Freezing Behavior of *Prunus*, Subgenus *Padus*, Flower Buds

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Abstract. Flower buds of *Prunus serotina* Ehrh. produced high temperature exotherms (HTEs) and low temperature exotherms (LTEs). Supercooling in *P. serotina* occurred during full dormancy in December and early January but disappeared thereafter, whereas no supercooling was observed in *P. padus* L. or *P. virginiana* L. Both intact and detached inflorescences of *P. serotina* supercooled and froze as a unit and not as individual florets. Exotherms in dehydrated parts occurred at lower temperatures than in hydrated parts. Dormant buds of *P. serotina* lost the detectable exotherms when kept at -7°C for 2 days, while buds stored at 3°C exhibited LTEs between -20 and -26°C. Dormant Morescences of *P. serotina* were filled with elongated procambium and pith cells. In contrast, *P. padus* and *P. virginiana* had differentiated xylem vessel elements (XVE) the entire length of the inflorescence and did not supercool. Bud scales and bud axis of *P. serotina* were the flower parts where water apparently migrated during storage at 3 and -7°C. This was not observed for *P. padus* and *P. virginiana* flower buds. The rate of water migration from the inflorescences to bud scales and axis probably plays a role in the freezing behavior of *P. serotina*.

Native *Prunus* species are widely distributed in North America. *P. virginiana* is distributed in the Boreal forest and other northern latitudes, while *P. padus*, *P. maackii* Rupr., and *P. serotina* are found in more southern latitudes (Rajashekar and Burke, 1978). Flower buds of many *Prunus* species avoid freezing by supercooling, thereby avoiding bud injury (Quamme, 1974). However, *Prunus* taxa with racemose inflorescences usually do not supercool (Rajashekar and Burke, 1978). *Prunus serotina* is a member of the subgenus *Padus*, which also includes *P. padus*, *P. virginiana*, and *P. pensylvanica* (Rehder, 1950). This subgenus is characterized by flower buds borne laterally on spurs with a large racemose inflorescence that freeze without deep supercooling (Ashworth, 1984). According to Rajashekar and Burke (1978) and Burke and Stushnoff (1979), the twigs and flower buds of all of the above species, except *P. serotina*, froze without supercooling. Only one flower bud of *P. serotina* did not supercool, while the twigs supercooled to -38°C (Rajashekar and Burke, 1978).

Water in the flower bud primordium can supercool if it is isolated from ice crystals or other ice nucleators. Buds that supercool have physical features that prevent ice propagation from the bud scales and bud axis into the bud primordium. The presence or absence of xylem vessel elements is apparently the main factor that determines whether supercooling in *Prunus* species can occur. Supercooling can occur in dormant flower bud primordia if xylem vessels do not form a continuous conduit between the dormant primordium and bud axis (Ashworth, 1984).

Anatomical observations of several members of the subgenus *Padus* showed that the racemose inflorescence has large pith cells with large intercellular spaces (Ashworth, 1984). Xylem vessels in *P. padus* and *P. virginiana* were found to extend the length of the raceme and into the pedicels of individual florets

(Ashworth, 1984). Supercooling is prevented in such species by the propagation of ice through xylem and pith into the inflorescence. However, the fact that *P. emarginata* Hook is another species with eight to 10 florets born on short racemose (Rehder, 1950) that deep supercooled during winter (Kadir and Proebsting, unpublished) prompted us to study the hypothesis that not all taxa with racemose flowers freeze without supercooling. The objectives of this study were to further study the freezing behavior and the anatomical characteristics of *P. serotina* flower buds and compare them with those of *P. padus* and *P. virginiana*.

Materials and Methods

Plant materials. Shoots were collected weekly during early December to mid-March of 1989-90 and 1990-91 from trees of *P. padus*, *P. serotina*, and *P. virginiana* in the Interregional Repository of Virus Free Fruit Trees (IR-2) collection of virus tested fruit trees at the Washington State Univ., Irrigated Agriculture Research and Extension Center, Prosser, Wash. Shoots were immediately placed into plastic bags, on ice, and freeze tested within an hour, unless otherwise stated.

Differential thermal analysis (DTA). About 10 to 15 flower buds of *P. serotina* and five flower buds each of *P. padus* and *P. virginiana* were detached from shoots along with 1 to 2 mm of bark tissue and placed on both sides of a thermoelectric module with 71 thermocouple junctions sandwiched between two conductive plates (Andrews et al., 1983). A thermal conducting paste (Omega Engineering, Stamford, Conn.) was applied to the surface of some plates but it did not improve the LTEs signals compared to the plates without the paste. Moist paper towels were placed on the surface of the plates to induce HTEs but they did not change the HTE temperatures. Buds were held in place with aluminum foil.

Some flower buds were subjected to artificial hardening or dehardening treatments by placing prepared plates in a freezer at -7°C or in a cold room at 3°C for 2 to 3 days. Plates were transferred to the laboratory in a thermos bottle to prevent thawing of the -7°C samples during transfer. After preconditioning

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Abbreviations: DTA, differential thermal analysis; HTEs, high temperature exotherms; LTEs, low temperature exotherms; XVE, xylem vessel elements.

Fig. 1. Longitudinal sections of dormant inflorescence of (A) *P. padus* ($\times 23.4$) and (B) *P. serotina* ($\times 50.4$) sampled during Dec. 1990.

treatments, plates were suspended in a double-walled, polystyrene box in a freezer. Samples were cooled to -33°C at a cooling rate of $4^{\circ}\text{C}/\text{h}$. The -7°C samples were placed in the -7°C equilibrated polystyrene box and the 3°C samples were placed in the polystyrene box at 0°C .

Freezing was recorded as exothermic events on a multiple-channel strip-chart potentiometer (Linseis L7060, Princeton Junction, N.J.). The temperature within the box was monitored with a 11-mm diameter (36 gauge) copper-constantan thermocouple. Frozen samples were removed from the freezer after it had cooled to -33°C and, then placed in a 100% relative humidity (RH) chamber at room temperature (23°C) for 24 h before flower buds were observed for tissue oxidation.

Hydration and dehydration. Twigs of *P. serotina* and *P. padus* with 10 to 15 flower buds were collected and kept in plastic bags on ice until used. For hydration treatments, eight to 10 cm stem sections with intact buds were vacuum infiltrated at 90.0 kPa at room temperature for 4 to 6 h. Buds were detached from the twigs and placed on both sides of the thermoelectric module as described. Detached inflorescences without bud scales or bud axis were also placed on plates. Plates were placed in the polystyrene box in the freezer, cooled to -33°C at a cooling rate of $4^{\circ}\text{C}/\text{h}$ before being removed, and placed in 100% RH at room temperature for 24 h.

Buds and detached inflorescences of *P. serotina* that were

used for dehydration tests were collected on the same day. Inflorescences were dried at room temperature to 50%, 83%, 87%, and 92%, while buds were air dried to 64% and 83% of their original water content before being prepared for the DTA test. The extent of supercooling of the flower bud population was expressed as the temperature at which 50% (LTE₅₀) of the intact and detached inflorescences were injured.

Anatomical observation. Twigs of *P. serotina*, *P. padus*, and *P. virginiana* buds were collected at 2-week intervals during December and weekly during March. Excised flower buds with their scales removed were vacuum infiltrated with formalin-acetic acid at 90.9 kPa at room temperature and for 5 to 7 min and then dehydrated with ethyl-alcohol series. The development of XVE was observed in the bud axis and the raceme of the inflorescence using 15 μm serial sections embedded in paraffin and stained with safranin-fast green (Johanson, 1940).

Water movement. Twigs with intact buds of *P. serotina* and *P. padus* were collected with samples for DTA. Twigs were kept in plastic bags on ice, then some were transferred to a room at 3°C and some to a freezer at -7°C for 2 days before being dissected under a dissecting microscope in the cold room and in a freezer at subfreezing temperatures, respectively. Bud scales and inflorescences were excised. Water content was then determined by drying the samples at 90°C for 24 h and was expressed as percentage of fresh weight.

Fig. 2. Typical DTA profile of dormant flower buds and detached inflorescence of (A) *P. padus* and (B) *P. virginiana* sampled 8 Dec. 1990 and cooled to -33C at 4C/h. The tracing reflects the freezing of multiple flower buds.

Table 1. The effect of hydration and forcing on viability of *P. padus* flower buds and inflorescence frozen at 4C/h to -33C. Samples collected 17 and 24 Jan. 1991.

Treatment	Buds viable (%)
Control	100
Hydrated bud	100
Hydrated inflorescence	89
Forced bud	0
Forced inflorescence	0
LSD <i>P</i> = 0.05	5

Results and Discussion

Exotherm analysis. Species belonging to the subgenus *Padus* have racemose inflorescences with numerous florets (Fig. 1). Neither flower buds nor the detached inflorescences of *P. padus* and *P. virginiana* deep supercooled during DTA tests (Fig. 2).

Fig. 3. DTA profile of dormant *P. serotina* flower buds and detached inflorescence sampled 8 Jan. 1991 and cooled to -33C at 4C/h. The tracing reflects the freezing of 10 to 15 flower buds.

Fig. 4. DTA profile of *P. serotina* flower buds sampled 10 Jan. 1991 then stored at 3 or -7C for 2 days. Buds were cooled to -33C at 4C/h. The tracing reflects the freezing of 10 to 15 flower buds.

Table 2. The effect of hydration and dehydration on the temperature at which 50% (LTE₅₀) of the intact flower buds and detached inflorescences of *P. serotina* were injured.

Water content (% fresh wt)	Temperature (°C)	
	Bud	Inflorescence
	LTE ₅₀	LTE ₅₀
50	---	-20.4
64	-20.5	---
83	-18.5	-17.2
87	---	-15.4
92	---	-11.6
100	-13.2	-9.7
LSD <i>P</i> = 0.05	0.97	0.97

Flower buds of *P. padus* (Fig. 2A) produced HTEs between -5.5 and -8C, while those of *P. virginiana* (Fig. 2B) produced large HTEs at -7.2 to -9C. In both species, the detached inflorescences freeze at lower temperatures than the intact

Fig. 5. Longitudinal section of procambium cells within the raceme of dormant *P. serotina* flower buds sampled mid-December without xylem vessel elements present ($\times 1000$).

Fig. 6. longitudinal section of xylem vessel elements within the raceme of dormant *P. padus* flower buds sampled mid-December ($\times 1000$).

ones, indicating that bud scales and the bud axis enhance ice nucleation at higher temperatures. The presence of XVE in the flower bud facilitates the spread of ice formed in the vessels at -10°C to the supercooled water in the primordium (Ashworth, 1984) and prevents deep supercooling of the primordia. The number of exotherms produced in this study from the freezing

of the inflorescences coincided with the number of inflorescence per plate. Exotherms of detached inflorescences occurred as individual peaks, while intact inflorescences produced continuous peaks. Removing bud scales and axis caused an individual inflorescence to freeze at a relatively different temperature.

Both *P. padus* and *P. virginiana* survived -33°C during dor-

Fig. 7. Longitudinal section of xylem vessel elements within the raceme of developed *P. serotina* flower buds sampled early March (x1000).

Fig. 8. Water content (percent fresh weight) of the inflorescences of *P. serotina* and *P. padus* stored at 3 or -7C for 2 days during Winter and Spring 1990-91.

mancy, as indicated by visual observations. Buds of *P. padus* sampled 24 Jan. and hydrated by vacuum infiltration for 4.5 h and stored at 100% RH for 12 h, survived -33C (Table 1). However, buds collected 17 Jan. and forced at room temperature (23C) for 7 days did not survive. Apparently, during forcing, flower buds became more sensitive to subfreezing temperatures than dormant, hydrated flower buds. Therefore, an increase in water content of a dormant flower bud alone was insufficient to cause bud deacclimation and injury by subfreezing temperatures.

Rajashekar and Burke (1978) reported that twigs from *P. serotina* produced LTEs, but that the flower bud did not supercool. Our results showed that the freezing behavior of *P. ser-*

otina was dissimilar to that of *P. padus* or *P. virginiana*. Dormant flower buds (10 to 15) of the racemose *P. serotina* supercooled like nonracemose *Prunus* species (Proebsting and Andrews, 1981). The time of sampling and number of buds per test were critical in producing LTEs indicative of supercooling. Not all flower buds per run had developed the ability to supercool, thus using more than one bud was critical in producing LTEs. Most of the LTEs of *P. serotina* were produced only during December and early January. Flower buds and inflorescences of *P. serotina* produced small and sharp LTEs (Fig. 3). Flower bud LTEs occurred between -20 and -26C, and the detached inflorescences produced LTEs between -17 and -22C. Surprisingly, the number of LTEs of *P. serotina* did not coincide with either the number of flower buds or the inflorescences on the plates. These results suggest that, during dormancy, either not all the inflorescence produced LTEs, the DTA system was not sensitive enough to detect all the LTEs at that time of the season, or simultaneous exotherms occurred.

No LTEs to -33C were detected from buds of *P. serotina* frozen for 2 days at -7C during dormancy, while those stored at 3C supercooled and produced LTEs between -20 and -26C (Fig. 4). In buds kept at -7C for 2 days, water probably migrated from flower primordia to ice crystals between the scales (Quamme, 1978); thus, the primordia either supercooled beyond -33C (our technique could not detect LTEs beyond -33C) or the small amount of water remaining in the inflorescences, when frozen, could not be detected by DTA.

As the season advanced beyond 30 Jan. 1991, LTEs were no longer observed in *P. serotina* flower buds, only detached inflorescences produced LTEs. Therefore, different parts of the bud were studied to determine which part enhanced supercooling of the detached inflorescences. Inflorescences attached to either the bud axis or the scales did not produce LTEs (data not shown). Detached individual florets from the raceme and

the individual raceme did not supercool either. Thus, dormant inflorescences froze as a unit and not as individual florets.

Hydration and dehydration. Both hydrated and dehydrated flower buds of *P. serotina* produced LTEs (Table 2). Detached inflorescences produced only LTEs, since HTEs are associated with freezing of water in bud scales and bud axis (Graham and Mullin, 1976). Inflorescences dehydrated to 50% water content produced a LTE_{50} at -20.4 (Table 2), while hydrated samples produced LTE_{50} near -9.7. The LTEs were higher the higher the water content of inflorescences between 64% to 92%. Intact buds that had been dehydrated to 64% water content produced LTEs at lower temperatures than buds at 83% or 100% water content. These results suggest that dehydration enhanced supercooling of the inflorescence in intact buds and caused a decrease in exotherm temperatures compared to the hydrated samples. This finding agrees with earlier reports on sweet cherry [*P. avium* (L.) L.] and peach [*P. persica* (L.) Batsch] flower buds (Andrews and Proebsting, 1987; Quamme, 1983).

Anatomical observations. In dormant *P. serotina* flower buds sampled mid-December, the length of the bud was filled with elongated procambium and small pith cells with very small intercellular spaces (Fig. 5). XVEs were restricted to the base of the flower bud axis (not shown). In contrast, dormant flower buds of *P. padus* sampled mid-December had differentiated XVE in the raceme (Fig. 6), which presumably prevented both the buds and the inflorescences from supercooling. XVE played a major role in regulating the freezing behavior of *P. padus* and *P. virginiana*. XVEs of *P. serotina* started to develop and grow into the inflorescence during early March when buds developed into the first swelling stage (Fig. 7). These observations do not agree with an earlier report that dormant *P. serotina* flower buds had XVEs developed in the inflorescence (Ashworth, 1984). Our observations would explain the seasonal differences in supercooling observed in this study.

Water movement. Frozen inflorescences of *P. serotina* had lower water content than *P. padus* and dehydrated more during storage at -7°C than at 3°C (Fig. 8). No obvious changes in water content for *P. padus* inflorescences were observed during storage at 3 or -7°C. This result suggests that water in *P. serotina* flower buds migrated out from the inflorescence during storage at -7°C to freeze around and in the scales and bud axis, thus decreasing water content of the inflorescences. In contrast, water in frozen buds of *P. padus* did not migrate during 2 days of storage at -7°C; therefore, no significant decrease in water content of the inflorescences was observed. Apparently *P. serotina* bud scales and axes serve as an ice sink, as they do in *Prunus* species having nonracemose inflorescences (Quamme, 1978), unlike in *P. padus* and *P. virginiana*. Inflorescences of *P. serotina* air dried for 40 min at room temperature lost 50%

of their water content; while only 12% and 17% was lost from *P. padus* and *P. virginiana*, respectively.

In summary, XVE is the main factor that prevented supercooling in *P. padus* and *P. virginiana*. However, flower buds of *P. serotina* did not develop XVE in the inflorescences until early March, yet supercooling was prevented earlier; therefore, a factor other than XVE prevented supercooling of the intact inflorescences. The detached inflorescences of *P. serotina* supercooled beyond 30 Jan. 1991. Supercooling was enhanced by dehydration, and buds kept at -7°C for 2 days either supercooled beyond -33°C or the small amount of water that froze was not detected by the DTA technique. Not all flower buds supercooled during dormancy or the LTEs were not big enough to be detected by DTA, thus using more than one flower bud is essential to record LTEs.

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