Plant Physiol. (1990) 92, 718–725
0032-0889/90/92/0718/08/$01.00/0

Received for publication May 25, 1989
and in revised form October 23, 1989

The Formation and Distribution of Ice within Forsythia Flower Buds[1]

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ABSTRACT

Differential thermal analysis detected two freezing events when dormant forsythia (*Forsythia viridissima* Lindl.) flower buds were cooled. The first occurred just below 0°C, and was coincident with the freezing of adjacent woody tissues. The second exotherm appeared as a spike between −10 and −25°C and was correlated with the lethal low temperature. Although this pattern of freezing was similar to that observed in other woody species, differences were noted. Both direct observations of frozen buds and examination of buds freeze-fixed at −5°C demonstrated that ice formed within the developing flowers at temperatures above the second exotherm and lethal temperature. Ice crystals had formed within the peduncle and in the lower portions of the developing flower. Ice also formed within the scales. In forsythia buds, the developing floral organ did not freeze as a unit as noted in other species. Instead the low temperature exotherm appeared to correspond to the lethal freezing of supercooled water within the anthers and portions of the pistil.

Water within the dormant flower buds of a number of woody species has been observed to supercool to low temperatures prior to freezing (6, 8–10, 13, 14). In these species, survival of the developing floral organs depended upon avoiding ice formation and the accompanying stresses. When the freezing of water in these tissues was examined using thermal analysis techniques, two distinct freezing events were detected. The first occurred a few degrees below 0°C and corresponded to the freezing of water within the bud scales and subtending axis tissues. The second freezing event, often referred to as the low temperature exotherm, corresponded to the freezing of a fraction of supercooled water and was closely correlated to the temperature at which the flower bud was killed (6, 13, 14). The temperature of this second freezing event typically ranged from −15 to −30°C, depending upon the species examined and the stage of cold acclimation (1, 6, 8, 10, 13, 14). In species with multiple florets within a single bud, multiple low temperature exotherms have been observed, each corresponding to the lethal freezing of an individual floret (6, 8, 10, 14).

The distribution of ice in overwintering flower buds from several species has been examined using both fresh specimen and freeze-fixed tissue (2, 5, 9, 10, 15, 18). Ice crystals were not uniformly distributed in these tissues, but were segregated into specific locations within the bud. Large crystals were observed within the bud scales and in the subtending bud axis tissue (2, 5, 9, 10, 15, 18). There was no evidence of ice formation within the developing floral organ at temperatures above the lethal low temperature. These observations were consistent with results obtained from thermal analysis experiments where the initial freezing event occurred in the scales and subtending tissues, while water within the developing floral organ supercooled to much lower temperatures before freezing (2, 15).

Experiments with *Prunus* and *Rhododendron* species have suggested that the developing floral organs lacked tolerance to ice formation (6, 8, 15). When ice formation was initiated within these tissues, the entire floral organ froze as a unit and was killed. In addition, if florets were inoculated with ice crystals, buds were killed at temperatures above those normally lethal (15). These observations led researchers to conclude that some form of barrier must be present at the tissue level to prevent the spread of ice into the developing floral organ (1, 6, 8, 10, 15). Although the nature of this barrier has not been established, various features of the overwintering buds have been implicated (1, 6, 10, 15).

The purpose of the present study was to further characterize the freezing of water in overwintering forsythia flower buds. Nus and co-workers (13) observed a low temperature exotherm near −23°C when forsythia buds were frozen. They reported that this freezing event was associated with a dramatic loss of tissue viability and based on the similarity of their observations to previous reports suggested that this represented the freezing of a fraction of supercooled water. Although thermal analysis results for forsythia were similar to those reported for other species which deep supercool, it was not clear if other aspects of bud freezing behavior were similar. In addition, it was hoped that by comparing observations on forsythia to other species, common features which facilitate supercooling could be identified.

MATERIALS AND METHODS

Forsythia (*Forsythia viridissima* Lindl.) flower buds were obtained from a planting on the Purdue University campus, West Lafayette, IN. Shoots of the current season's growth were harvested, placed into plastic bags and brought to the laboratory immediately prior to the start of each experiment. When shoots were harvested before leaf fall, the leaves were excised with a scalpel to facilitate subsequent handling.

[1] Purdue University Agricultural Experiment Station Article No. 12040.

Thermal Analysis

The temperature at which water froze in forsythia flower buds was determined using thermal analysis. Shoot pieces, approximately 8 cm long, were placed into stoppered individual glass test tubes. The base of the shoot piece was placed in 0.5 mL of deionized water containing a small chip of ice, and the test tubes were placed in a circulating glycol bath (Neslab Instruments, Portsmouth, NH). Bath temperature was initially 0°C, and following a 30 min equilibration period, was lowered at 2°C/h. The junction of a 30-gauge copper-constantan thermocouple was attached to the surface of individual buds with a small piece of masking tape. Temperatures were monitored at 30 s intervals with a datalogger interfaced to a computer (Dianachart Inc., Rockaway, NJ). The freezing of water was detected as an abrupt increase in sample temperature, and exotherm temperature was noted as the sample temperature immediately prior to that increase.

Differential Thermal Analysis

The freezing of water in buds was also characterized using DTA.[2] The technique was a modification of that described by Quamme *et al.* (16). Excised flower buds or isolated floral tissues were placed into small aluminum foil containers along with the junction of a 40-gauge copper-constantan thermocouple. Oven dried tissue was used as a reference. The 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 in a −80°C freezer. Block temperature and cooling rate were controlled using a resistance heater and temperature programmer. Samples were cooled at 2°C/h.

Hardiness Evaluations

To estimate the freezing resistance of forsythia flower buds, shoot pieces were placed into stoppered individual glass test tubes as described for thermal analysis. Bath temperature was initially 0°C and following a 30 min equilibration period was lowered at either 2 or 5°C/h. When appropriate test temperatures were reached, a sample of 10 replicate test tubes were transferred to an ice bath and held overnight. Test temperatures varied and were altered throughout the year to bracket the estimated lethal temperature. Following the laboratory freeze-thaw protocol, specimens were held in sealed test tubes for 2 d at room temperature. Buds were then bisected and rated visually for survival.

The response of tissues within the forsythia flower bud to a freezing stress were examined using the vital stain, 2,3,5-triphenyltetrazolium chloride. Following the freezing stress, buds were bisected with a razor blade and placed in 0.8% 2,3,5-triphenyltetrazolium chloride in a 50 mM phosphate buffer (pH 7.4). Tissues were incubated overnight at room temperature and evaluated with the aid of a dissecting microscope.

[2] Abbreviations: DTA, differential thermal analysis; FAA, formalin-acetic acid-alcohol fixative; SEM, scanning electron microscopy.

Freeze Fixation

Shoot pieces were placed into stoppered individual glass test tubes as described for thermal analysis. Following a 30 min equilibration period, bath temperature was reduced at 2°C/h to −5°C. After an additional 1 h incubation period, a precooled solution of 8% formaldehyde (v/v) was added to each test tube and the specimens fixed while frozen as described by MacKenzie *et al.* (12). Sufficient fixative was added to completely submerge the tissue. In addition, a chip of ice was added to ensure that the fixative would remain at equilibrium concentration with the unfrozen solution within the tissue. Specimens were held in the fixative for 7 d at −5°C. The formaldehyde solution was then replaced with 50% ethanol and held for an additional 30 min. Specimens were returned to room temperature and dehydrated using a graded ethanol series. Once in absolute ethanol, buds were bisected longitudinally with a razor blade. After an additional exchange in absolute ethanol, specimens were critical point dried, and sputter coated with gold-palladium. Prepared specimens were viewed with a JEOL JSM-840 SEM at 5 or 10 kV.

Fixation of Unfrozen Tissues

Control tissues were fixed in a FAA fixative (11). Buds were excised from the current season's growth and vacuum infiltrated with fixative. Specimens remained in the fixative for seven days at room temperature and were subsequently prepared for SEM as described previously.

Freeze Substitution

Forsythia flower buds were excised from plants in the field and immediately quench frozen into melted Freon 12 at −150°C. Buds were excised from unfrozen tissues and on subsequent days following a natural freeze. At the time the frozen buds were excised the air temperature was −4.8°C ± 0.2°C and −15°C ± 0.2°C, respectively, as measured with copper-constantan thermocouples and a portable monitor. In each instance, tissues were excised and subsequently handled with tools equilibrated with the outside air temperature. Once frozen into melted Freon 12, specimens were rapidly transferred into liquid nitrogen and held until subsequently processed. The quench frozen samples were freeze substituted for seven days as previously described (3). Following three rinses in absolute ethanol, buds were bisected longitudinally with a razor blade. After an additional exchange in absolute ethanol, the samples were prepared for SEM as previously noted.

Examination of Fresh Specimen

Dormant forsythia shoots, approximately 15 cm long, were excised from the current season's growth and the basal ends wrapped with a moistened tissue. Samples were placed in an aluminum foil envelope and put into a dewar flask. Tissue temperature was monitored with a copper-constantan thermocouple. The dewar flask was placed in a −15°C freezer and incubated overnight. The following day, buds were excised at −15°C and placed on a cold surface and bisected longitudinally. Buds were viewed using a dissecting microscope. Al-

though initially frozen, buds would thaw during the examination. The location of large ice crystals and the voids which their formation created could be readily observed.

RESULTS

Forsythia flower buds developed during the summer in the leaf axils and overwintered to produce flowers the following spring. Although more than one bud can be borne at a single leaf axil, each individual bud contained a single developing floral organ which produced a single flower. By early autumn, buds had differentiated such that the anthers, pistil, and rudimentary sepals and petals could be distinguished (Fig. 1).

In the early fall the dormant flower buds had only limited freezing resistance. Resistance increased as fall progressed and when fully hardened, buds survived exposure to −20°C. DTA of hardy excised flower buds in midwinter distinguished two distinct freezing events (Fig. 2). The first was initiated at approximately −7°C, while the second appeared as a sharp spike between −20 and −25°C. In some instances a small sharp peak was closely associated with the second exotherm (Fig. 2). In thermal analysis experiments where buds remained attached to an 8 cm long shoot, the first exotherm was detected just below 0°C and was coincident with the freezing of water in the attached woody tissue. This treatment did not appear to affect significantly the temperature of the second freezing event in cold hardy buds (data not presented).

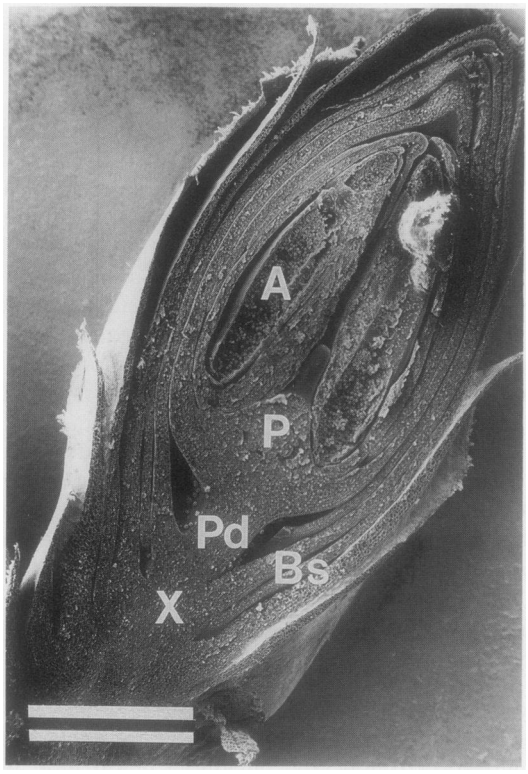


Figure 1. SEM of dormant forsythia flower bud harvested in October prior to the first frost. Bud was bisected longitudinally, coated, and viewed at 10 kV. Note developing floral organ with anthers (A), pistil (P), and peduncle (Pd). Also note the surrounding bud scales (Bs) and subtending tissue designated as the bud axis (X). Scale = 1 mm.

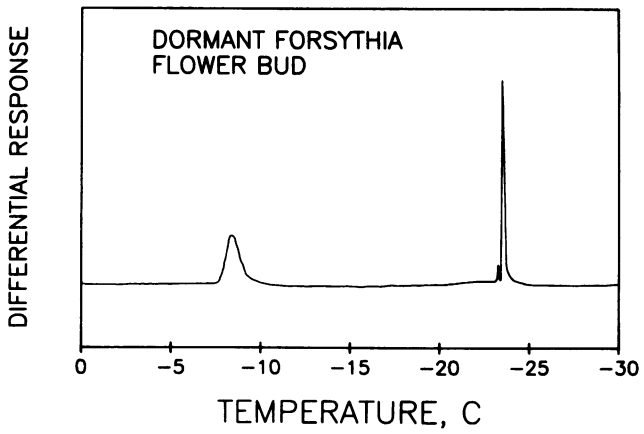


Figure 2. DTA of dormant forsythia flower bud. Bud was excised in January and cooled at 2°C/h during analysis.

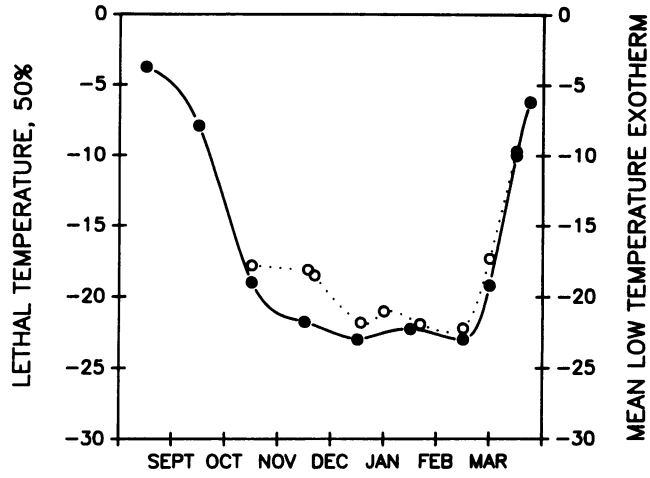


Figure 3. Seasonal changes in cold hardiness of forsythia flower buds. Plot includes both the lethal temperature for 50% survival (●—●) as determined by controlled freezing tests and the mean temperature of the low temperature exotherm (O····O) as determined by thermal analysis.

Both bud freezing resistance and the temperature at which the second freezing event was initiated varied seasonally (Fig. 3). In late summer, buds had only limited cold hardiness, and a distinct low temperature exotherm could not be detected. As the fall progressed, bud cold hardiness increased and deep supercooling was observed. The temperature of the low temperature exotherm was correlated with the lethal low temperature of forsythia buds throughout the remainder of the season (Fig. 3).

The relationship between the low temperature exotherm and the lethal temperature was further evaluated by cooling buds to temperatures which were estimated to represent the median temperature of the low temperature exotherm. The freezing of water within individual buds was monitored and a low temperature exotherm was detected in 26 of the 63 buds examined. Buds were then thawed and evaluated individually for survival using tetrazolium chloride staining. Of the 26 buds in which a low temperature exotherm was detected, 25 buds were dead. In contrast, only 12 of the 37 buds

in which no low temperature exotherm was observed were dead. The lack of a complete one to one relationship in the later buds may have resulted from failure to detect low temperature exotherms or that a portion of the buds were dead prior to the beginning of the experiment.

Although the results of this experiment suggested that bud tissues were killed by the freezing of a fraction of supercooled water, it was not clear in which tissue the freezing event occurred. Examination of buds treated with tetrazolium chloride immediately following thawing revealed that although the anthers and pistil were killed, tissues in the lower portion of the developing floral organ and in the rudimentary sepals were densely stained (data not presented). DTA of dissected floral tissues demonstrated that both isolated floral organs and isolated anther tissues could supercool to approximately −20°C prior to freezing.

To examine the distribution of ice in forsythia buds, frozen buds were bisected and examined with a dissecting microscope. Large ice crystals were observed within the lower portions of the bud (Fig. 4). Both the limited contrast between ice and adjacent tissues and the limited magnification range of the dissecting microscope made viewing difficult. However, by viewing frozen tissues as the ice melted, and by using dissecting tools to extract large crystals, the presence of ice crystals within the bud scales and in the lower portions of the floral organ was noted.

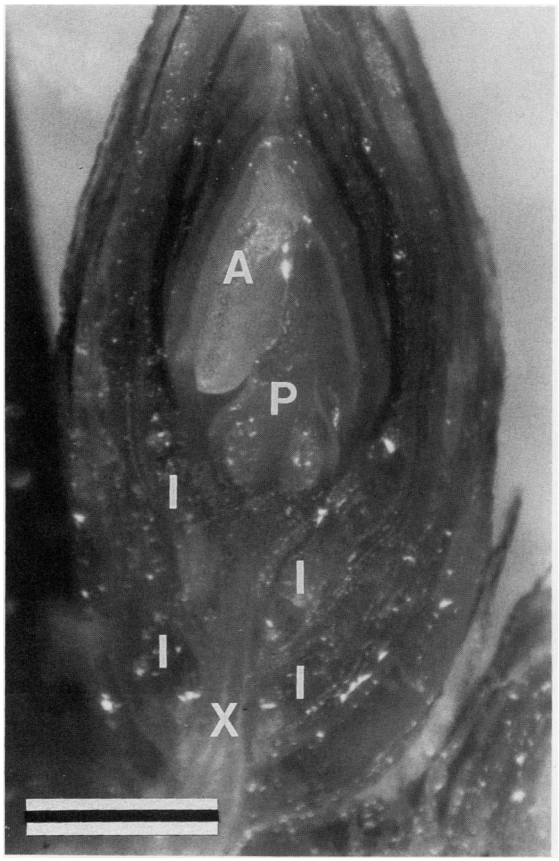


Figure 4. View of frozen forsythia bud. Bud was frozen, bisected longitudinally, and photographed prior to melting. Ice crystals (I) can be noted in the lower portions of the bud. Also note the anther (A), pistil (P), and subtending axis tissue (X). Scale = 1 mm.

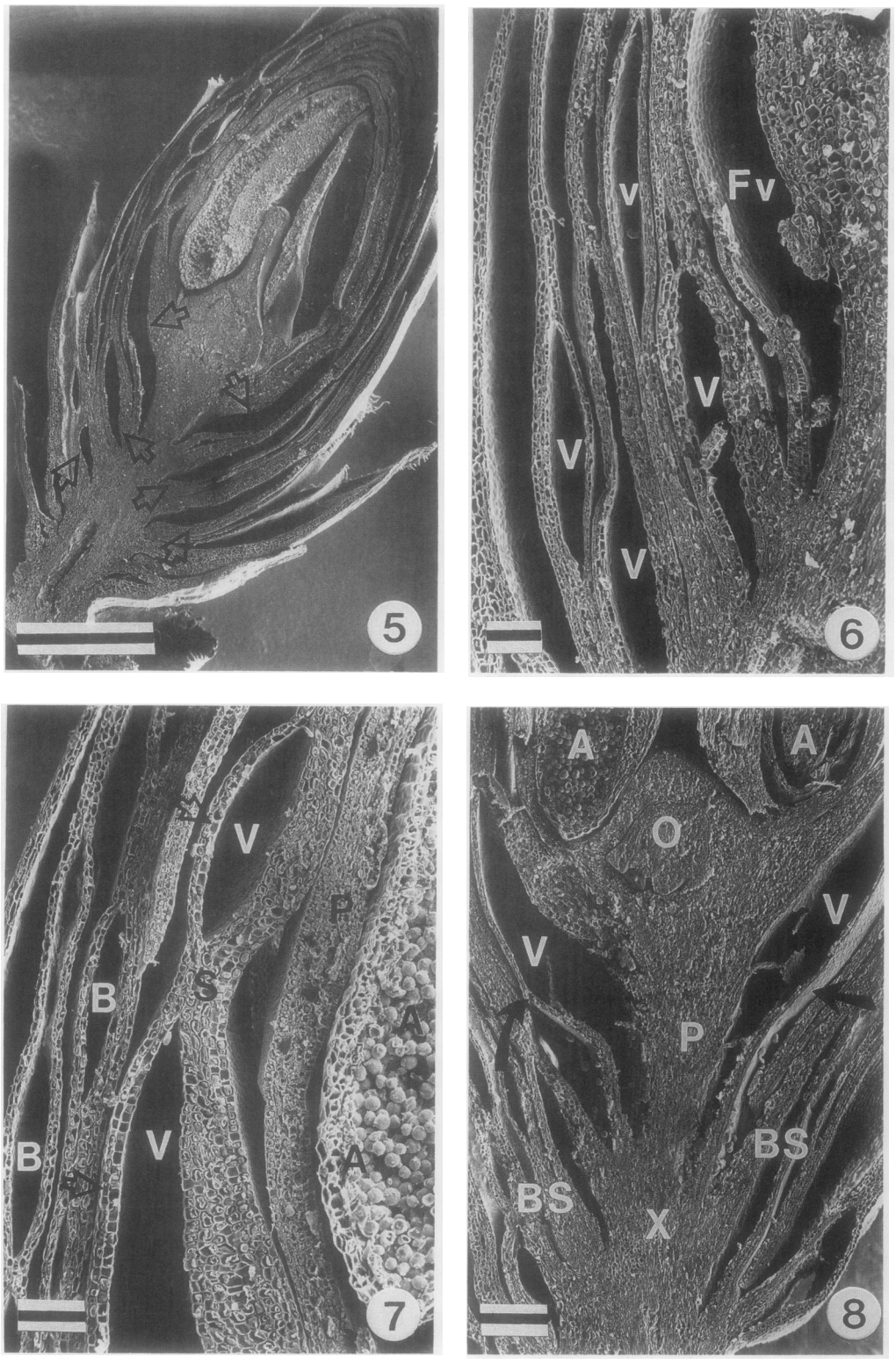
Isothermal freeze-fixation was used to further examine the distribution of ice crystals within forsythia flower buds. This technique permitted examination at higher magnification, and tissues could be viewed without the obstruction of extracellular ice crystals. Buds were frozen and fixed at various times throughout the year and compared to conventionally fixed tissues. When forsythia flower buds were frozen at −5°C and chemically fixed while frozen, several changes in bud structure were noted (Fig. 5). Prominent voids were observed within the bud scales and in the lower portions of the developing floral organ. Previous work on peach flower buds had demonstrated that these tissue voids corresponded to the location of large extracellular ice crystals (4). This was also confirmed in forsythia by the dissection and examination of frozen buds. The freeze-fixation protocol apparently preserved the morphology of the tissue in the frozen state. Therefore, areas where large extracellular ice crystals had formed appeared as voids.

The distribution of voids within the freeze-fixed forsythia buds suggested that ice had not formed uniformly throughout the bud, but instead formed in localized sites. In the bud scale tissues, voids were observed within the scales (Fig. 6). By examining the surfaces of the bud scales, and noting that the surrounding scale tissue joined above and below the voids, it was concluded that these voids had resulted from crystals forming within individual scales, rather than between scales. Voids were observed throughout the bud scale tissues (Figs. 5–10). Larger voids were apparent in the basal portions of the bud scales (Fig. 5) but smaller voids were observed at various locations in the scales (Figs. 5–7), including the uppermost portions of the tissue (Fig. 10). In all instances, the bud scale tissue was split and cell layers were separated.

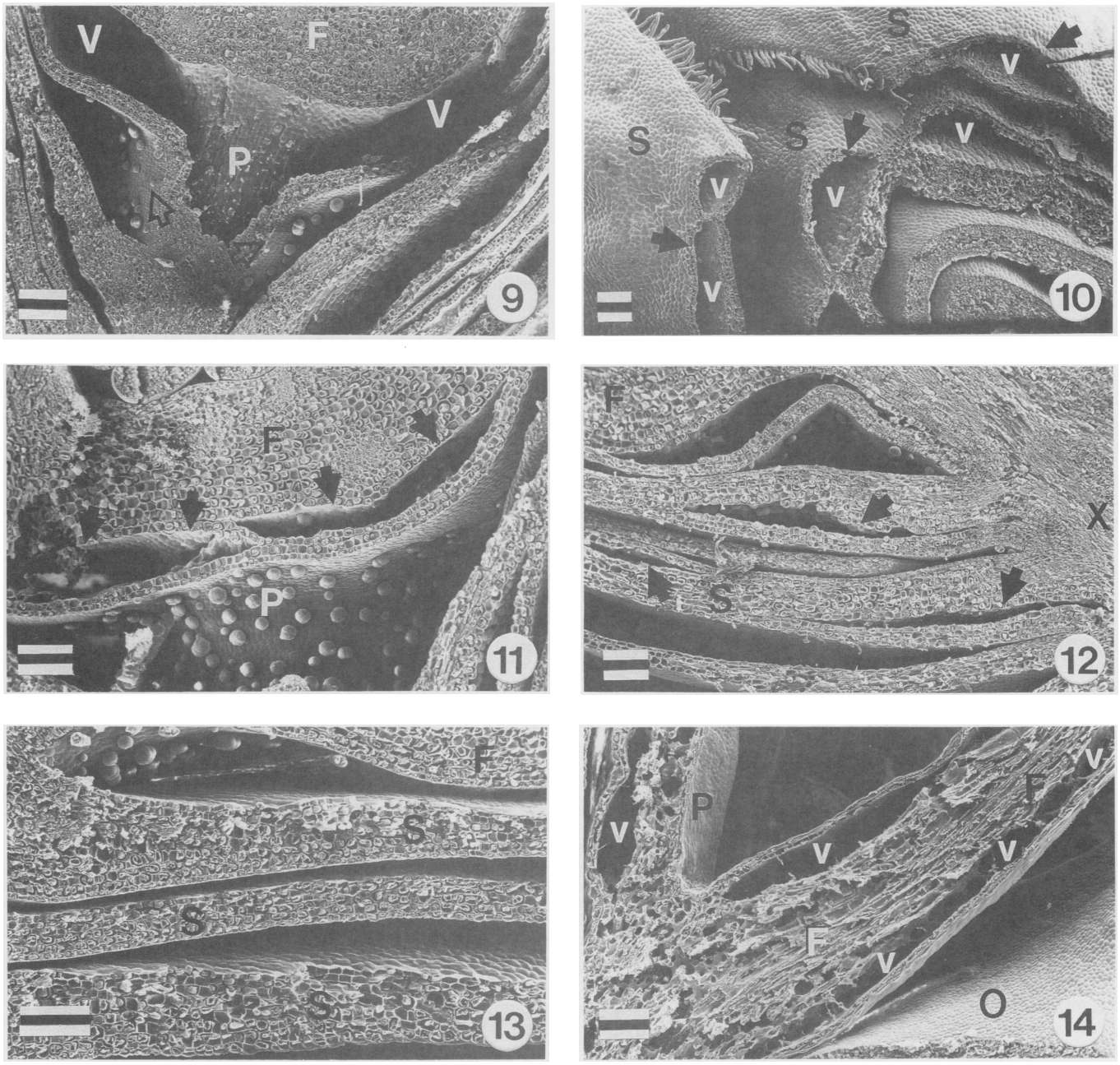
The largest voids appeared in the lower portions of the developing floral organs and in the peduncle (Figs. 5, 8, and 9). These voids appeared as blisters just below the epidermal layer (Fig. 8) and separated the upper two to three cell layers from the subtending tissue (Figs. 7–9). Smaller voids were also observed further up into the developing sepals (Fig. 7). Again, a blistering and separation of the outer two to three layers of cells from the adjacent tissue was noted. Neither intercellular voids nor other evidence of ice formation were observed within the developing petals, pistil and anthers until bloom.

The observations reported were consistent throughout the dormant season. Buds were sampled at monthly intervals and freeze fixed at −5°C. In all instances, the pattern and distribution of voids was similar. These observations were not artifacts of the freeze-fixation treatment or the laboratory freezing protocol. Buds which were harvested at subzero temperatures and processed using freeze substitution techniques appeared similar (data not presented). In addition, direct observations of frozen buds also corroborated these observations, although with far less visible detail.

The formation of large extracellular ice crystals apparently led to persistent mechanical disruption within forsythia flower buds. Buds harvested and conventionally fixed on two sampling dates prior to the first natural freeze appeared intact



Figures 5–8. SEM of dormant forsythia flower buds. Buds were freeze-fixed at −5°C, bisected longitudinally, coated, and viewed at 10 kV.
Figure 5. Overview of bud freeze-fixed in December. Note prominent voids within both the lower portions of the developing floral organ and the bud scales (*arrows*). Scale = 1 mm.
Figure 6. View of voids (V) within the bud scales of a bud freeze-fixed in December. Scale tissue surrounds the void indicating that ice crystals formed within the scales rather than between scales. Also note large void (Fv) within the floral organ. Scale = 100 μm.
Figure 7. View of large voids (V) within the developing floral organ of a bud freeze-fixed in December. Voids present in the lower portions of the developing flower and in the rudimentry sepals (S). Note blistering of epidermal layer (*arrows*). Voids were also observed within the adjacent bud scales (B), but not in the developing anther (A) and petal (P) tissue. scale = 100 μm.
Figure 8. View of large voids (V) within the peduncle (P) and lower portions of the developing floral organ. Bud was freeze-fixed in October. Note separation of the epidermal layer (*arrows*). Smaller voids can also be viewed within the bud scales (Bs). Ovary (O), bud axis (X), and portion of the anthers (A) also in view. Scale = 200 μm.



Figures 9–14. Scanning electron micrographs of forsythia flower buds. Buds were bisected longitudinally, coated, and viewed at 10 kV.

Figure 9. View of peduncle region of a bud freeze-fixed at −5°C in December. Note separation of epidermal tissue (*arrows*) from the subtending peduncle (P) and floral (F) tissues and the creation of a large void (V). Scale = 100 μm.

Figure 10. View of upper portions of the bud scales of a bud freeze-fixed at −5°C in January. Note voids (V) within the scale tissues (S) and separation of cell layers (*arrows*) due to ice crystal formation. Scale = 100 μm.

Figure 11. View of lower portion of developing flower bud fixed in December with FAA. Epidermal surface of the peduncle (P) and longitudinal section through the developing floral organ (F) visible. Note splits (arrows) in floral tissue separating the epidermis from the subtending cells. Scale = 100 μm.

Figure 12. View of bud scale (S) tissues from bud fixed in December with FAA. Portion of bud axis (X) and developing floral organ (F) visible. Note splits (*arrows*) within scales. Scale = 100 μm.

Figure 13. View of bud scale (S) tissues from bud fixed with FAA in early October, prior to the first frost. Note absence of splits and voids within the scales. Portion of the developing floral organ (F) also visible. Scale = 100 μm.

Figure 14. View of lower portions of the anther filament (F) and petal tissue (P) of −5°C freeze-fixed forsythia blossom at full bloom. Note voids (V) separating the epidermal layer from the subtending tissues and evidence of mechanical disruption of the tissue by ice formation. Outer surface of ovary (O) also visible. Scale = 100 μm.

with no evidence of voids or splits within the tissue (Fig. 1). In contrast, specimen collected at these same two time periods and freeze-fixed at −5°C exhibited the characteristic pattern of voids described earlier. This suggested that the voids were produced during the freezing treatment, and were not the result of a developmental modification of bud structure to accommodate ice crystal growth. It was also noted that once field grown plants had been exposed to a natural freeze, thin splits and small voids were visible even within conventionally fixed buds. The location of these tissue disruptions corresponded to areas where large voids had been observed in freeze-fixed tissues. Small voids were observed in the lower portions of the developing flower and in the peduncle region (Fig. 11). These voids occurred just below the epidermal layer and separated the outer two to three cell layers from the subtending tissues. Splits and small voids were also observed within the bud scale tissues (Fig. 12). The splits ran parallel to the axis of the scale and the location of these splits was apparently not related to any preformed structure within the tissue (Fig. 13). The wounds in both the floral organ and the scales persisted throughout the winter and could be observed at full bloom. No apparent wound healing response was noted. It is doubtful that these wounds were artifacts of specimen preparation. The wounds were not observed prior to the first natural freeze and were subsequently observed in all specimens. In addition the pattern and extent of the splits and small voids were observed in fresh unfixed specimens, in chemically fixed specimens and in freeze substituted specimens. Instead it appeared that these wounds were formed during freezing and that ice probably reformed in these locations during subsequent freeze-thaw cycles.

As bud development proceeded in the spring, forsythia flower buds became progressively more susceptible to low temperatures (Fig. 3). This loss of winter hardiness was accompanied by a decrease in the extent of supercooling and eventually a complete loss of the supercooling characteristic. During the deacclimation process, the distribution of ice within the tissue was similar to that observed in cold hardy buds. Examination of freeze fixed buds still revealed the presence of large voids within the bud scales and in the lower portions of the developing flower and in the peduncle region (data not presented). Voids or other evidence of extracellular ice formation were not observed within the pistil. The only apparent difference noted were voids observed within the anther filaments and in the petal tissues at bloom (Fig. 14). Ice had apparently formed below the epidermis and the growth of ice crystals separated the outer two to three cell layers from the subtending tissues. As noted in other tissues of the forsythia bud, the formation of large extracellular ice crystals led to a mechanical disruption of the tissue (Fig. 14).

DISCUSSION

Water within cold-acclimated forsythia flower buds froze as two distinct components. The first freezing event was initiated near the melting point, while the second occurred at lower temperatures and corresponded to the freezing of a fraction of supercooled water. Parallel thermal analysis experiments and cold hardiness determinations demonstrated that the second freezing event was correlated with the lethal temperature of the bud tissue. These observations were similar to those reported for buds from a number of woody plant species (1, 6, 8, 10, 14).

The freezing of water in forsythia buds was also spatially separated. An examination of both frozen buds and freeze-fixed tissues noted that ice crystals were segregated into specific locations within the bud rather than being uniformly distributed throughout the tissue. Upon freezing, large extracellular ice crystals formed within the bud scale tissues and in the lower portions of the developing floral organ. The formation of these crystals apparently corresponded to the initial freezing event noted in thermal analysis experiments. Based on the large size of these ice crystals, it was inferred that once freezing was initiated, water could migrate from adjacent tissues to the growing ice crystals. This led to the formation of large voids within the freeze-fixed buds and a blistering of the epidermal surface.

The segregation of ice into discrete locations within bud tissues has been reported in a number of woody species (2, 5, 9, 10, 15, 17, 18). In these buds, ice was observed within the scales, crown, and other tissues adjacent to, but outside the developing floral organs. This pattern of freezing has been termed "extraorgan" freezing (9), and the segregation of ice during freezing appeared to be critical for the survival of the developing flower. It was envisioned that once ice formation was initiated within the bud scales, water would be withdrawn from the developing floral organ and crystallize out in the bud scales and subtending tissues. In some species, dehydration of the floral tissues would preclude ice formation, whereas in others, sufficient dehydration would not occur during cooling and an isolated fraction of water within the floral organ would supercool. If in these later species, ice formation was initiated within the supercooled floral tissues, ice would rapidly spread throughout and the developing flower would be killed as a unit. The developing floral organs of these species apparently lacked any tolerance to ice formation (6, 8, 9, 10, 15). This led investigators to propose that some form of barrier must exist to prevent the spread of ice into the floral tissues. It was envisioned that this barrier must be organized at the tissue level and effectively isolate the supercooled water in the floral organs from the ice in adjacent tissues. The exact nature of this barrier has not been established although several possibilities have been suggested (1, 6, 10, 15).

Although both the deep supercooling phenomena and the segregation of ice into discrete zones has been observed in forsythia, the response to freezing was distinct from earlier reports. Unlike other species which exhibit supercooling, ice was observed within the developing floral organs of forsythia at −5°C. Ice crystals formed in the lower portions of the developing flower and in the peduncle. The formation of ice in these regions led to blistering of the epidermal layer and a disruption of bud structure. These wounds persisted throughout the season and were visible at bloom. Apparently these wounds had no adverse effect on bud viability.

These observations on the freezing of forsythia buds have further complicated concepts on the supercooling of water in overwintering flower buds. Since ice had formed within the developing floral organ at −5°C, it was not clear what fraction of water within the bud was responsible for the low tempera-

ture exotherm. One possibility would be the water within the anthers and pistil. Several lines of evidence support this idea. It was noted that injury to these tissues was correlated with the occurrence of the low temperature exotherm. Furthermore, at full bloom when buds no longer exhibited supercooling, evidence of ice formation within the anther filaments was noted. In addition, DTA experiments with dissected anthers noted a single freezing event at temperatures similar to the low temperature exotherm observed when intact buds were frozen (data not presented). However, these DTA studies were not conclusive. Both equipment limitations and the presumed differences in heat transfer between isolated anthers and anthers enclosed within bud scales, prevented quantitation of the amount of water freezing in these experiments. In addition, each forsythia bud has two anthers of similar size mounted on separate filaments. Since two distinct low temperature exotherms were not observed then ice would need to be initiated in both anthers simultaneously.

If in fact water within the anthers and pistil supercooled to low temperatures prior to freezing and they were subsequently killed when freezing was initiated, then what prevented ice from spreading from adjacent parts of the flower and nucleating ice formation? In other species, it was proposed that some form of barrier separated the developing floral organ from ice in adjacent tissues, and that once introduced into the floral tissues ice would spread rapidly. Some feature of the forsythia bud permitted a fraction of water to supercool to low temperatures despite the presence of ice in adjacent portions of the flower. One possibility was that the nucleation barrier was organized at the cellular level as in the xylem ray parenchyma cells of many hardwood trees (4, 7). This appeared unlikely since the shape of the low temperature exotherm in forsythia buds indicated that when freezing was initiated, a fraction of supercooled water froze abruptly. This response would not be anticipated if cells were freezing as individual units. The low temperature exotherm in hardwood tissues occurred over a broader temperature range as a population of individual cells froze independently (4, 7).

While unable to address many of the previously mentioned questions, it was clear that ice formed within the developing floral organs of overwintering forsythia buds, and that this pattern of ice formation was distinct from that reported in other species which exhibit deep supercooling (2, 6, 9, 10, 15). Further work will be required to establish what features of the forsythia bud influence the formation and spread of ice within the tissue.

ACKNOWLEDGMENTS

The author would like to thank Tom Willard for technical assistance; Deb Altman for assistance in preparing the manuscript; and Dr. Charles Bracker and Dick Bentlage of the Purdue University Electron Microscopy Center for their help and cooperation.

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