Table 1. Modal LTE and lethal temperatures of 'Meteor' tart cherry flower buds subjected to deacclimating conditions.

Collection date (1988)	Days of deacclimation	Modal LTE temperature ^z (°C)	LT ₅₀ ^y (°C)
18 Jan.	0	-27	NA ^x
	2	-27	NA
	4	-22	NA
	6	-21	-18.2
	8	-17	-16.0
1 Feb.	10	-15	-15.7
	0	-28	-29.3
	2	-25	NA
	4	-21	-22.5
	6	-19	-17.9
14 Feb.	8	-16	-14.5
	10	-6	-8.0
	0	-27	-26.4
	2	-22	-18.2
	4	-18	-16.2
29 Feb.	6	-17	-15.0
	8	-9	-9.0
	0	-24	-24.0
	2	-18	-15.9
14 Mar.	4	-16	-14.0
	0	-24	-22.2
	2	-10	-7.7

^zModal LTE temperature = most frequently observed LTE temperature (integer).

^yLT₅₀ = lethal temperature killing 50% of flower primordia.

^xNA = not available.

Table 2. DTA of 184 single buds frozen in various stages of deacclimation during 1987-88, demonstrating the synchronous cessation of supercooling of primordia within a bud.

	No. LTEs/bud (lower than -6°C)				
	0	1	2	3	4
No. individual buds with no. of LTEs given	71	1	5	56	51

Fig. 2. Variability of LTE temperatures of flower primordia within individual 'Meteor' tart cherry buds. Each symbol represents the standard deviation of the primordium LTEs within a single bud, in regression against the mean LTE temperature for that bud. $Y = 4.17 + 0.28X + 0.005X^2$, $r^2 = 0.28$.

been noted after deacclimation in other *Prunus* crops (Andrews et al., 1983b; Quamme, 1974).

An association between 'Meteor' flower bud endodormancy and flower primordium supercooling after deacclimation was apparent in this study. Deacclimated primordia ceased to supercool earlier in 1986-87, a year of accelerated chill unit ac-

Fig. 3. Variability of LTE temperatures among 'Meteor' tart cherry flower buds evaluated on a given date. Each symbol represents the standard deviation of the mean bud LTE temperatures for a sample of eight buds in regression against the mean LTE temperature for all buds frozen on that date. Samples in which LTEs were detected from all buds are represented by (●), and samples in which some buds did not exhibit LTEs are represented by (▲). $Y = 4.35 + 0.12X$, $r^2 = 0.36$.

Fig. 4. Chill unit accumulation (Utah model) 1986-87 (-----) and 1987-88 (——), and BBI of 'Meteor' tart cherry, 1987-88 (●).

Table 3. A comparison of 'Meteor' tart cherry flower bud dormancy and supercooling during 1987 and 1988.

	Year	
First sampling date at which:	1987	1988
Vegetative budbreak occurred within 2 weeks	23 Jan.	10 Feb.
Flower buds reached stage 3 within 2 weeks	15 Jan.	25 Jan.
LT ₅₀ temperature increased to -15°C		
after 2 days deacclimation	2 Mar.	14 Mar.
after 4 days deacclimation	16 Feb.	29 Feb.
after 10 days deacclimation	2 Feb.	1 Feb.
LTEs (lower than -6°C) decreased to fewer		
than two per bud		
after 2 days deacclimation	16 Feb.	14 Mar.
after 4 days deacclimation	16 Feb.	14 Mar.
after 10 days deacclimation	14 Jan.	1 Feb.

cumulation, than in 1987-88. The rapid increase in the BBI during mid- to late Jan. 1988 approximately coincided with the termination of flower bud endodormancy, a decrease in detectable exotherms from deacclimated buds, and the appearance of XVEs in deacclimated primordia. After this time, the response of buds to favorable growth temperatures was more gradual.

Supercooling in stone fruit flower buds is complex. Changes in the LTE temperature have been associated with the level of

hydration (Andrews and Proebsting, 1987; Quamme, 1978, 1983) and the osmotic potential and sucrose content of the flower primordia (Quamme and Gusta, 1987). Maintenance of the primordium supercooling characteristic may be due to the presence of a dry region or barrier to water redistribution in the bud axis (Quamme, 1978) or by the lack of a functional vascular trace between the flower bud axis and the primordium (Ashworth, 1982, 1984). However, dormant primordia possessed a limited ability to supercool even in the presence of ice nucleation. Quamme (1978) and Quamme and Gusta (1987) detected an innate ability of dormant peach primordia to supercool to -10°C , and Rajashekar (1989) showed that a fraction of water in peach primordia could remain supercooled when nucleated. An absence of mature XVEs in the upper bud axis and primordia of 'Meteor' tart cherry was generally associated with deep supercooling, but the relationship was imperfect. Changes in the flower bud that prevent primordium supercooling do not appear to entirely depend on XVE maturation.

The association of 'Meteor' flower bud endodormancy and primordium supercooling demonstrated here should be considered in attempts to manipulate stone fruit flower bud cold hardiness.

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