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Stem and Flower Bud Hardiness of Deciduous Azaleas[1]

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Abstract

Stem and flower bud hardiness of five deciduous azalea (*Rhododendron* sp.) clones were compared on six dates during the dormant period of 1992–1993. Visual evaluation and a specific conductance technique were compared on four dates as methods of evaluating freezing injury of stems. With a single exception, stems were always more hardy than the corresponding florets. Stems acclimated more rapidly in the fall and were from 3–15C (5–27F) more hardy than florets on two November sampling dates. Stems and florets of all clones achieved their maximum hardiness levels in January. With the exception of 'Spicy Lights' , the maximum midwinter hardiness obtained by florets was 2–4C (4–7F) less than that of the corresponding stems. Midwinter stem hardiness was greatest in 'White Lights' and 'Mandarin Lights' [–40C (–40F)] while 'Spicy Lights' exhibited the greatest floret hardiness [–40C (–40F)]. Florets deacclimated substantially more than stems between January 25 and March 17. Rates of deacclimation in stems and flower buds were similar between March 17 and April 14, but stems were still significantly more hardy than florets on April 14. Visual ratings and specific conductance measurements provided similar estimates of hardiness in most, but not all cases. Use of visual observation for evaluating freezing injury of azalea stems is recommended based upon the relative ease and efficacy of this technique.

Significance to the Nursery Industry

Limited cold hardiness is one factor precluding more widespread use of azaleas and rhododendrons in much of the northern United States. To date, breeding programs at the University of Minnesota Landscape Arboretum and the University of Helsinki have successfully developed a number of cold-tolerant cultivars. This has been accomplished, however, without a clear understanding of the distinct hardiness characteristics of stems and flower buds. Results of this study indicate that both stems and flower buds of the clones tested possess sufficient cold hardiness to survive typical minimum winter temperatures in central Minnesota, but that flower buds can be substantially more vulnerable to injury than stem tissues. Characterization of flower bud hardiness of potential germplasm should enhance efforts to breed new azalea cultivars and broaden the range of flowering shrubs available to northern gardeners.

Introduction

Most species and cultivars of deciduous azalea, members of the Ericaceous genus *Rhododendron*, lack sufficient cold hardiness to survive in northern climates. In Minnesota, cold injury ranges from loss of a portion of the flower buds to severe stem die-back or plant death. Breeders at both the University of Minnesota Landscape Arboretum (18) and the University of Helsinki, Finland (29) are working to develop cold-tolerant varieties. Because the spring floral display is the primary ornamental attribute of deciduous azaleas, most cold hardiness research to date has focused on cold tolerance of overwintering flower buds (7, 20). Graham and Mullin (7) reported that overwintering azalea florets supercool to avoid ice formation at temperatures as low as –40C (–40F). However, because regenerative tissues of the stem are crucial to plant survival, (31), hardiness characteristics of both stems and flower buds need to be assessed. Flower buds and vegetative tissues may differ in timing and/or rates of acclimation and deacclimation as well as maximum midwinter hardiness levels (4, 17). Thus, laboratory freezing tests performed throughout the dormant period would provide a better estimate of the relative tolerance of these organs than could be obtained from a single, midwinter determination (11, 14, 15, 16, 19).

Assessing injury following freezing treatments is a vital part of laboratory procedures for determining plant cold hardiness (3). Visual observation of tissue injury is the method used by many investigators. Although relatively simple to perform, visual evaluations are qualitative in nature and require that samples incubate for one to two weeks following treatment to allow time for oxidative browning of injured tissue. The exosmotic method provides a relatively rapid and quantitative alternative to visual observation for estimating freezing injury (3). With this technique, severity of cold injury is based upon electroconductivity measurements of solute leakage from damaged tissues following incubation in distilled water. Although used extensively for many years on a range of species, the reliability of this technique has varied. Siminovitch et al. (26) noted that although good correlations between electroconductivity readings and plant survival have been reported, release of electrolytes from nonliving tissues can complicate evaluation of critical injury. Stergios and Howell (27) reported that the electroconductivity method was suitable for grape (*Vitis labrusca.* L.) wood, but worked poorly for cherry (*Prunus cerasus* L.) and raspberry (*Rubus strigosus* Michx.) wood and strawberry (*Fragaria* sp.) crowns. Lawes et al. (13) found that visual and specific conductance determinations of stem hardiness in kiwifruit (*Actinidia* sp.) did not agree closely. To our knowledge, the efficacy of this technique for determining injury of azalea stems has not been reported.

The primary objective of this study was to compare the cold hardiness of azalea stems and florets throughout the dormant period in order to assess the relative vulnerability of these organs to cold injury. A second objective was to compare the effectiveness of visual evaluations of injury with specific conductance measurements of electrolyte leakage as methods of determining cold hardiness of azalea stems.

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Table 1. Comparison of cold hardiness levels (C) of stem tissues as determined by visual observation of tissue injury or electroconductivity (EC) measurements of solute leakage.

Sampling date	Assessment method	'Spicy Lights'	'White Lights'	'Mandarin Lights'	800104	570091
11/16/92	Visual	–25	–34	–31	–31	–34
	EC	–22	–28	–34	–34	–34
12/01/92	Visual	–34	–37	–34	–34	–37
	EC	–32	–34	–34	–34	–37
1/05/93	Visual	–38	–38	–40	–38	–36
	EC	–38	–34	–38	–38	–38
1/25/93	Visual	–38	–40	–38	–38	–38
	EC	–40	–38	–38	–38	–38

Materials and Methods

Five deciduous azalea clones from the University of Minnesota azalea breeding program were selected for comparison of stem and flower bud hardiness. Three of the clones, 'White Lights', 'Spicy Lights' and 'Mandarin Lights', are introductions from the Minnesota program. Clone 800104 is a sibling to the cultivar 'Golden Lights'. The fifth plant, clone 570091, is the female parent of 800104 and has been used extensively as a parent in the program.

Six plants of each clone were growing in a field plot at the Minnesota Landscape Arboretum in Chanhassen, Minnesota [(44°50' N latitude; USDA Hardiness Zone 4a (26)]. Shoots of the current year's growth with flower buds were collected from all six plants of each clone on the following dates during 1992–93: November 16 and 30, January 4 and 25, March 17, and April 14. All material was prepared in the laboratory within two hours of collection. Shoot sections 2.5 cm (1 in) long were prepared for determinations of stem hardiness. Flower buds were prepared with 1 cm of subtending stem left attached. Stem sections and flower buds were placed in polyethylene bags with moist paper towelling serving as an ice nucleating agent. Eight stem sections and five flower buds of each clone were put in each bag. A copper-constantan thermocouple was inserted into the center of a single flower bud in each bag and the bags were placed in a programmable, ultra-low temperature freezer. Samples were held overnight in the freezer at a temperature approximating the previous evening's minimum temperature. One bag of stem sections and flower buds was held under refrigeration at 2C (36F) to serve as a control.

The following day, the freezer temperature was decreased at a rate of 5.6C (10F) per hr which is comparable to rates used previously for determining hardiness of azalea flowers (8). Sample temperatures were monitored on a strip-chart recorder. The range of treatment temperatures was varied by season to bracket the estimated lethal temperature. Bags were removed from the freezer at 2 or 3C (4 or 5F) intervals, depending upon the sampling date, and the samples were allowed to thaw under refrigeration at 2C (36F) for 24 hr. The flower buds and four of the eight stem sections were incubated in the polyethylene bags at ambient room temperature [20–23C (68–73F)] for 7 days prior to visual evaluation. Flower buds were dissected and the percent floret survival within each bud calculated. Individual flower buds typically contained between 5 and 10 individual florets. Damage to the corolla and ovaries was easily distinguished from healthy tissue in the florets. Stem sections were sliced longitudinally and visually evaluated under a binocular microscope (5, 11, 24, 27, 30). The initial freezing injury observed in stems was typically a browning of cells at the xylem/pith interface and was considered nonlethal. Stems exhibiting brown discoloration and breakdown of cells in the cambium, xylem, or phloem were classified as dead.

Data are presented in the lowest survival temperature (LST) format (24). Flower bud LSTs reported here are the minimum temperatures at which ≥ 50% of the florets survived. This rating is widely used and is based on the assumption

Table 2. Comparison of results obtained with visual evaluation and electroconductivity (EC) measurements of solute leakage for determining cold hardiness of azalea stem sections sampled on January 25, 1993.

	Temperature treatments (C)										
Cultivar	Control	–26	–28	–30	–32	–34	–36	–38	–40	–42	–44
'Spicy Lights'											
Visual[z]	1111	1111	1111	1111	1111	1111	1111	1111	0000	0000	0000
EC[y]	25 (1.3)	30 (2.2)	27 (0.8)	29 (0.7)	25 (1.5)	28 (2.2)	37 (1.8)	30 (0.3)	32 (1.6)	44 (2.9)	55 (4.9)
570091											
Visual	1111	1111	1111	1111	1111	1111	1111	1111	0000	0000	0000
EC	20 (1.5)	21 (0.9)	22 (1.2)	20 (0.7)	23 (0.1)	25 (1.4)	24 (1.2)	22 (1.8)	30 (2.7)	35 (1.8)	38 (1.8)

[z]Visual ratings of viability (1 = alive, 0 = dead) of four individual stem sections per temperature treatment.

[y]Specific conductance expressed as (EC reading following treatment / EC reading following autoclaving) × 100. Values represent the mean of 4 samples (± SE).

that 50% floret survival will provide an acceptable floral display. Stem LSTs are the minimum temperatures at which ≥ 50% of the stem sections were uninjured.

The four remaining stem samples were evaluated for injury using an electroconductivity technique (27). Samples were individually sealed in 10-ml glass vials containing 7 ml of deionized water and shaken for 20 hr to facilitate electrolyte leakage from injured tissues (27). Initial conductivity of the water was then measured using a conductivity bridge (Barnstead Model PM-70CB). Samples were autoclaved [121C (250F) at 103 kPa (15 psi)] for 1 hr to kill the tissues and reshaken for 17 hr. Conductivity was measured a second time and the specific conductance of each sample was calculated as (initial conductivity / final conductivity) × 100.

To determine LST values using the electro-conductivity technique, specific conductance data were compared using the Student-Newman-Keuls multiple range test. The lowest test temperature at which the average specific conductance did not differ significantly from that of the control ($P < 0.05$ significance level) was considered to be the LST.

Results and Discussion

Comparison of methods of evaluating injury. The LST values for stem tissues determined by the two evaluation methods are presented in Table 1. LSTs provided by the two methods were identical in 9 of 20 clone-sampling date combinations and varied by one test temperature increment [2 or 3C (4 or 5F)] in 10 of the remaining 11 cases. Stem injury was easily detected via visual observation under the microscope. Although some variation in injury among samples was occasionally observed as temperatures approached lethal levels, the killing point, typically, was easily identified (Table 2). Specific conductance measurements were often difficult to interpret due to the absence of a clear demarcation between values from living and injured samples (Table 2). Stergios and Howell (27) pointed out that the effectiveness of individual evaluation methods can vary with the physiological condition of the plant throughout dormancy. In our study, differences in cold hardiness levels indicated by the two methods occurred for at least two of the clones on every sampling date, suggesting that the disparities were not due to a transient ineffectiveness of one of the methods. Gu et al. (9) reported that estimates of cold hardiness of broad-leaved evergreens based upon specific conductance were typically 2–3C (4–5F) lower than visual estimates. As seen in Table 1, results of the two methods did not vary consistently for azaleas.

Although the differences in cold hardiness indicated by the two viability tests were less for azaleas than has been reported for some other species, the relatively labor-intensive nature of the specific conductance method combined with its inferior discernment of injury limit the usefulness of this technique for azalea hardiness determinations. The ease of visual detection of injury and the general agreement with results determined from specific conductance measurements increased our confidence in the visual rating system.

Stem and flower bud hardiness. Substantial hardening of both stems and flower buds of all clones occurred by November 16 (Fig. 1). Freezing temperatures are thought to be required for induction of the second and most extensive stage of cold acclimation in woody plants (31). In our study, daily minimum air temperatures were at or below freezing through-

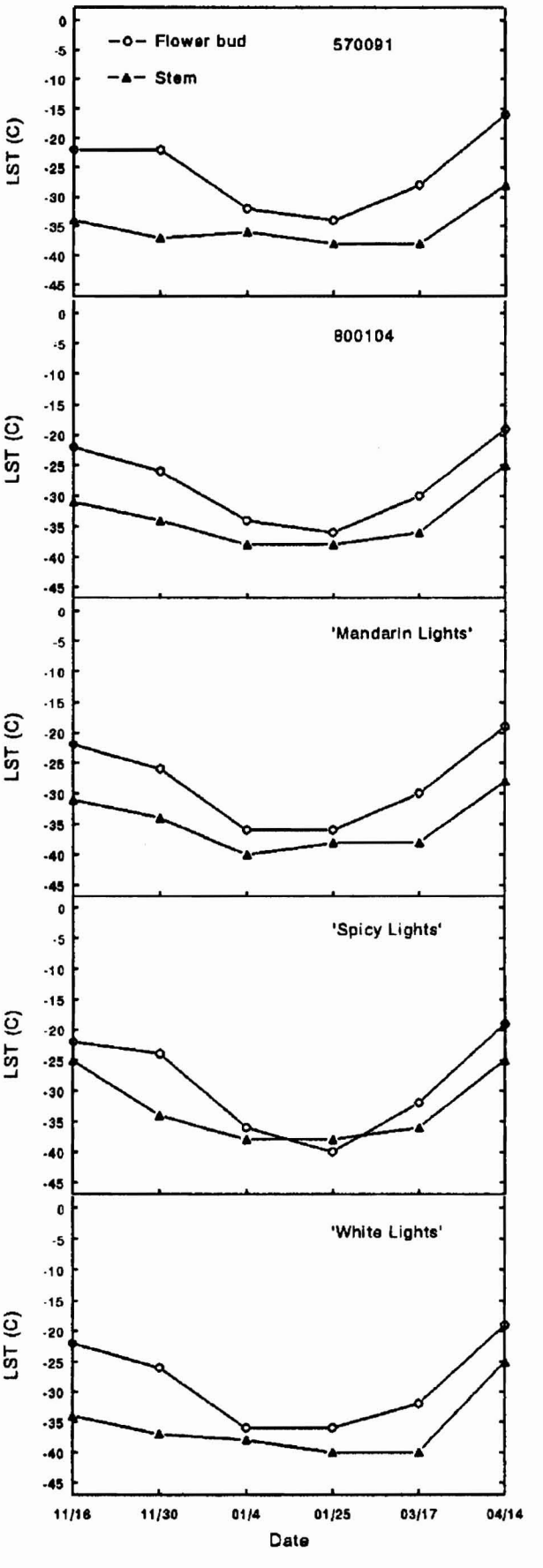

Fig. 1. Laboratory determinations of cold hardiness (LST) of stems and flower buds of *Rhododendron* taxa on 6 sampling dates during the winter of 1992–1993.

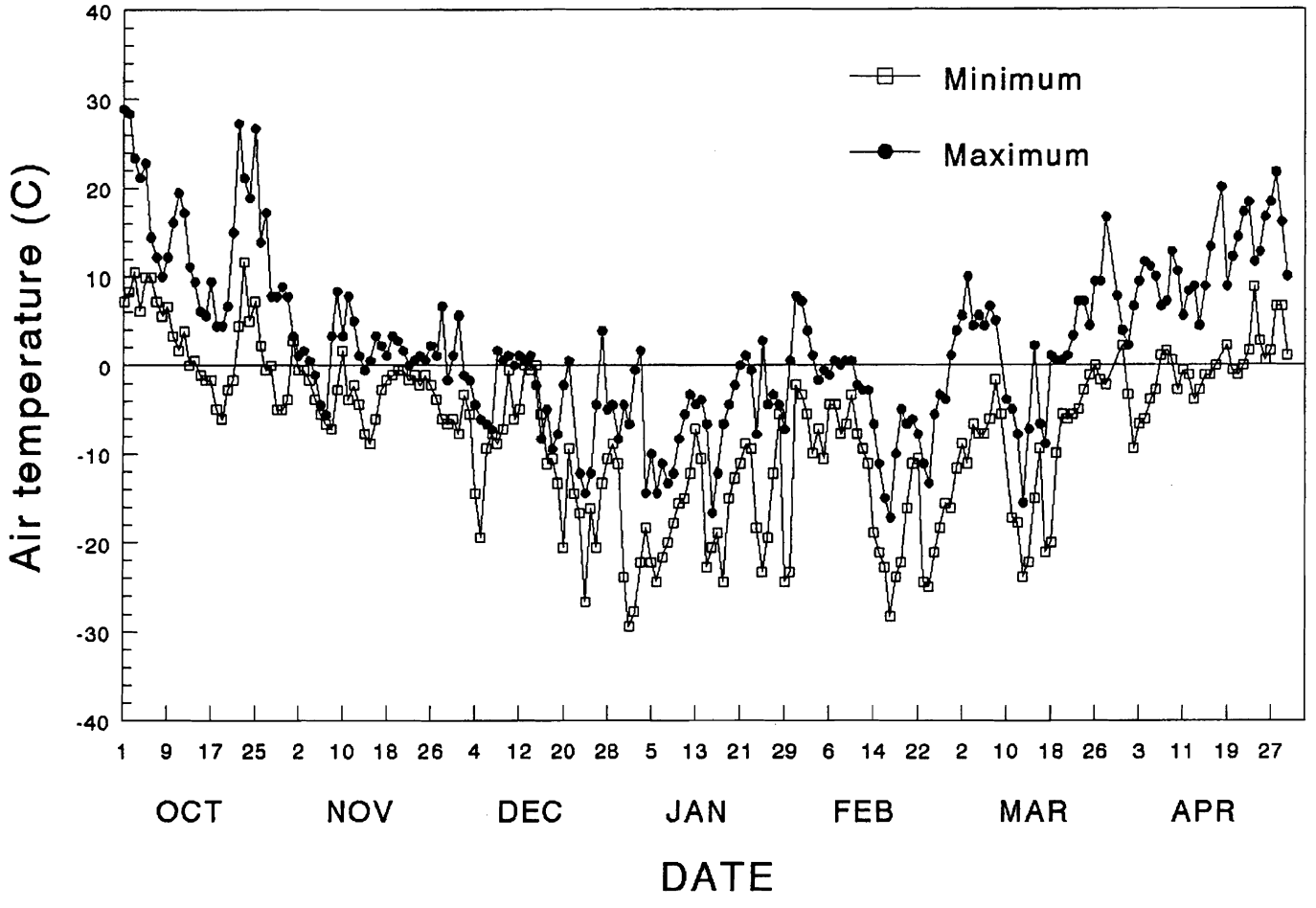

Fig. 2. Daily maximum and minimum air temperatures (C) at the University of Minnesota Landscape Arboretum during the winter of 1992–1993.

out most of the month prior to the first sampling (Fig. 2). The prevalence of daytime temperatures above the freezing point throughout the period preceding sampling did not appear to impede hardening. This observation agrees with the model developed by Anisko et al. (1) for prediction of cold hardiness of woody plants. They found that photoperiod and chill accumulation were better predictors of hardiness of six deciduous tree species than weekly temperature maxima measured prior to freezing tests. Cold acclimation of azalea flower and stem tissues in the fall also appears to be regulated predominantly by day length and minimum temperatures.

Florets acclimated later than stems and were, depending upon the clone, 3–15C (5–27F) less hardy than stems on the November 16 and 30 freezing dates. Relative cold hardiness of flower buds and stem tissues during the fall acclimation period varies among woody plant species and even between cultivars of some species. Flower buds of peach [*Prunus persica* (L.) Batsch.] trees can be more hardy than stems in early November (22). In lowbush blueberries (*Vaccinium angustifolium* Ait.), relative hardiness of stems and flower buds in the fall varied with clone and by bud position on the stem within a clone (4). The consistent results we obtained with 5 different clones indicates that hardening of azalea florets in the fall is delayed relative to stems. Despite this disparity, florets did obtain a substantial degree of hardiness by 16 November, withstanding –22C (–8F). Pellett et al. (20) postulated that the supercooling ability of deciduous azalea flower buds responds rapidly to temperature changes once some critical stage of acclimation is reached and that this stage was surpassed by late-November in provenances of three azalea species grown in Vermont. Graham and Mullin (7) reported that flower buds of seven deciduous azalea taxa grown in Minnesota hardened substantially by late-September and were comparable in hardiness to the five clones tested in the present study by late-November. A comparison of freezing test results with historical temperature data (not shown) from the Minnesota Landscape Arboretum, Chanhassen, indicates that stems and florets of these clones are capable of acclimating sufficiently to withstand typical minimum temperatures during the fall in central Minnesota.

In midwinter (January 25), only 'Spicy Lights' exhibited floret hardiness greater than that of stems. The maximum midwinter floret hardiness of the other four clones was 2–4C (4–7F) less than that of corresponding stems (Fig. 1). Sakai et al. (24) reported similar findings when looking at midwinter hardiness of a number of broadleaf evergreen *Rhododendron* species. Florets of 'Spicy Lights' and stems of 'White Lights' and 'Mandarin Lights' withstood –40C (–40F) in January, the maximum hardiness level detected in this study. Some field injury of flower buds was detected on the January 4 sampling date, with the percentage of injured florets ranging from 6% for 'Spicy Lights' to 36% for the clone 570091. This injury likely occurred on January 1 when a minimum air temperature of –29C (–21F) was recorded. The hardiness levels measured in the January 4 freezing test indicated that the majority of florets were sufficiently hardy to

survive the 1 January minimum temperature. Variation in hardiness among florets within an inflorescence and between inflorescences has been reported previously for deciduous azaleas (6, 7, 20) and may relate to variations in the water-ice relationships established between individual florets and bud scales (7).

Freezing tests indicate stems of the five azalea clones possess sufficient hardiness to withstand midwinter minimum temperatures in central Minnesota. These results corroborate observations of field performance at the Minnesota Landscape Arboretum where all five clones have been grown for a minimum of 15 years without serious injury or plant mortality. Tests also showed that at least a portion of the florets of these clones is capable of obtaining an adequate level of midwinter hardiness. This does not preclude, however, the possibility of midwinter injury following periods of above freezing temperatures. Pellett et al. (20) reported that flower buds of three deciduous azalea species dehardened rapidly in midwinter in response to increasing mean daily temperatures on the three days preceding sampling. Hardiness of peach buds can also change rapidly in midwinter when air temperatures approach 0C (32F) (20). Vegetative tissues also may deacclimate in response to warm temperatures in midwinter. Hong and Sucoff (10) reported that xylem parenchyma of apple (*Malus pumila* Mill.) twigs dehardened rapidly in response to above freezing temperatures. Although the effect of temperature fluctuations on midwinter deacclimation of tissues was not specifically examined in this study, it is worth noting that daytime air temperatures at or above 0C (32F) occurred on 2 of 3 days and 3 of 4 days prior to the January 4 and January 25 samplings, respectively, without a substantive loss of hardiness in either stems or flower buds. This might be explained by the fact that nighttime temperatures during these two periods were consistently well below the freezing point and conducive to reacclimation. Alternatively, the azalea clones may not have accumulated sufficient chilling hours to overcome endodormancy and, consequently, were unresponsive to above-freezing temperatures. The latter hypothesis is supported by the model of Anisko et al. (1) which indicated that total chill and heat accumulation played a greater role in determining plant hardiness than did either weekly maximum or minimum air temperatures measured shortly prior to hardiness testing. The effect of diurnal temperature fluctuations on azalea deacclimation and rehardening in midwinter merits closer study.

While little or no loss of stem hardiness was detected on March 17, florets had decreased in hardiness by 4–8C (7–14F), depending upon the clone. Graham and Mullin (7) also reported decreases in azalea floret hardiness by the third week of March in Minnesota. Floret deacclimation may have occurred in response to warm daytime temperatures in early March (Fig. 2). However, daily minimum air temperatures remained below 0C (32F) prior to March 17 and daytime maxima had returned to sub-freezing levels during the week prior to sampling. These facts suggest that florets deacclimated more than stems in response to warm temperatures and/or were less capable of reacclimating when temperatures again declined. The differential response of these two organ systems may relate to their respective freezing avoidance mechanisms. Xylem ray parenchyma cells of many temperate zone woody plants, including members of the genus *Rhododendron*, deep supercool to avoid lethal ice formation (2, 24, 25). *Rhododendron* florets withstand freezing via a combination of extraorgan freezing (water migrates out of florets to ice sinks in the budscales) and deep supercooling of water remaining in the floret (12). Graham and Mullin (7) found a strong correlation between the loss of hardiness and increased floret water content. They postulated that as temperatures warmed, water migrated back into florets from the bud scales resulting in a loss of hardiness. They did not report measurements of stem hardiness or stem water content. However, the absence in the xylem of a temperature dependent equilibrium between water in the ray parenchyma cells and extraorgan ice sinks as proposed for florets might account for the ability of living xylem tissue to avoid deacclimation in response to transient warm temperatures.

Both stems and florets of the five azalea clones deacclimated substantially by April 14 (Fig. 1). Woody plant tissues can deharden rapidly in response to warm air temperatures once chilling requirements have been satisfied (1, 22). Deacclimation of *Cornus sericea* L. stems was detected when daily maximum and minimum air temperatures remained above the freezing point for several days (30). In this study, mean daily minimum and maximum air temperatures the week prior to the April 14 sampling were –0.5 and 9C (31 and 48F), respectively. Stems were still 6–12C (11–22F) more hardy than florets on April 14. Despite this differential, flower buds maintained ample hardiness to avoid injury under all but the most extreme spring conditions in central Minnesota.

Overall, azalea stems were hardier than corresponding flower buds in 29 of 30 clone-sampling date combinations. Differences in hardiness between vegetative and reproductive structures have similarly been reported for peaches (22), evergreen rhododendrons (23, 24), and blueberries (4). The azalea clones tested all possessed sufficient cold hardiness of vegetative tissues to insure plant survival. Future breeding efforts with this germplasm should focus on increasing flower bud hardiness to improve bloom following severe winter conditions.

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