

Seasonal Variation in Low-temperature Tolerance of *Vaccinium angustifolium* Ait.

Paul E. Cappiello¹ and Scott W. Dunham²

Department of Plant, Soil, and Environmental Sciences, University of Maine, Orono, ME 04469-5722

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Abstract. Seven *Vaccinium angustifolium* clones were tested for low-temperature tolerance over two dormant seasons. Flower primordia in the pseudoapical bud were damaged at higher temperatures than were stem tissue and primordia of the fourth floral bud. The flower primordia located at the stem tip also reacclimated earlier and seemed to show a stronger response to abrupt spring warming than did other tissues tested. Given the lowest survival temperatures determined and the ambient temperatures recorded, we recommend that the physiological and economic aspects of cryoprotectants and flower-delaying treatments be studied further.

The lowbush blueberry in northeastern North America is a managed wild crop that produces ≈20 million kilograms of fruit each year in Maine alone. The crop is composed mainly of *Vaccinium angustifolium*, with a minor amount of *V. myrtilloides* Michx. In commercial production, fruit is produced in alternate years. The first year's vegetative growth produces flower buds for the following season's crop; in the second year, the flower buds produce flowers and fruit that is harvested in late summer. There has been an increase in per-acre production of lowbush blueberry over the past two decades as a result of improved pest control and fertility programs. However, the increases in production have been inconsistent from year to year, partially because of problems arising from low-temperature damage. Inconsistent yield is a problem facing growers attempting to enlarge their markets.

Low-temperature tolerance of vegetative and reproductive tissues of *Vaccinium* spp. has been investigated. Hancock et al. (1987) reported significant variation in frost tolerance of flowers of several highbush blueberry (*V. corymbosum* L.) cultivars. They reported that bud position and stage of flower opening significantly influenced the amount of damage sustained during two spring frosts. Gupton (1983) also reported that frost tolerance of flowers decreased as anthesis progressed in rabbiteye blueberry (*V. ashei* Reade) cultivars. Patten et al. (1991) compared low-temperature tolerance of rabbiteye, highbush,

and southern highbush blueberry (*Vaccinium* spp.). Rabbiteye was the most cold sensitive and showed a linear increase in low-temperature-induced damage with progressive opening of flowers. Using *V. corymbosum* 'Rancocas', Biermann et al. (1979) reported that bud position on the stem and floret moisture content played important roles in determining lowest survival temperatures (LST) of reproductive tissues during the dormant season. Quamme et al. (1972) found that two *V. angustifolium* clones were significantly more cold hardy than nine other *Vaccinium* species and hybrids.

We conducted a series of experiments during Winter 1991–92 and 1992–93 to understand better the patterns of low-temperature tolerance in *V. angustifolium*. Our specific goal was to determine the minimum survival temperatures of vegetative and reproductive structures over the dormant season.

Materials and Methods

For the initial study, four clones of *V. angustifolium* were identified in a commercial production field in Ellsworth, Maine. Beginning in November and continuing through May, 30 stem samples per clone were collected monthly, placed in a cooler on crushed ice, and brought to Orono, Maine, for analysis. Stems collected had an inflorescence bud at each of the top four nodes (positions 1–4, numbered basipetally). Each stem was sealed in a 15-mm glass culture tube before transferring to the freezing apparatus. Samples were not surrounded with a moist medium as in other studies (Lindstrom and Dirr, 1991; Warmund et al., 1989), because an initial test series indicated that the moist medium did not affect LST estimate. All samples were processed as described within 6 hours of harvest.

Prepared samples were placed in a microprocessor-controlled low-temperature freezer (model 40-9.4; Scientemp, Adrian, Mich.) for freeze tests. A nickel-constantan thermocouple was imbedded under the bark of

a reference sample for monitoring stem tissue temperatures. The study was conducted using four clones during Winter 1991–92 and on three clones during Winter 1992–93.

Tissue was acclimated in the chamber at 3C for ≈3 h. Then the chamber temperature was decreased to –3C over 2 h, held for ≈4 h, and decreased again at a rate of 3C/h to a minimum of –40C. Five stem samples of each clone were removed at 5C intervals from –5C to –40C and were placed immediately in a chamber held at 3C for ≈24 h. Then they were held at ≈24C for 48 h before evaluation.

Following cold treatment, samples were sectioned and visually evaluated under a binocular microscope for damage to stem tissue and flower primordia. Inflorescence buds 1–4 and a 1-cm stem segment (located 1 cm below the stem tip) were sliced longitudinally for evaluation. Stem tissue was evaluated for damage as described by Fuchigami et al. (1971), Sakai et al. (1986), and Lindstrom and Dirr (1991). After 48 h of incubation at 24C, oxidative browning of phloem and cambial tissues was easily distinguished. Stems exhibiting such signs were classified as dead. The number of stems surviving each temperature treatment was recorded. Flower primordia, visible from a single median section of each flower bud, were counted, and the percent survival was determined. Survival was determined by the absence of browning in the corolla and visible ovaries. As with evaluations of stem tissue damage, the damage to the corolla and ovaries was easily distinguished from healthy tissue in the florets.

Statistical analysis was performed using Duncan's multiple range test (SAS Institute, 1985) ($\alpha=0.05$), following arcsin transformation of percent survival data. Data are presented in the LST format (Lindstrom and Dirr, 1991; Sakai et al., 1986). The LST designated in this report is the lowest temperature treatment that resulted in 100% stem or flower primordia survival. Some previous reports on cold hardiness of woody taxa have reported LST based on survival percentages ranging from 40% to 66% (Biermann et al., 1979; Pellett et al., 1991; Sakai et al., 1986). For our study, 100% survival was chosen because it requires less interpolative manipulation of data to estimate the value, and it does not assume linearity of response to the temperature treatments. There was little linearity of response with *V. angustifolium*. Once the chamber temperature decreased to the temperature where damage occurred, there was a rapid drop in survival from one temperature to the next in all cases using *V. angustifolium* clones. As noted in other similar studies (Lindstrom and Dirr 1991), the lack of variability within clones precluded the necessity for any additional statistical analysis. In all cases, the 100% survival at the LST was significantly different than the survival percentage at the next lowest temperature as determined by Duncan's multiple range test.

Results and Discussion

A significant seasonal pattern of low-

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¹Assistant Professor.

²Crop Technician.

temperature tolerance was observed for all clones during both studies (Tables 1–4). There was a significant difference in flower primordia cold tolerance based on bud position on the stem (Table 3). There was also a similar trend

of seasonal low-temperature tolerance exhibited by flower primordia and stem tissue during both test years.

The LST values for the inflorescence bud at position 1 (tip of stem) indicated that all

clones reached peak cold hardiness on or around either the January or February sampling dates (Table 1). Of the seven clones tested, two clones (1992–93 study) reached an LST of –35°C. In comparison, flower primordia in the fourth bud of all clones were able to withstand –35°C (Table 2). Fourth flower bud primordia of clone 6 were able to survive –40°C in Feb. 1993.

For buds in the first position, greatest loss of low-temperature tolerance occurred between the 10 Mar. and 13 Apr. sampling in the 1991–92 study (Table 1). For all four clones, there was a minimum 15°C increase in the LST between these two sampling dates. This abrupt change in LST was not observed during the 1992–93 study. One explanation for the later, more gradual deacclimation of the clones during 1992–93 is the more moderate warming trend during that spring (Figs. 1 and 2). The rapid rise in minimum and maximum temperatures in mid-Mar. 1991 likely played a role in the change in LST. Several previous studies have indicated that warming stem and floral tissues before low-temperature tolerance testing drastically decreases tolerance. Pellett et al. (1991) showed a positive correlation between minimum survival temperature and daily mean temperatures for the 3 days immediately before testing. They indicated that this short-term dehardening may be more important than ecotype in determining low-temperature survival in certain *Rhododendron* species. In addition, Warmund et al. (1989) reported that, after chilling requirements were met and 12 h of incubation at 16°C, *Rubus* spp. had a significant loss of low-temperature tolerance. Daily maximum temperatures for the 3 days before the 13 Apr. 1992 sampling date were 25.2, 20.7, and 23.8°C and followed a record high of 31.2°C ten days earlier (Fig. 1). In addition, there was an ≈20°C rise in the temperature minima for that same time. Our study does not permit us to ascertain the independent effects of the elevated day or night temperatures.

Primordia in the fourth bud position showed greatest loss of low-temperature tolerance between the 13 and 23 Apr. 1992 sampling. The delayed loss of low-temperature tolerance indicates that these tissues may be less sensitive to spring temperature fluctuations than primordia in the first bud position. This later deacclimation is consistent with the basipetal opening of inflorescence buds of field-grown plants. Later deacclimation of the more basally located buds occurred to a lesser degree during 1992–93. Again, this likely is due to the more moderate temperature changes exhibited during that spring.

The difference in low-temperature tolerance of floral tissues based on bud position on the stem is evident from the fact that the two buds at the stem tip were significantly less cold hardy than buds 3 and 4 (Table 3). Preliminary work on these same clones indicated that bud 4 reaches peak cold hardiness on the stem and that buds located lower on the stem achieve no greater low-temperature tolerance. This trend agrees with the one described by Biermann et al. (1979), who reported that *V. corymbosum*

Table 1. Minimum temperature (°C) resulting in 100% survival of *Vaccinium angustifolium* flower primordia of the tip inflorescence bud.

Clone	Sampling date							
	12 Nov.	16 Dec.	17 Jan.	19 Feb. ^z	10 Mar.	13 Apr.	23 Apr.	11 May
1991–92 Study ^y								
1	–20	–20	–25	–20	–20	–5	–2	–2
2	–20	–20	–25	–20	–20	–5	–2	–2
3	–20	–25	–30	–30	–25	–10	–2	–2
4	–20	–20	–30	–30	–30	–10	–5	–5
1992–93 Study ^x								
5	–25	–25	–30	–30	–25	–25	–15	–5
6	–25	–25	–30	–35	–30	–25	–20	–15
7	–20	–25	–35	–35	–25	–25	–15	–5

^zPlants covered by snow in Feb. 1992 and 1993 and in Mar. 1993.

^yn = 5.

^xn = 3.

Table 2. Minimum temperature (°C) resulting in 100% survival of *Vaccinium angustifolium* flower primordia of the fourth bud.^z

Clone	Sampling date							
	12 Nov.	16 Dec.	17 Jan.	19 Feb. ^y	10 Mar.	13 Apr.	23 Apr.	11 May
1991–92 Study ^x								
1	–25	–25	–30	–35	–30	–25	–10	–10
2	–25	–25	–30	–35	–30	–25	–15	–5
3	–25	–30	–35	–35	–35	–25	–10	–10
4	–20	–25	–30	–35	–30	–25	–10	–5
1992–93 Study ^w								
5	--- ^v	–30	–35	–35	–30	–30	–20	–10
6	--- ^v	–30	–35	–40	–30	–30	–25	–15
7	--- ^v	–25	–30	–35	–30	–25	–20	–10

^zBud 4 counting from stem tip.

^yPlants covered by snow in Feb. 1992 and 1993 and in Mar. 1993.

^xn = 5.

^wn = 3.

^vData not available.

Table 3. Survival percentage for *Vaccinium angustifolium* flower primordia exposed to –40°C as affected by bud position.^{z,y,x}

Bud		Sampling date						
position ^w	12 Nov.	16 Dec.	17 Jan.	19 Feb.	10 Mar.	13 Apr.	23 Apr.	11 May
1	5 a	25 a	16 a	20 a	4 a	4	0 a	0 a
2	6 a	34 a	22 b	26 a	10 a	19 b	10 b	0 a
3	35 b	45 b	41 c	47 b	28 b	25 bc	18 b	0 a
4	--- ^v	64 c	45 c	51 b	34 b	38 c	31 c	0 a

^zAll data from 1992–93 study.

^yData for all three clones combined.

^xMean separation within columns by Duncan's multiple range test ($\alpha = 0.05$).

^wPosition 1 = tip bud.

^vData not available.

Table 4. Minimum temperature (°C) resulting in 100% survival of *Vaccinium angustifolium* stems.

Clone	Sampling date							
	12 Nov.	16 Dec.	17 Jan.	19 Feb.	10 Mar.	13 Apr.	23 Apr.	11 May
1991–92 Study ^z								
1	–25	–30	–40	–40 ^y	–40	–35	–20	–15
2	–20	–30	–35	–35	–30	–25	–20	–15
3	–25	–30	–40	–40 ^y	–35	–35	–20	–15
4	–25	–30	–40 ^y	–40 ^y	–40	–35	–20	–20
1992–93 Study ^x								
5	–25	–35	–35	–35	–35	–30	–20	–15
6	–25	–35	–35	–40 ^y	–35	–30	–25	–15
7	–20	–30	–40 ^y	–40 ^y	–30	–25	–20	–15

^zn = 5.

^yActual readings less than –40°C.

^xn = 3.

buds showed increasing cold hardiness the lower they were located on the stem.

Throughout the two seasons of the study, stem tissue attained equal or greater low-temperature tolerance than bud tissue in either the first or fourth position on the stem (Table 4). The single exception was in clone 6 on the Feb. 1993 sampling date. Of the seven clones tested, five reached a cold hardiness level below the lower limit of the equipment. Those clones are indicated as having an LST of less than -40°C . LST of the first flower bud primordia equaled that of stem tissue on only four of the 56 clone-sampling date combinations. Those similarities all occurred on the 12 Nov. sampling dates, when the required killing temperature was well below record lows.

Compared to primordia in the fourth bud, stem tissue again attained a greater or equal degree of cold hardiness; however, in this case, $\approx 40\%$ of the clone-sampling date combinations

showed the same LST for flower primordia and stem tissue. Stems and buds (in position 4) showed a similar spring deacclimation pattern. Stems of three of the four clones tested during 1991–92 showed a 15°C increase in LST rating between 13 and 23 Apr. 1992.

Low-temperature-induced midwinter damage to lowbush blueberry is unlikely to occur in any tissues other than reproductive tissue in the topmost bud. Major blueberry growing regions rarely experience temperatures below those indicated as LSTs for stems and buds located in positions 3 and 4. Thus, other complicating factors, such as moisture and wind, are involved in the injury to those tissues observed in the field for some years.

Our flower primordia data indicate that buds in the top position maybe subjected to temperatures near or below the listed LST. Buds in the fourth position, however, exhib-

ited LSTs below minimum recorded temperatures throughout the studies. Spring field evaluations of the clones indicated no winter injury to buds or stems. This result would be expected for stems and buds in the lower positions because ambient temperatures never dropped below the LSTs. First bud survival at ambient temperatures below the LST likely is due to snow cover. On the two occasions that ambient temperatures dropped below the LST, the plants were covered with snow and, therefore, presumably were insulated sufficiently.

The documented patterns of fall hardening, mid-winter hardiness, and spring dehardening of these tissues will provide a foundation on which to base future studies. Given the LSTs determined and ambient temperatures recorded, we recommend that physiological and economic aspects of cryoprotectants and flower-delaying treatments be studied. Alternatively, selection of clones with greater low-temperature tolerance, delayed spring deacclimation, or both maybe pursued as a solution to low-temperature injury in lowbush blueberry.

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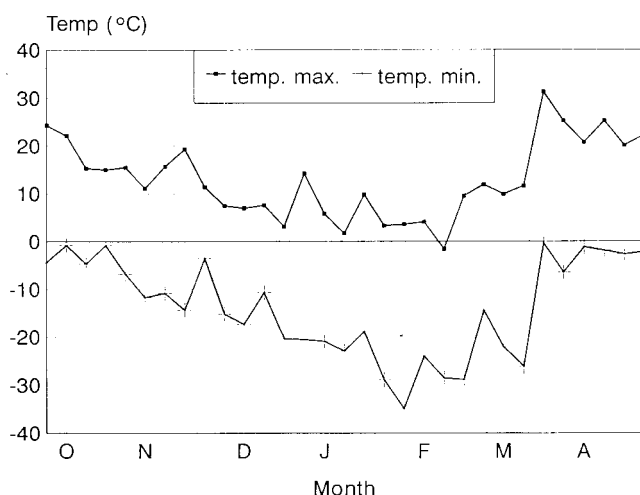


Fig. 1. Weekly minimum and maximum temperatures in 1991–92

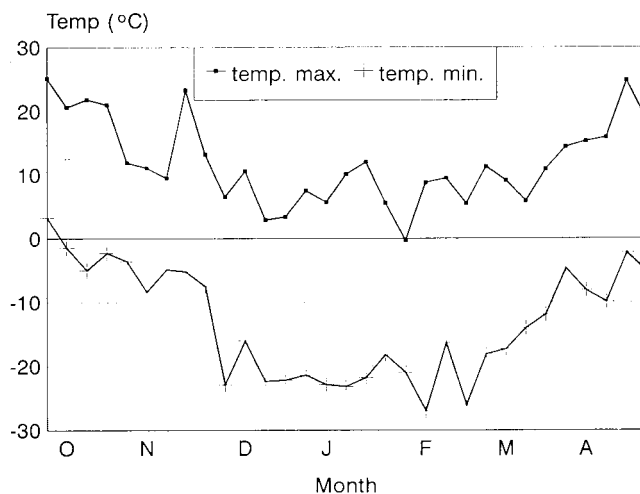


Fig. 2. Weekly minimum and maximum temperatures in 1992–93