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Xylem Development in *Prunus* Flower Buds and the Relationship to Deep Supercooling

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ABSTRACT

Xylem development in eight *Prunus* species was examined and the relationship to deep supercooling assessed. Dormant buds of six species, *P. armeniaca*, *P. avium*, *P. cerasus*, *P. persica*, *P. salicina*, and *P. sargentii* deep supercooled. Xylem vessel elements were not observed within the dormant floral primordia of these species. Instead, discrete bundles containing procambial cells were observed. Vascular differentiation resumed and xylem continuity was established during the time that the capacity to deep supercool was lost. In *P. serotina* and *P. virginiana*, two species which do not supercool, xylem vessels ran the length of the inflorescence and presumably provided a conduit for the spread of ice into the bud. The results support the hypothesis that the lack of xylem continuity is an important feature of buds which deep supercool.

The dormant flower bud primordia of several woody plant species avoid freezing injury by deep supercooling (2-5, 9, 10, 12-15). Water remains supercooled within the bud primordia despite the formation of ice crystals in the bud scales and adjacent tissues. Therefore, buds which deep supercool must possess structural and/or physiological features which prevent the spread of ice from adjacent tissues into the primordia. Water within xylem vessel elements freezes above −10 °C (7). Once initiated, ice readily propagates through the vessels to other parts of the plant (7, 8). Therefore, it was predicted that xylem vessels could not form a continuous network connecting the primordia to the remainder of the plant. If such a network did exist, ice would propagate via the xylem and nucleate the water within the primordia.

Anatomical studies with dormant peach flower buds demonstrated that the primordia did not contain xylem vessel elements (1). Instead the vascular tissues were composed of procambial cells. These cells were elongated, contained cytoplasm, and lacked secondary wall thickenings. Differentiation of the procambium and the development of xylem continuity occurred in the spring during blossom development. Vascular continuity in 14 *Prunus* species was indirectly assessed by following the movement of a water soluble dye. A relationship between the lack of vascular continuity and deep supercooling was observed (2). The results of these studies led to the proposal that the lack of xylem continuity between the dormant primordia and the remainder of the plant was an important feature permitting buds to deep supercool (1, 2). The purpose of this study was to further test this hypothesis. Direct observations on vascular development in *Prunus* flower buds were made to assess the relationship to deep supercooling.

MATERIALS AND METHODS

Twigs, 20- to 40-cm long from the current season's growth, were harvested on a monthly basis, beginning September 1982 through April 1983. *Prunus armeniaca* L. and *P. persica* (L.) Batsch were collected from the West Virginia University Farm in Kearneysville, WV. *P. avium* L. and *P. cerasus* L. were collected from a local commercial orchard. *P. salicina* Lindl. was collected from the Appalachian Fruit Research Station. *P. serotina* Ehrh., *P. virginiana* L., and *P. sargentii* Rehd. were collected from the National Arboretum, Washington, DC. Upon collection, twigs were put into plastic bags and placed into an insulated box containing crushed ice. Samples were transported to the laboratory and stored on ice until processed.

Anatomical Studies. Flower buds were excised from twigs and the bud scales removed. The top 1 to 2 mm of primordia or inflorescence was cut off to facilitate fixation and embedding. Buds were vacuum infiltrated and fixed with formalin-acetic acid, dehydrated with an alcohol-tertiary butyl alcohol series and embedded in paraffin (6). Ten μm longitudinal and cross sections were cut using a rotary microtome and stained with safranin-fast green. Serial sections were examined with light and fluorescent (440 to 510 nm exciter filter, 520 nm barrier filter) microscopy. Observations were made on four buds of each species at each sampling date.

Differential Thermal Analysis. The freezing of water within buds was characterized using DTA.[1] The technique was a modification of that described by Quamme, *et al.* (11). Excised flower buds were placed into small aluminum foil containers along with the junction of a 40-gauge copper-constantan thermocouple. Freeze-dried tissue was used as a reference. Output of the thermojunctions was monitored with a strip chart recorder (0.5 mv/full scale). Samples were placed into glass test tubes which were fitted into holes bored into an aluminum block. The block was placed into a −70°C deep freeze. Block temperature and cooling rates were controlled using a resistance heater and temperature programmer. Samples were cooled at 5°C/h.

RESULTS

The eight *Prunus* species examined in this study could be placed in three distinct categories based on floral morphology. The flower buds of both *P. persica* and *P. armeniaca* had a single floral primordium enclosed within the bud scales (Fig. 1). The buds of *P. avium*, *P. cerasus*, *P. salicina*, and *P. sargentii* had multiple primordia. The umbel contained 3 to 4 floral primordia each on a separate pedicel and enclosed by a single set of bud scales (Fig. 2). Both *P. serotina* and *P. virginiana* had a racemous inflorescence (Fig. 3).

The anatomy and morphology of the vascular system varied

[1] Abbreviation: DTA, differential thermal analysis.

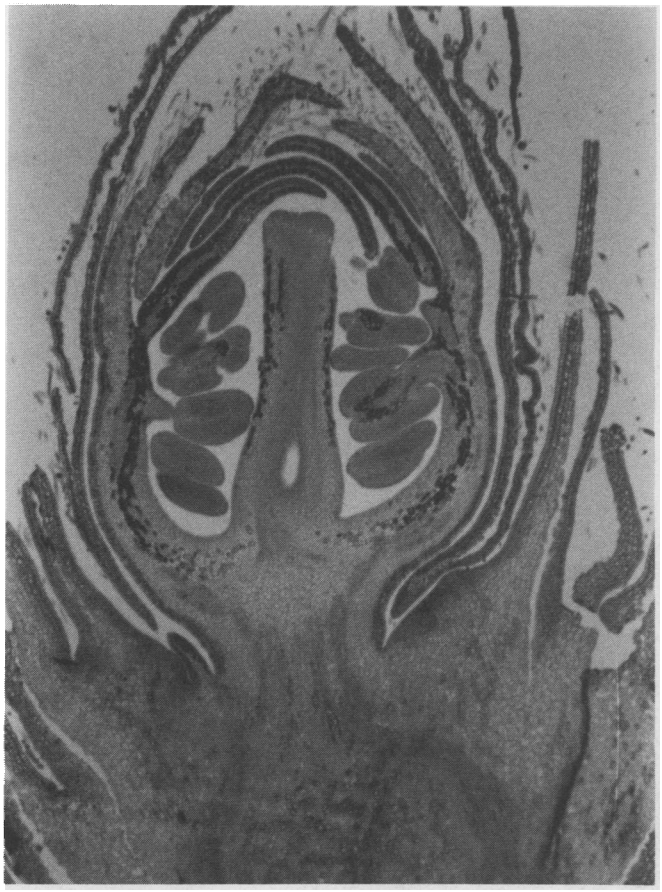

FIG. 1. Longitudinal view of a dormant flower bud of *P. persica* sampled in December. Bud contained a single floral primordium.

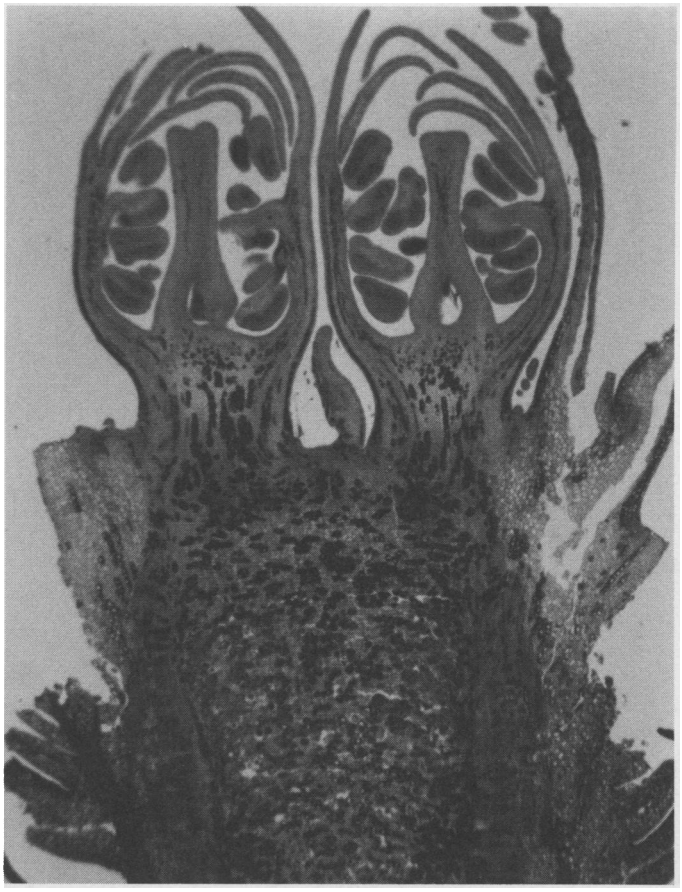

FIG. 2. Longitudinal view of a dormant flower bud of *P. avium* sampled in December. Bud contained multiple floral primordia.

among the types of inflorescences. Buds having a single primordium (*P. persica* and *P. armeniaca*) had a vascular ring in the base of the bud axis. Higher in the axis and within the flower pedicel, a ring of discrete vascular bundles was observed. These bundles diverged in the primordium and were observed in the anther filaments, pistil, and rudimentary sepals and petals. Vascular traces within the primordia were recognizable in the earliest samples. Only procambial cells were observed within the bundles in both the primordium and the upper portion of the bud axis. These cells were elongated, contained cytoplasm, and lacked secondary wall thickenings. The cells within the vascular tissues remained procambial during the fall and winter. In January, the first signs that vascular differentiation was resuming were noted. Cross sectional views of procambial cells within the primordia demonstrated that a slight increase in cell wall thickness and autofluorescence had occurred. An increase in cell vacuolation was also noted. Xylem vessel elements were not observed within the primordia until the March sampling date. At that time, vessel elements were observed throughout and xylem continuity was established between the primordia and the remainder of the plant. A further proliferation of xylem vessels occurred later in the spring.

The vascular systems in *P. cerasus*, *P. avium*, *P. salicina*, and *P. sargentii* were similar. A vascular ring was observed in the base of the bud axis, and further up, a ring of discrete bundles was present. The vascular bundles diverged to form a ring of bundles leading to each individual primordium. The bundles transversed the pedicel and were observed throughout the individual primordium. As in *P. persica* and *P. armeniaca*, the vascular strands were recognizable in September. The strands within the primordium and upper portions of the bud axis were composed of procambial cells (Fig. 4). Xylem vessels were not observed within the primordia of any of these four species through December. In January, vascular differentiation appeared to resume in *P. sargentii*. Vessels were observed in the axis tissue adjacent to the primordia. A continuous conduit of xylem was observed up to the base of the pedicel. Differentiation continued throughout the spring. In both *P. avium* and *P. salicina*, xylem continuity between the primordia and adjacent tissues was established between the February and March sampling dates (Fig. 5). Prior to that time, no xylem vessel elements were observed within the bud primordia in either species. *P. cerasus* was the last of the species to undergo xylem differentiation. Observations made up to and including the March sampling date failed to detect xylem vessel elements within the bud primordia. Only procambial cells were observed within the vascular strands. Subsequently, xylem differentiation occurred; in April, vessels were observed throughout the primordia and xylem continuity was established.

The dormant buds of *P. armeniaca*, *P. avium*, *P. cerasus*, *P. persica*, *P. salicina*, and *P. sargentii* had all differentiated so that the stamens and pistil were recognizable (Figs. 1 and 2). Cells within the floral primordia were small and tightly packed. The cells shared common walls and little intercellular space was observed.

The vascular system in the racemous inflorescence (*P. serotina* and *P. virginiana*) was different than that observed in the other *Prunus* species. Xylem vessels ran the length of the raceme (Fig. 6). Vascular traces diverged and entered the individual primordia. The primordia had not differentiated to the extent of the other *Prunus* species examined. Stamens and pistils were not recognizable until spring. The base of the individual floral primordia was close to the vessels in the raceme. The vascular tissue

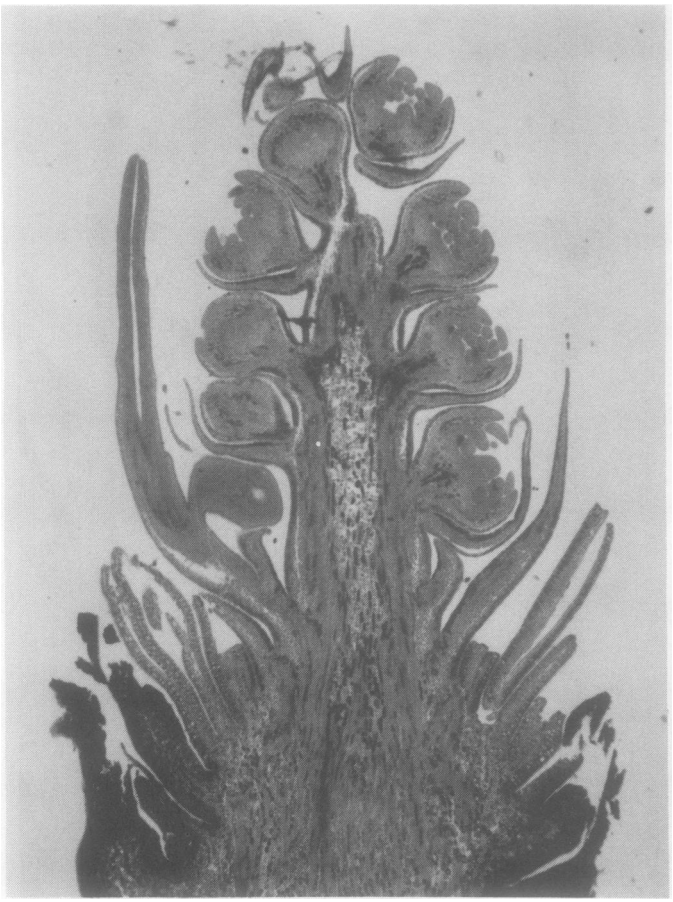

FIG. 3. Longitudinal view of a dormant inflorescence of *P. virginiana* sampled in December. Numerous individual floral primordia are borne on a raceme.

FIG. 4. Longitudinal view of vascular bundle within a dormant flower primordium of *P. avium*. Vascular tissue composed of procambial cells. Sample taken in December (× 1000).

observed in the dormant primordia was composed primarily of procambial cells. However, vessel elements were frequently observed in the pedicels of individual primordia. In both *P. serotina* and *P. virginiana*, the center of the raceme was composed of a large pith. Cells in the pith were larger than adjacent cells in the cortex (Fig. 6). These cells were not tightly packed and large intercellular spaces were observed. In addition, the cells within the pedicels of individual floral primordia were larger and had

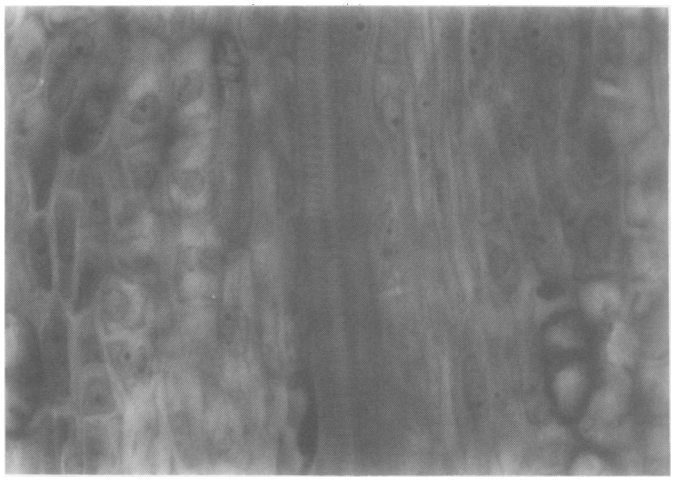

FIG. 5. Longitudinal view of xylem vessel elements within developing floral primordium of *P. avium*. Sample taken in March (× 1000).

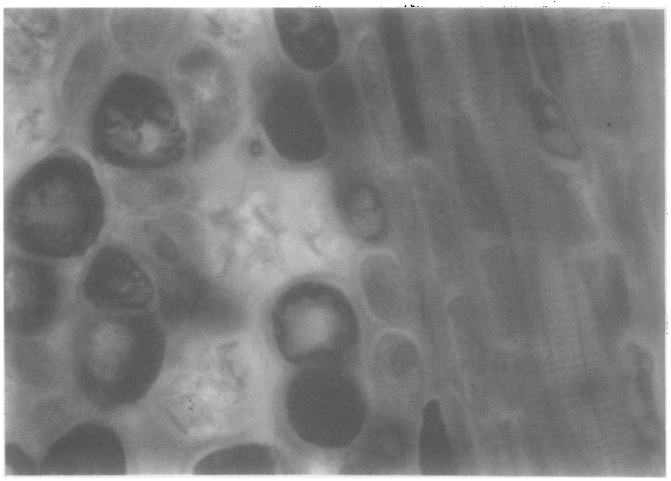

FIG. 6. Longitudinal view of vessels and pith within the raceme of *P. virginiana*. Sample taken in December (× 1000).

larger intercellular spaces than those observed in the other *Prunus* species.

DTA of dormant *Prunus* buds produced three distinct patterns. Two exotherms were observed when buds having single floral primordia (*P. armeniaca*, and *P. persica*) were frozen. A high temperature exotherm occurred between −5 and −10°C and corresponded to the freezing of water in the bud axis and scales (1). The low temperature exotherm occurred between −15 and −25°C and corresponded to the freezing of water within the primordia (Fig. 7). In buds having multiple primordia (*P. avium*, *P. cerasus*, *P. salicina*, and *P. sargentii*), a single high temperature exotherm and multiple low temperature exotherms were observed (Fig. 7). In the racemous inflorescence (*P. serotina* and *P. virginiana*), only the high temperature exotherm was observed (Fig. 7).

The ability of *P. armeniaca*, *P. avium*, *P. cerasus*, *P. persica*, *P. salicina*, and *P. sargentii* to deep supercool varied seasonally. Supercooling was not observed in September and October, while in November and December all buds deep supercooled. The time period when species lost the capacity to deep supercool varied. *P. sargentii* buds did not deep supercool in January. The capacity to supercool was lost in *P. persica*, *P. avium*, *P. armeniaca*, and *P. salicina* sometime between the February and March sampling dates. *P. cerasus* retained the supercooling characteristic the longest. Supercooling was lost between the March and April sampling dates. Low temperature exotherms were never observed

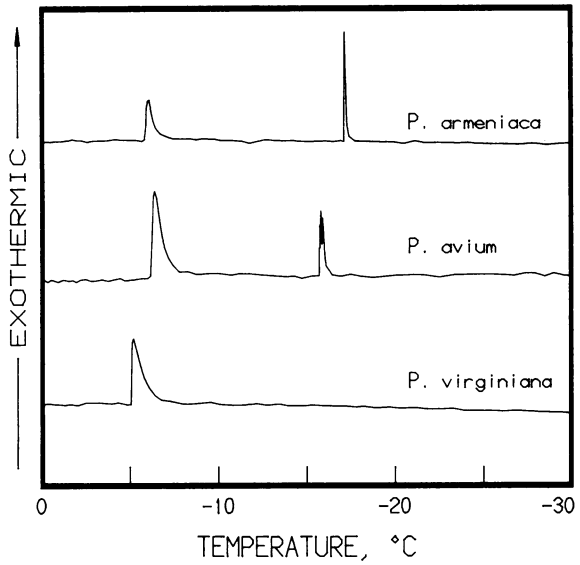

FIG. 7. DTA of dormant *Prunus* buds. Representative runs of buds: with a single primordium (*P. armeniaca*); with multiple primordia (*P. avium*), and with a racemous inflorescence (*P. virginiana*). Buds were run in December and cooled at 5°C/h.

in either *P. serotina* or *P. virginiana*. The high temperature exotherms were observed throughout the year at approximately the same temperature.

DISCUSSION

Water in plant tissues will not supercool unless heterogeneous ice nucleating substances are absent and the spread of ice from adjacent tissue can be prevented. The properties of a tissue which facilitate supercooling are undoubtedly a composite of several features and include physiological, structural, and morphological features. Presumably each component is critical and the loss of any one would prevent deep supercooling. One component is the relationship between xylem development and deep supercooling. It had been proposed that deep supercooling could not occur in dormant bud primordia if xylem vessels formed a continuous conduit connecting the dormant bud primordia with the remainder of the plant (1). If xylem continuity was established, ice could propagate via the vascular system and nucleate the water within the primordia. Previous work has supported this concept. Light microscopy of peach buds demonstrated that dormant bud primordia lacked xylem vessels (1). In addition, xylem continuity was indirectly assessed by observing the movement of a water soluble dye into the buds of 14 *Prunus* species. Dye was not observed to enter the primordia of any of the nine species which exhibited deep supercooling (2). In contrast, dye was observed within the primordia of those species which did not supercool. The results suggested that xylem continuity had not been established in the dormant buds of the *Prunus* species which deep supercool, but was established in species which lacked the supercooling characteristic. The present study corroborated the previous results. Direct observations on six *Prunus* species which deep supercool demonstrated the lack of xylem continuity during dormancy. Xylem vessels were never observed in the primordia of buds which supercooled. The lack of xylem continuity appears to be a common feature of *Prunus* species which supercool. In each species, xylem continuity was established during the same time interval that the capability to supercool was lost. The combined evidence supports the contention that the lack of xylem continuity is an important feature of buds which deep supercool.

Several members of the genus *Prunus* do not supercool. Burke and Stushnoff (4) observed that these species all had a racemous inflorescence. It was apparent from the anatomical observations, that xylem vessels ran the length of the raceme and were observed in or near the pedicels of the individual primordia. In addition, a large pith was observed within the raceme. The pith contained larger cells and intercellular spaces than was observed within the individual primordium. Presumably, both the xylem and the pith would facilitate the propagation of ice into the inflorescence and prevent supercooling.

In the present study, it was noted that the establishment of xylem continuity and the loss of the capacity to deep supercool occurred during the same time interval. It would be tempting to speculate that the establishment of xylem continuity would provide a conduit for the spread of ice into the bud primordia and would lead to the irreversible loss of the capacity to deep supercool. However, the large time interval between sampling dates in the present study did not provide sufficient precision to establish causality. Further experimentation will be required to test this idea.

The present study supports the hypothesis that the lack of xylem continuity between the primordia and adjacent tissues is an important feature of buds which supercool. The lack of xylem continuity, while apparently critical, is but one of presumably numerous features of the tissue which permit deep supercooling. Therefore, it follows that although xylem continuity would not be expected in tissues which deep supercool, tissues which lack xylem continuity would not necessarily exhibit deep supercooling. In the latter case, other yet unidentified features of the tissue may prevent supercooling.

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