Dead *Prunus* Flower-bud Primordia Retain Deep-supercooling Properties

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Abstract. Differential thermal analysis (DTA) was used to measure deep supercooling in flower buds of *Prunus dulcis* Mill., *P. armeniaca* L., *P. davidiana* (Carr.) Franch., *P. persica* (L.) Batsch, three sweet cherry (*P. avium* L.) selections, and 'Bing' cherries (*P. avium* L.) during Winter 1990-91 and 1991-92. Low temperatures in Dec. 1990 killed many flower buds. After the freeze, dead flower primordia continued to produce low-temperature exotherms (LTEs) at temperatures near those of living primordia for >2 weeks. In Feb. 1992, cherry buds that had been killed by cooling to -33C again produced LTEs when refrozen the next day. As buds swelled, the median LTE (LTE₅₀) of dead buds increased relative to that of living buds, and the number of dead buds that produced LTEs decreased. LTE artifacts from dead flower primordia must be recognized when DTA is used to estimate LTE₅₀ of field-collected samples.

Flower-bud primordia of *Prunus* species avoid freezing by supercooling water in the bud tissue (Burke and Stushnoff, 1979; Quamme, 1974). Differential thermal analysis (DTA) is a reliable and consistent method to determine the critical temperature for flower buds of cultivated *Prunus* species. The typical DTA profile of *Prunus* species consists of two exotherms: high-temperature exotherms and low-temperature exotherms (LTEs). LTEs have been associated with primordia death (Burke and Stushnoff, 1979; Graham and Mullin, 1976; Quamme, 1974). When dormant flower buds are killed, subsequent LTE₅₀ estimates should be based on surviving-bud hardiness. When flower buds are killed, their tissues turn brown within 24 h of thawing. By artificially freezing and visually detecting dead buds, it is easy to base LTE₅₀ estimates on surviving-bud behavior. It has been reported that dead azalea (*Rhododendron kosterianum* Schneid.) primordia supercooled (George et al., 1974), while injured blueberry (*Vaccinium corymbosum* L.) buds produced no LTEs several days before DTA (Biermann et al., 1979). After the advective freeze that swept through south-central Washington during the last 2 weeks of Dec. 1990, we observed that there were more buds producing LTEs than there were living buds in the sample. LTE₅₀ mea-

surements after the freeze were based on the distribution of LTEs from living and dead flower primordia. The objective of this study was to use DTA to measure the extent of supercooling of living and dead flower buds of several *Prunus* species.

Flower buds of the following *Prunus* species were collected at the Washington State Univ. Irrigated Agriculture Research and Extension Center, Pullman, Wash.: *P. davidiana* (ornamental), *P. dulcis* (almond), *P. persica* (peach), *P. armeniaca* (apricot), and 'Bing' cherry (*P. avium*) (Rehder, 1951). The samples were collected before, during, and after the Dec. 1990 freeze. Samples were placed in plastic bags and stored at 3C for 2 to 3 days before being prepared in a walk-in refrigerator for DTA. In addition, during February of the mild Winter 1991-92, samples were collected from 'Bing' cherries and the trees of 7144-3, 7147-13, and 7309-4 sweet cherry selections planted in 1983 at the research orchard.

Ten to 15 flower buds were detached from their twigs with 1 to 2 mm of bark tissue and placed on both sides of thermoelectric module plates (Andrews et al., 1983). Buds were held

in place with aluminum foil. Plates were frozen as reported by Kader and Proebsting (1992). *Prunus* flower buds were removed from the freezer the next day and placed in 100% relative humidity at room temperature before being tested for tissue browning.

Duplicate bud samples were collected from the field and observed under a microscope to estimate the extent of flower-bud injury. In 1991-92, there was no damage to cherry flower buds in the field. In Feb. 1992, DTA of living (from the field) and dead (from the samples that were exposed to -33C in the freezer) primordia were compared. Dead samples were removed from the freezer and placed on a new (dry) plate, and DTA was repeated. DTAs were run on both samples as before.

Data are expressed as LTE₅₀, the temperature at which 10% (LTE₁₀) and 90% (LTE₉₀) of flower primordia were injured, and the percentage of primordia that produced LTEs.

During the Dec. 1990 freeze, the temperature dropped to -21.7C and damaged *P. dulcis*, *P. davidiana*, *P. avium*, *P. persica*, and *P. armeniaca* flower buds. Flower buds of some species were injured before 20 Dec., in the early days of the cold wave. During late December, when 25% to 96% of flower buds was injured, DTA recorded almost the same percentage of LTEs at temperatures similar to those of noninjured flower buds measured before the freeze. Whether buds were living or dead, they supercooled. Ninety-six percent of *P. dulcis* flower buds was injured on 26 Dec., yet DTA recorded LTEs similar to those of noninjured flower buds (Table 1). *Prunus persica* and *P. armeniaca* flower buds supercooled to lower temperatures on 20 Dec. and 2 Jan., respectively, than noninjured buds in early December. Injured buds also produced only slightly fewer LTEs than noninjured buds. On 2 Jan., *P. avium* flower buds (39% injured) also produced a similar number of LTEs at similar temperatures as noninjured buds.

Living and dead flower buds of the three cherry selections and 'Bing' cherries supercooled during Feb. 1992 (Table 2). Water that remained in dead flower primordia froze at higher temperatures than in living flower buds. As buds developed in late February, LTE₅₀ increased for living and dead primordia. On 26 Feb., when living buds were swelling, dead

Table 1. Median low-temperature exotherm (LTE₅₀), temperature that kills 10% (LTE₁₀) and 90% (LTE₉₀) of *Prunus* flower buds, percentage of buds supercooled [LTE/primordium (LTE/P)], and percentage of buds injured before and after the advective freeze that began late Dec. 1990.

Measurement date	Species	Injured buds (%)	Mean LTE $\pm$ SD ($^{\circ}$ C)			LTE/P (%)
			LTE ₁₀	LTE ₅₀	LTE ₉₀	
13 Nov.	<i>P. dulcis</i>	0	-12.3 $\pm$ 0.2	-18.3 $\pm$ 1.7	-20.3 $\pm$ 0.2	66 $\pm$ 2.8
26 Dec.	<i>P. dulcis</i>	96 $\pm$ 4.9	-12.6 $\pm$ 0.3	-17.4 $\pm$ 0.4	-20.8 $\pm$ 0.2	49 $\pm$ 4.2
11 Dec.	<i>P. armeniaca</i>	0	-21.0 $\pm$ 0.5	-22.2 $\pm$ 0.2	-22.8 $\pm$ 0.1	83 $\pm$ 2.8
2 Jan.	<i>P. armeniaca</i>	69 $\pm$ 1.1	-22.0 $\pm$ 0.7	-23.2 $\pm$ 0.3	-24.5 $\pm$ 0.7	94 $\pm$ 7.1
15 Jan.	<i>P. armeniaca</i>	63 $\pm$ 2.1	-18.5 $\pm$ 0.3	-21.6 $\pm$ 2.2	-23.3 $\pm$ 1.7	78 $\pm$ 4.2
20 Dec.	<i>P. avium</i>	0	-21.8 $\pm$ 0.3	-23.5 $\pm$ 0.4	-24.7 $\pm$ 0.2	86 $\pm$ 2.8
2 Jan.	<i>P. avium</i>	39 $\pm$ 2.1	-20.4 $\pm$ 0.1	-21.9 $\pm$ 0.2	-22.6 $\pm$ 0.4	73 $\pm$ 1.4
30 Nov.	<i>P. davidiana</i>	0	-11.7 $\pm$ 0.6	-18.8 $\pm$ 0.4	-22.5 $\pm$ 0.7	89 $\pm$ 1.4
22 Dec.	<i>P. davidiana</i>	37 $\pm$ 7.8	-11.0 $\pm$ 0.1	-15.4 $\pm$ 0.6	-19.0 $\pm$ 0.0	96 $\pm$ 4.9
26 Dec.	<i>P. davidiana</i>	35 $\pm$ 3.5	-12.4 $\pm$ 0.4	-16.4 $\pm$ 0.1	-23.5 $\pm$ 0.7	87 $\pm$ 2.8
12 Jan.	<i>P. davidiana</i>	47 $\pm$ 4.6	-15.4 $\pm$ 0.3	-21.6 $\pm$ 2.1	-24.0 $\pm$ 1.4	88 $\pm$ 2.8
5 Dec.	<i>P. persica</i>	0	-17.5 $\pm$ 0.7	-20.0 $\pm$ 0.2	-20.3 $\pm$ 0.3	76 $\pm$ 4.2
20 Dec.	<i>P. persica</i>	25 $\pm$ 5.7	-19.5 $\pm$ 0.7	-21.6 $\pm$ 0.6	-22.0 $\pm$ 0.4	67 $\pm$ 2.1

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Table 2. Median low-temperature exotherm (LTE₅₀) (mean ±SD) for living (L) and dead (D) flower buds of three sweet cherry selections and 'Bing' cherries during Feb. 1992.

Date (Feb.)	LTE ₅₀ (°C)							
	7144-3		7147-13		7309-4		Bing	
	L	D	L	D	L	D	L	D
11	-19.8 ± 2.3	-18.5 ± 0.8	-18.3 ± 0.3	-17.7 ± 1.2	-16.3 ± 0.3	-12.7 ± 0.3.	-16.9 ± 0.1	-13.8 ± 0.4
20	-18.5 ± 0.8	-17.8 ± 0.6	-16.8 ± 0.0	-16.5 ± 1.2	-15.8 ± 0.0	--z	-16.3 ± 0.0	-10.9 ± 2.4
24	-16.6 ± 0.4	-14.7 ± 1.4	-16.2 ± 0.1	-1 2.5 ± 0.4	-12.7 ± 0.0		-15.0 ± 0.4	-13.0 ± 2.4
26	-14.5 ± 0.4	---	-14.1 ± 0.1	---	-13.3 ± 1.7	---	-12.6 ± 0.0	---

No LTEs.

primordia did not produce LTEs. Living flower buds of 7309-4 developed earlier than those of the other selections; thus, LTEs in dead buds disappeared earlier. Buds with living flower primordia produced more LTEs than dead buds, and the differences increased as the buds developed (data not shown).

We conclude that, when a dormant flower primordium is killed by freezing, the tissue water remains intact and compartmentalized so that ice crystals cannot grow. The DTA response remained normal for >2 weeks after dormant primordia were killed, a result indicating that water remained in the primordia without evaporating as the buds developed toward the stage at which they normally lose compartmentalization and the ability to supercool deeply. We suggest that factors, probably morphological, that permit compartmentalization are disrupted by freezing. When com-

partmentalization is lost, water in the primordium freezes as part of the first LTE.

When many primordia have been killed by freezing, subsequent DTAs are suspect. LTE₅₀ estimates will include LTEs from living and dead primordia, which will not respond "in the same manner to factors that influence bud hardiness. Under these circumstances, observing bud injury is advisable.

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