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Supercooling in Dormant Flower Buds of *Forsythia,* and the Correlation between Pistil Size and Bud Hardiness[1]

Cindy L. Flinn and Edward N. Ashworth[2]
Department of Horticulture and Landscape Architecture
Purdue University, West Lafayette, IN 47907-1165

Abstract

Experiments were conducted to determine if dormant buds of *Forsythia* taxa exhibit the deep supercooling characteristic. Specimens were collected from thirteen *Forsythia* taxa including: *F. suspensa* (Thunb.) Vahl, *F.* x *intermedia* cv. Spectabilis (Koehne), *F*. x *intermedia* cv. Lynwood (G.E. Peterson), *F. europaea* (Degen and Baldacci), *F. giraldiana* (Lingelsh), *F. japonica* (Makino) var. *saxatilis* (Nakai), *F. mandshurica* (Uyeki), *F. ovata* (Nakai), *F. suspensa* var. *fortunei* (Lindl.), *F. viridissima* (Lindl.), *F*. x *intermedia* cv. Arnold Giant (Sax), *F*. cv. Arnold's Dwarf, and *F*. cv. Meadowlark (Flint). Buds and attached stem segments, were cooled at 2C (3.6F) per hour, and the temperature at which freezing occurred was determined by thermal analysis. Typically, two distinct freezing events were detected within *Forsythia* buds. The first freezing event, or high temperature exotherm, occurred just below 0C (32F), while the second freezing event, or low temperature exotherm, occurred between –16C (3.2F) and –28C (–18.4F). The low temperature exotherm corresponded to the freezing of supercooled water within dormant buds, and the detection of low temperature exotherms in buds of all 13 *Forsythia* taxa indicated that deep supercooling is common among members of this genus. In nine of the 13 *Forsythia* taxa, the temperature of the low temperature exotherm was an accurate indicator of bud freeze-tolerance (LT_{50}), as determined by a laboratory freeze-stress protocol. The discrepancies noted in the other four taxa were apparently due to the occurrence of field freezing injury prior to conducting these laboratory studies. Evidence indicated a relationship between the extent of supercooling and the size of the pistil in dormant *Forsythia* buds.

Index words: freezing injury, cold hardiness, supercooling, *Forsythia*.

Species used in this study: *Forsythia suspensa, F*. x *intermedia, F. europaea, F. giraldiana, F. japonica* var. *saxatilis, F. mandshurica, F. ovata, F. suspensa* var. *fortunei, F. viridissima*.

Significance to the Nursery Industry

Temperate perennial landscape plants are exposed to harsh environmental conditions as they over-winter. Low winter temperatures can injure shoots, kill dormant flower buds, and desiccate leaves. Winter injury can cause losses in over-wintering nursery stock, and in established landscape plantings. In addition, low winter temperatures have an indirect effect on the nursery/landscape industry, since minimum winter temperatures generally dictate the regions in which species and taxa can be successfully grown. Breeding and selecting cold hardy landscape plants is a goal of many research programs. Such plant improvement programs often rely on laboratory freeze tests or survival during 'test' winters as a means to evaluate plant cold hardiness. The former method is labor intensive, whereas the latter approach requires numerous years of testing, and generally does not provide an indication of the extent to which different taxa vary in freeze-tolerance. An alternative approach is to utilize thermal analysis techniques as a rapid screen for bud hardiness. In this paper, we show that flower buds of *Forsythia* taxa survive freezing by supercooling and that measuring low temperature exotherms by thermal analysis could be used to evaluate flower bud cold hardiness.

Introduction

Low temperature injury in the over-wintering flower buds of many woody species is the result of ice formation within developing floral organs (8, 10, 20, 27, 31, 32, *among others*). Avoiding ice formation within these tissues is critical for winter survival, and two mechanisms that enable developing floral organs to avoid freezing, and thus facilitate winter survival, have been described (12, 24). These two mechanisms, referred to as deep supercooling and extra-organ freezing, are similar in that ice formation in tissues adjacent to the floral organs facilitates the redistribution of freezable water from freeze-sensitive pistils and stamens (4, 6, 12, 13, 21, 24, 28). In buds that deep supercool, a fraction of water within the floral organs remains liquid at temperatures well below freezing (2, 8, 10, 12, 20, 24, *among others*). Lethal injury in these buds occurs when the supercooled water freezes, nucleated either by the spread of ice from surrounding tissues, or by heterogenous or homogeneous ice nucleation within floral organs (2, 8, 10, 12, 20, 24, *among others*). The freezing of supercooled water within plant tissues can be detected as a low temperature exotherm by using thermal analysis. In contrast, tissues that undergo extra-organ freezing do not exhibit a low temperature exotherm and, depending upon the taxon, may survive temperatures as low as –60C (–76F) (8, 12, 16, 28), well below the homogenous ice nucleation point. During extra-organ freezing, all freezable water is apparently redistributed from the developing floral organ to ice sinks (8, 12, 28).

Over-wintering buds of many important horticultural species deep supercool as a mechanism of avoiding freezing

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[2]Graduate Assistant and Professor, respectively.

injury (10, 17, 18, 19, 20, 22, 23, 26, 27, 30, 31, 32). Consequently, the quest to improve freezing tolerance among these species has prompted many researchers to investigate the physiological and morphological features that facilitate deep supercooling (2, 3, 5, 7, 9, 11, 21, 25). These investigations have been aided by the identification of both supercooling and non-supercooling species within the genus *Prunus* (5, 8, 15, 16, 20). One primary difference between supercooling and non-supercooling *Prunus* taxa is flower morphology. Dormant flower buds of supercooling taxa typically have large, well-developed floral organs (5, 8, 16, 24) in contrast to the small, rudimentary organs on the racemose inflorescence of the non-supercooing *Prunus virginiana* and *P. padus* (5, 8, 16). This led to the hypothesis that the morphology and size of the dormant floral organs are associated with the supercooling characteristic. If this hypothesis is correct, selection for smaller dormant floral organs might facilitate the development of taxa that do not supercool, and might, therefore, survive temperatures below the homogeneous ice nucleation point (–40C, 40F).

The *Forsythia* genus is an excellent system in which to test the hypothesis that the size of dormant flowers is associated with the presence of the deep supercooling characteristic. The 13 *Forsythia* taxa examined in this study possess a wide range of flower sizes and levels of cold tolerance. At present only one taxon, *F.* x *intermedia* cv. Spectabilis has been examined for the deep supercooling characteristic (4, 17). Therefore, our objectives were to 1) determine whether deep supercooling is a common feature among the genus *Forsythia*, 2) determine the relationship between any observed low temperature exotherms and low temperature injury, and 3) investigate the relationship between the size of dormant pistils and the extent of supercooling.

Materials and Methods

Plant material. Thirteen *Forsythia* taxa were evaluated. Three of the taxa, *F. suspensa* (Thunb.) Vahl, *F.* x *intermedia* cv. Spectabilis (Koehne), and *F*. x *intermedia* cv. Lynwood (G. E. Peterson), were collected from plantings on the Purdue University campus, West Lafayette, IN. Specimens of the other 10 taxa, *F. europaea* (Degen and Baldacci), *F. giraldiana* (Lingelsh), *F. japonica* (Makino) var. *saxatilis* (Nakai), *F. mandshurica* (Uyeki), *F. ovata* (Nakai), *F. suspensa* var. *fortunei* (Lindl.), *F. viridissima* (Lindl.), *F*. x *intermedia* cv. Arnold Giant (Sax), *F*. cv. Arnold's Dwarf, and *F*. cv. Meadowlark (Flint) were obtained from the Arnold Arboretum, Jamaica Plain, MA. Specimens from the Purdue campus (Purdue taxa) were collected on January 15, 17, and 19, 1992. During collection, the terminal sections of each taxon were sealed in plastic bags with damp paper towels, packed in ice and transported to the laboratory within 1 h of collection. On February 5, 7, and 9, three separate shipments of *Forsythia* specimens were received via over-night express from the Arnold Arboretum (Arnold taxa). Collection and shipment of the 10 Arnold taxa were randomized, and duplicate sets of each taxon were shipped during the sampling period. Thus, each shipment contained terminal shoots of six to seven taxa that had been sealed in plastic bags with damp paper towels and stored at 4C (39F) prior to shipment without ice. Upon arrival, specimens from both locations were processed immediately by cutting stems into 5-cm (2-in) long sections, each with 3 to 10 flower buds. Stem sections were either resealed in plastic bags and stored at 4C (39F) for approximately 14 hr, or used immediately in thermal analysis experiments.

Thermal analysis. To determine whether dormant buds from each taxon exhibited the deep supercooling characteristic, a modified version of the thermal analysis procedure described by Ashworth (4) was used. All flower buds except one were excised from four processed stem sections of each Purdue taxon and from six stem sections per each Arnold taxon. A 30-gauge (0.25-mm-diameter) copper-constantan thermocouple was taped to the single bud remaining on each stem segment, and specimens were placed into stoppered test tubes containing 0.5 ml water and a chip of ice. The tubes were then cooled at 2C (3.6F) per hour to –40C (–40F) in a circulating ethanol/ethylene glycol (50:50 by vol) bath (components from Neslab Instruments, Newington, NH). Bud temperatures were recorded every 15 seconds with a computer-based data-acquisition system. The temperature preceding an abrupt increase in bud temperature was recorded as an exotherm, and these were averaged among the 12 replicates for each taxon.

Hardiness determinations. Approximately 14 hr after initiation of thermal analysis experiments, two twigs of each taxon were placed into each of 16 stoppered test tubes along with 0.5 ml of water. Bud temperature was monitored in two separate tubes using 30-gauge (0.25-mm-diameter) copper-constantan thermocouples. Seven tubes were clustered around each reference tube in a circulating bath identical to the one described in thermal analysis experiments. After a 30-min equilibration period at 1C (33.8F), a chip of ice was added to each test tube and specimens were cooled 2C (3.6F) per hour to a range of seven temperatures centered around the taxon's mean low temperature exotherm temperature for that replicate. Sampling temperatures for Purdue taxa spanned 8C (≈14F) with 1C (≈2F) intervals between the five mid-range temperatures. Arnold Arboretum taxa were sampled over a 6C (≈11F) range of 1C (≈2F) temperature intervals. After removal from the bath, buds were thawed in sealed tubes at 1C (33.8F) and subsequently bisected. A half of each bud was stained for viability in 6% aqueous tetrazolium chloride for 24 hr in the dark. Mortality, expressed as a percentage of the total buds sampled, was regressed as a function of temperature, and the resulting equation was solved for the temperature at which 50% of the ovaries were killed (LT_{50}).

Evaluation of low temperature exotherms as an estimate of hardiness. To evaluate whether mean low temperature exotherms are a measure of hardiness among *Forsythia* taxa, mean temperatures of low temperature exotherms were compared to LT_{50} estimates within taxon replications in a split plot design by analysis of variance (SAS PROC ANOVA). For each collection period, the different levels of taxa were blocked by collection date as whole plots, with mean low temperature exotherm temperature and LT_{50} as two levels of the subplot treatment. Significant differences between mean low temperature exotherm temperature and LT_{50} within a taxon were determined by critical t values based on standard errors of the split plot design (29).

Relationship between pistil size and low temperature exotherm temperature. To determine whether pistil size and corresponding low temperature exotherm temperatures were

correlated, a subset of the thawed buds from thermal analysis experiments were excised and fixed at room temperature for 7 days in formalin-acetic acid-alcohol (FAA) (14). Specimens were stored in fixative, and rinsed with distilled water prior to examination. The buds of Purdue taxa were bisected to expose the largest longitudinal pistil area. Buds from Arnold Arboretum taxa were either similarly bisected along the longitudinal axis, or were bisected to expose the largest transverse area. The cut surfaces of bisected specimens were sealed to a glass slide with a viscous solution of 5% Phytagel™ (Sigma Chemical Co., St. Louis, MO) to which 100 mM citric acid and 150 mM ascorbic acid had been added as antioxidants. After the Phytagel solidified, the slides were inverted and photographed with the aid of a dissecting microscope. Pistil images were then copied from 35mm negatives by hand-drawing on clear acetate sheets. The copied images were digitized and processed using an image acquisition and processing program. The relationship of these data with their corresponding LTE temperatures was then analyzed (SAS PROC CORR).

Results and Discussion

Survey of supercooling ability. Over-wintering buds of all the *Forsythia* taxa examined in this study exhibited deep supercooling. Thermal analysis of each taxa typically showed two distinct freezing events. The first exotherm, or high temperature exotherm, was a broad, rounded peak that occurred between 0 and –2C (32–29F) (data not shown). The second exotherm, or low temperature exotherm, was characterized by an abrupt, narrow peak ranging in temperature from –16.0 (3.2F) to –28.2C (–18.8F). Occasionally, among *F*. x *intermedia* 'Spectabilis' and 'Lynwood' buds, a third, smaller exotherm was observed almost coincident with the low temperature exotherm. When these buds were sectioned to identify the source of the third event, two pistils were found in each bud, one of normal size and shape, and the other much smaller and malformed. Only the temperature of the larger low temperature exotherm was used in data analyses. Another anomaly noted in approximately 30% of thermal profiles was the absence of low temperature exotherms. This was observed in all taxa and occurred at a frequency of zero to three buds per replication among Purdue taxa, and at a frequency of zero to four buds per replication among Arnold taxa. The frequency of buds without detectable low temperature exotherms was highest among the third replicated sampling of local taxa, which followed a severe freeze of –22C (–7.6F) on the eve of collection.

Mean low temperature exotherm temperatures ranged among taxa from –18.9C (–2F) for *F*. x *intermedia* 'Lynwood' to –26.7C (–16F) for *F. europaea* (Table 1). Means for the remaining taxa were distributed over a narrow range of 2.4C (4.3F) (Table 1), indicating that hardiness was similar for most of the taxa examined in these experiments.

Relationship between low temperature exotherms and flower bud hardiness. To evaluate whether low temperature exotherm temperatures were indicative of bud death, flower bud hardiness was estimated by assessing bud mortality after exposure to a narrow range of freezing temperatures. The resulting coefficients of determination were typically greater than 0.90, indicating that mortality rates were linearly related to sampling temperatures. An anomalous LT_{50} of –11C (12F) for the third replication of 'Lynwood' was greater than the range of sampling temperatures. Since predicted temperature values were only valid within that range, the erroneous LT_{50} was replaced with –18.5C (–1.3F), an estimate derived from analysis of covariance (PROC GLM, SAS). Comparison of mean LTEs and LT_{50}s within taxa revealed that the two estimates did not differ significantly for nine of the thirteen taxa examined ($P = 0.05$) (Table 1). Significant differences were detected, however, for the following taxa: *F. suspensa*, *F. giraldiana*, *F. mandshurica*, and *F. suspensa* var. *fortunei*, although the largest disparity was only 2.8C (5F) (Table 1).

Table 1. Relationship between mean low temperature exotherm (LTE) temperature and flower bud hardiness (LT_{50}) within 13 *Forsythia* taxa.

Taxa	Method of hardiness estimation: MLTE[z] (C)	LT_{50}[y] (C)	Significance
Purdue campus: January 15–19, 1992			
F. suspensa	–20.6 ± 1.5	–17.8 ± 0.9	*
F. x *intermedia* 'Spectabilis'	–20.2 ± 2.1	–18.8 ± 1.0	NS
F. x *intermedia* 'Lynwood'	–18.9 ± 1.1	–16.9 ± 0.4	NS
Arnold Arboretum: February 5–9, 1992			
F. 'Arnold's Dwarf'	–20.5 ± 0.2	–20.5 ± 0.6	NS
F. 'Arnold's Giant'	–20.9 ± 0.4	–20.0 ± 0.4	NS
F. europaea	–26.7 ± 0.4	–26.7 ± 0.5	NS
F. giraldiana	–22.6 ± 0.2	–21.4 ± 0.1	*
F. japonica var. *saxatilis*	–21.0 ± 1.3	–20.2 ± 0.2	NS
F. mandshurica	–22.0 ± 0.6	–20.1 ± 0.2	*
F. 'Meadowlark'	–21.7 ± 1.1	–21.3 ± 1.2	NS
F. ovata	–22.3 ± 0.8	–21.7 ± 0.4	NS
F. suspensa var. *fortunei*	–21.8 ± 0.6	–20.0 ± 0.1	*
F. viridissima	–20.8 ± 0.3	–19.8 ± 0.3	NS

[z]Mean of 12 LTE temperatures (three replications of four LTE temperatures) and standard deviation.

[y]Mean of two replicates of hardiness evaluation and standard deviation.

[*,NS]Significant, not significant; means within columns were separated using critical t based on standard errors of the split plot design. Critical $t_{05} = 2.29$ for Purdue taxa, 1.29 for Arnold Arboretum taxa.

Table 2. Comparison between pistil size[z] of *Forsythia* taxa collected from the Purdue campus and their corresponding mean low temperature exotherm (LTE) temperatures.

Taxon	Number of observations	x LTE (C)	x Area (mm^2)[y]
F. suspensa	10	–20.3	0.64a
F. x *intermedia* 'Spectabilis'	6	–20.1	0.75a
F. x *intermedia* 'Lynwood'	6	–18.9	0.60a

[z]Pistil size estimated by measuring the area of a longitudinal plane through a bisected pistil.

[y]Means within columns were separated by Bonferonni multiple comparisons test. Values with the same letter were not significantly different (P = 0.05).

Relationship of pistil size to low temperature exotherm temperature. Buds that exhibited low temperature exotherms in thermal analysis experiments were used to examine the relationship between hardiness and pistil size. Size was quantified for two dimensions. In some cases, buds were bisected along the longitudinal axis, and the exposed plane of the pistil was measured. The pistils of all Purdue taxa were measured along the longitudinal plane. The area of the longitudinal plane ranged from 0.46 to 0.94mm^2 (Fig. 1a) within these taxa. Although there was variability in pistil size among the buds sampled, the mean values for the three taxa sampled at Purdue were similar (P = 0.05) (Table 2). Correlation analysis revealed that the size of individual pistils, based upon measurements of the longitudinal plane, was not linearly related to the bud's corresponding low temperature exotherm temperature (r = –0.34, n = 22) (Fig. 1a).

The size of pistils in the Arnold Arboretum taxa were estimated by measuring the longitudinal and transverse plane. Some buds were bisected longitudinally, while the remainder were cut in the transverse orientation. The area along the longitudinal and transverse axis were measured (Table 3). Estimates of mean pistil size obtained by measuring a transverse plane through the pistil ranged from 0.41 to 0.72 mm^2 while estimates obtained by measuring the longitudinal plane ranged from 0.33 to 0.81 mm^2 (Table 3, and Fig. 1). Measurements along one axis did not reliably predict the measurement obtained in the alternate orientation (Table 3). For example, the longitudinally bisected pistils of *F. viridissima*, were among the smallest in the Arnold Arboretum sampling, but were one of the largest based upon a measurement of transverse area (Table 3).

Despite the limitations of these methods in estimating pistil size, low temperature exotherm temperatures were correlated with pistil size, as estimated by measurements along the longitudinal and transverse axis (Fig. 1). The relationship was stronger when pistil size was estimated using transversely bisected pistils (P = 0.001) than estimated along the longitudinal axis (P = 0.01).

The freezing characteristics of buds from the *Forsythia* taxa evaluated in this study were similar to that reported previously for 'Spectabilis' (4, 17) and other supercooling species having a solitary flower (2, 8, 19, 20, 21). Buds of every *Forsythia* taxon studied typically exhibited two freezing events. The first, associated with the formation of ice within the bud scales and the lower portion of the floral axis, was not related to bud hardiness (data not presented). The second freezing event, or low temperature exotherm, coincided with

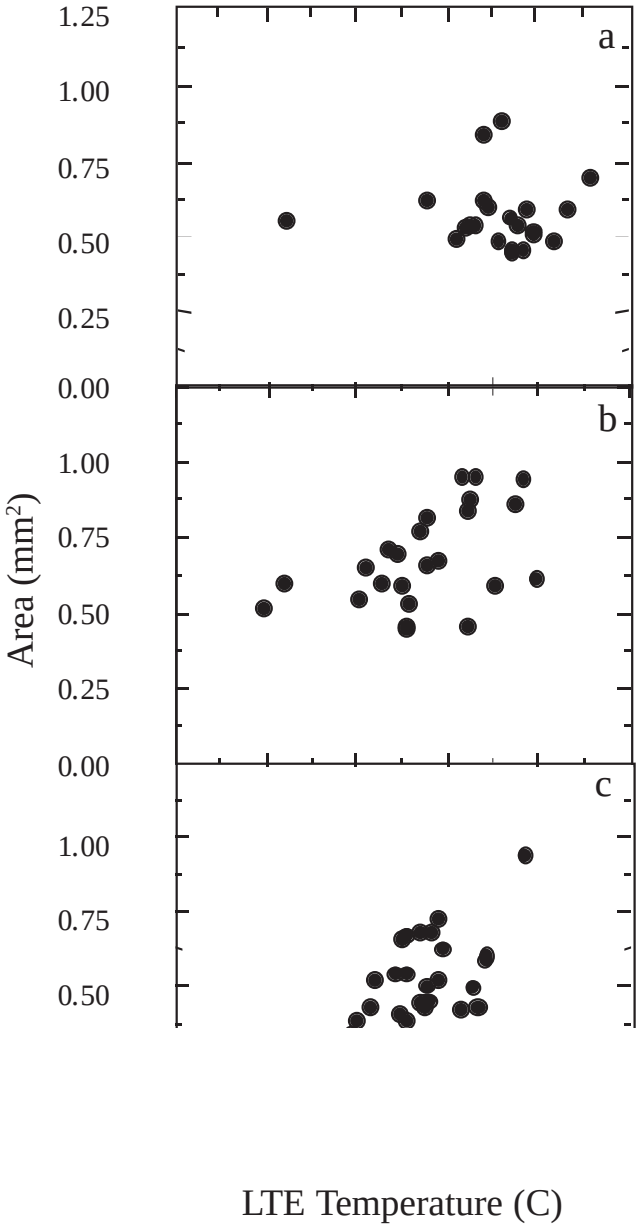

Fig. 1. Relationship between pistil size and low temperature exotherm (LTE) temperature. Although there was no correlation among longitudinal sections of the three Purdue taxa (a) (r = –0.34), longitudinal (b) and transverse (c) sections of the 10 Arnold Arboretum taxa were correlated (r = 0.30; a = 0.05 and r = 0.46; a = 0.001, respectively).

the freezing of supercooled water within portions of the overwintering floral organ.

The absence of low temperature exotherms in the thermal profiles of some individual buds had been previously observed in 'Spectabilis' (4), and among peach (*Prunus persica* (L.) Batsch.) buds (2). In the latter species, profiles without low temperature exotherms were associated with flower buds that had been killed prior to thermal analysis, leading Ashworth (2) to conclude that a viable portion of the bud axis was crucial to supercooling. Two lines of evidence indicate that the absence of low temperature exotherms in thermal profiles of *Forsythia* buds during the current study may have resulted from field injury prior to sampling. First, spot-checking of experimental buds revealed that injury was some-

Table 3. Comparison between pistil size[z] of *Forsythia* taxa collected from the Arnold Arboretum and their corresponding mean low temperature exotherm (LTE) temperatures.

Taxon	Longitudinal			Transverse		
	Number of observations	x LTE (C)	x Area (mm^2)[y]	Number of observations	x LTE (C)	x Area (mm^2)
F. 'Arnold's Giant'		—	—[x]	5	–20.6	0.72b
F. 'Arnold's Dwarf'	5	–19.7	0.81cd	—	—	—
F. europaea	4	–26.4	0.60abcd	—	—	—
F. giraldiana	4	–23.2	0.62abcd	4	–23.1	0.48ab
F. japonica var *saxatilis*	3	–20.2	0.86d	2	–22.0	0.54ab
F. mandshurica	3	–22.5	0.33a	3	–23.2	0.50ab
F. 'Meadowlark'	5	–21.9	0.69abcd	—	—	—
F. ovata	3	–22.0	0.61abcd	4	–22.3	0.41a
F. suspensa var *fortunei*	3	–22.1	0.42ab	3	–21.6	0.44a
F. viridissima	2	–21.3	0.47abc	2	–21.1	0.64b

[z]Pistil size estimated by measuring the area of either a longitudinal or a transverse plane through a bisected pistil.

[y]Means within columns were separated by Bonferonni multiple comparisons test. Values with the same letter were not significantly different ($P = 0.05$).

[x]Data were not available.

times visually undetectable. Consequently, some field-injured buds were inadvertently included among the test specimens in thermal analysis experiments. Second, an increase in the number of profiles devoid of low temperature exotherms was observed among local taxa immediately after a severe freeze in the West Lafayette area. Therefore, the lack of low temperature exotherms among thermal profiles of *Forsythia* taxa may have been an indication of undetected field injury.

The observation that prior field injury affects the results of thermal analysis experiments may explain the significant differences between mean low temperature exotherm temperatures and LT_{50} among *F. suspensa*, *F. giraldiana*, *F. suspensa* var. *fortunei*, and *F. mandshurica* (Table 1). Since LT_{50}s were qualitative measures of mortality, discrimination between death due to experimental treatment and field injury was not possible. The possibility that a portion of the buds had been freeze-killed prior to our investigations was supported by the observation that LT_{50}s were typically higher than the corresponding mean low temperature exotherm temperatures for taxa from both locations (Table 1). Furthermore, when mortality rates for a given replication were adjusted downward to exclude the percentage of buds that were devoid of low temperature exotherms, the resulting LT_{50} estimates were not different from mean low temperature exotherm temperatures. Therefore, since it appears that hardiness was underestimated by LT_{50}s, mean low temperature exotherm temperatures may have provided a more accurate estimate of flower bud hardiness among the 13 *Forsythia* taxa examined.

Discrepancies between LT_{50} and mean low temperature exotherm temperatures were reported at various times throughout dormancy in flower buds of sweet cherry (*Prunus avium* L.) (1) and 'Spectabilis' (4). Although the magnitude of the disparities was as large as the maximum disparity in the current study, the researchers found that the linear relationship between the estimates over time was not adversely affected (1, 4). Therefore, they arrived at a similar conclusion, as did we, that mean low temperature exotherm temperatures provided an accurate assessment of flower bud hardiness.

Previously, researchers had observed that conifer (28) and *Prunus* taxa (5, 8, 16, 24), which differed in flower bud hardiness, also varied in morphological features. Generally, the hardiest taxa had the smallest floral organs, and low temperature exotherms were often not detected when these buds were frozen. In the current study, a correlation between pistil size and the extent of supercooling was noted only among some *Forsythia* taxa (Fig. 1b–c), even though all 13 taxa supercooled (Table 1). What mechanism might account for the correlation between flower bud hardiness with pistil size? One possibility is related to the potential speed that water can be withdrawn from the floral organ and crystallized into extra-organ ice. The smaller floral organs of the hardier buds contain less water and have a greater surface area. This combination would facilitate a more rapid redistribution of water from floral organs to ice sinks in adjacent tissues than would occur in larger buds (6, 8, 12, 16, 21, 24, 25, 28). This hypothesis is consistent with observations reported for buds of *P. pennsylvanica* L., which were typically hardy to –25C (–13F) (8). Following exposure to sub-lethal freezing temperatures, buds of this species no longer exhibited a low temperature exotherm, but were able to withstand temperatures of –80C (–112F) without apparent injury (8). The exposure to sub-lethal freezing temperatures had apparently enabled sufficient water to leave the floral tissues during extra-organ freezing so that supercooling and ice nucleation within the floral organ were avoided, and buds survived to much lower temperatures. The corollary of this hypothesis would be that larger floral organs cannot lose sufficient water during extra-organ freezing, and are thus prone to injury at warmer temperatures.

The strength of the correlation between *Forsythia* hardiness and pistil size was likely affected by the method used to quantify pistil size in the current study. We did not measure the volume of the pistil, but instead estimated pistil size by measuring the cross-sectional area of pistils that had been bisected along either the longitudinal or transverse axis. Such estimates assume that pistils of different taxa have similar shape. Unfortunately, this does not appear to be true, since a ranking of pistil size among taxa varied depending upon whether estimates were based upon longitudinal or transverse estimates. In addition, accurate and repeatable quantification of longitudinal area was affected by both the ability to cut directly through the middle of each bud, and the ability to delineate the proximal end of the pistil where it adjoined with the bud axis. Fortunately, the boundaries of transverse

pistil areas were less subjective, and were determined by the circumference of the pistil.

In summary, all 13 *Forsythia* taxa examined in the present study exhibited the supercooling characteristic. Due to the freezing characteristics of *Forsythia* buds, thermal analysis has the potential to be a useful technique for evaluating bud cold hardiness, since mean low temperature exotherm temperature provided an accurate assessment of hardiness in most of the taxa examined. A correlation between pistil size and bud hardiness was also observed in this study. However, breeding for reduced pistil size in *Forsythia* would not necessarily result in hardier taxa. This was illustrated by our observation that the hardiest taxon in our study, *F. europaea*, did not have the smallest pistils (Table 3). Thus, other features of *Forsythia* buds affect hardiness, and these features need to be identified.

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