

## Seasonal Changes in the Cold Hardiness of the Flower Buds of Highbush Blueberry with Varying Species Ancestry

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### Abstract

Many blueberry cultivars contain genetic material from *Vaccinium* species adapted to southern and northern regions. In this study, we compared the flower bud hardiness of several cultivars with diverse species backgrounds during the fall, winter and spring. Twigs were collected from field-grown plants and frozen to a range of temperatures to determine the temperature resulting in 50% bud mortality. The hardest cultivars were 'Sierra', 'Patriot' and 'Elliott', as well as the new cultivars 'Aurora' and 'Liberty'. 'Bluecrop' and 'Brigitta' were intermediate in hardiness, and 'Legacy' was the least hardy cultivar tested. Results suggest that hardiness of cultivars cannot be predicted accurately from their species composition.

Low temperature injury reduces highbush blueberry (*Vaccinium corymbosum* L.) production in many regions. Injury can occur in the autumn as tissues are acclimating, in mid-winter when tissues are fully acclimated, or in the spring during deacclimation. Flower buds are typically less hardy than twig or cane tissues (4). Flower buds and shoot tissues begin acclimating to cold in October, but may not attain maximum hardiness until December or January (3,5). Flower buds can begin deacclimating soon after maximum hardiness is reached, and under Michigan conditions, injury can occur in the late winter or spring (3). In contrast, cold injury to blueberries in western Washington most often occurs in the late fall and winter (8).

Many *Vaccinium* species have been utilized by blueberry breeders to develop cultivars adapted to diverse climates (1,10,14). Lowbush blueberry (*V. angustifolium*) has been used to develop hardy half-high cultivars for cold areas. Lowbush and lowbush/highbush hybrids acclimate earlier than highbush cultivars, and tolerate early winter cold as a result (15), but some also deacclimate quickly and lose hardiness in the late winter.

Native southern species have also been hybridized with northern highbush types to develop "southern highbush" types (1,7). While most of these hybrids were generated for areas with very low-chilling hours and warm winters, some have chilling requirements between 600-800 hours and could have varying degrees of cold tolerance. Recent releases that fall into this "intermediate" category are 'Legacy', 'Ozarkblue' and 'Sierra'.

Predicting hardiness of cultivars from their genetic makeup alone appears difficult. In our informal observations of cultivar trials in Michigan, flower buds of 'Sierra' have rarely been damaged by low temperature, while flower buds of 'Legacy' and 'Ozarkblue' are commonly damaged. Flower buds of most pure northern highbush types such as 'Elliott' and 'Bluecrop' survived most winters, while a high percentage of the flower buds of 'Brigitta' were damaged in most years. Ehlenfeldt et al. (9) reported that two cultivars containing southern species ('Legacy', 'Ozarkblue') were less hardy than northern highbush cultivars in mid-winter, but they deacclimated at comparable rates. The stage of bud opening or development provides an accurate estimate

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of the degree of deacclimation (16).

The overall cold hardiness of blueberries is affected by rates of acclimation, degree of tolerance during deep winter rest and rates of deacclimation. The variation in cold damage observed among highbush cultivars may relate to differences in all three of these parameters. To test this hypothesis, we compared the flower bud hardiness of a diverse array of blueberry cultivars in the fall, winter and spring of 2003 and 2004.

### Materials and Methods

During the first season, 60 twigs, at least 10 cm long and with three or more flower buds, were removed on 25 Nov. 2003, from 'Bluecrop' (4 plants), 'Brigitta' (5 plants), 'Patriot' (4 plants), 'Sierra' (3 plants), and 'Legacy' (2 plants) at Grand Junction, Mich., and from 'Bluecrop' (5 plants), and 'Aurora' and 'Liberty' (1 plant each) at the Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, Mich. Twigs were placed in plastic bags with a moist paper towel, transported in an ice-filled cooler to East Lansing, Mich, and prepared within 24 hr for controlled temperature freezing as described previously (13,17).

To determine freezing tolerance, three twigs from each cultivar were bundled together in moist cotton cheese cloth, wrapped in aluminum foil, and placed in an Ultralow Freezer (ScienTemp, Adrian, Mich.) programmed to cool at a rate of 2 °C per hour. Three bundles (replicates) were removed when temperatures recorded by a thermocouple placed next to the bundles reached -12, -16, -20, and -24 °C. Three control bundles were also held at 3 °C. After freezing, the bundles were held at 3 °C for 1 d and 20 °C and 100% relative humidity for 5 d (to allow expression of visible symptoms). Buds were cross-sectioned and examined under a dissecting microscope to determine the number that were green (live) and brown (dead). The temperature resulting in 50% flower primordia mortality ( $LT_{50}$ ) was

calculated using the modified Spearman-Kärber method (2). Additional twigs were sampled on 19 Jan. and 1 Mar. 2004 from the same bushes and four bushes of 'Elliott' at both locations. Twigs were handled and assessed as described above, except that twigs sampled in Jan. were removed at temperatures of -17, -21, -25, -29, and -33 °C, and those sampled in Mar. were frozen to -9, -13, -17, -21 and -25 °C. Because the same cultivars were not sampled on each date, ANOVA was performed on  $LT_{50}$  data for each date and location, and cultivar means were separated using an LSD test ( $P = 0.05$ ). Data for each cultivar were also analyzed separately to compare sampling times.

In the second season (2004-2005), twigs were only sampled from plants at Grand Junction. Samples were collected from the same bushes as in year 1, as well as four bushes each of 'Ozarkblue' and US 245. US 245 is 75 % 'Bluecrop' and 25% *V. darrowii*, one of the primary species used to develop low-chill southern highbush cultivars (6). Sampling dates and freeze temperatures were 17 Nov. 04 (-8, -12, -16, -20, -24, -28, -32 °C), 24 Jan. 05 (-16, -20, -24, -28, and -32 °C), and 10 Mar. 2005 (-10, -14, -18, -22, -26 °C). Post-freezing handling and evaluation were the same as in year 1. Since the same cultivars were sampled on each date, data were analyzed as a factorial design, with Time and Cultivar as factors.

Daily minimum and maximum temperatures were recorded by automated weather stations at each location. Average daily temperatures were calculated as:  $(\min + \max)/2$ . The average daily temperatures for the seven days prior to each sampling were computed.

### Results

At SWMREC in 2003-2004, 'Aurora' was the hardiest cultivar in the late fall, winter and early spring, followed by 'Liberty', 'Elliott' and 'Bluecrop' (Table 1). 'Aurora' did not significantly vary in hardiness across dates ( $P > 0.05$ ), while 'Liberty' and 'Bluecrop' were

**Table 1.** Flower bud hardness of blueberry cultivars at SWMREC in Benton Harbor, Mich., as determined by controlled freezing tests.

Cultivar (LSD <sub>0.05</sub> across dates)	LT <sub>50</sub> (°C) <sup>z</sup>		
	25 Nov. 2003	19 Jan. 2004	1 Mar. 2004
Aurora (ns) <sup>y</sup>	-25.3 a <sup>x</sup>	-26.8 a	-25.7 a
Liberty (3.2)	-21.4 b	-25.8 a	-25.0 ab
Elliott (ns)	nt <sup>w</sup>	-23.3 ab	-23.7 ab
Bluecrop (2.0)	-17.4 c	-19.5 b	-21.3 b
LSD <sub>0.05</sub>	3.6	4.5	4.0

<sup>z</sup>Temperature resulting in 50% flower bud mortality<sup>y</sup>Means across columns not significantly different<sup>x</sup>Letters indicate significant differences between means in columns<sup>w</sup>Cultivar not tested

significantly less hardy in November than on later sampling dates. ‘Elliott’ was not tested in the fall. The average daily temperatures for the 7 days prior to the Nov, Jan, and Mar sampling dates were 9, -4, and 1 °C, respectively.

At Grand Junction in 2003-2004, ‘Sierra’, ‘Patriot’, and ‘Elliott’ were the most hardy overall with no significant changes in LT<sub>50</sub> across seasons (Table 2). ‘Legacy’ was the least hardy in all seasons, and had significantly higher LT<sub>50</sub> values in the spring than the fall or winter. In fact, Legacy was 10 degrees less hardy than any other cultivar in March. ‘Brigitta’ and ‘Bluecrop’ were intermediate in hardness, with ‘Bluecrop’ being significantly

less hardy in the fall. ‘Brigitta’ also had its lowest LT<sub>50</sub> in the fall, but the value was not significantly different than the other seasonal values. The average daily temperatures at Grand Junction for the 7 days prior to the Nov., Jan., and Mar. sampling dates were 9, -4, and 0 °C, respectively.

In 2004-2005, LT<sub>50</sub> data showed a highly significant date x cultivar interaction. A Tukey’s HSD test was used to separate the large number of means (Table 3). ‘Sierra’, ‘Patriot’ and ‘Elliott’ were again the most hardy overall, but they were significantly less hardy in the spring and fall than winter. ‘Legacy’ was again the least hardy in all seasons, but with

**Table 2.** Flower bud hardness of blueberry cultivars at Grand Junction, Mich., as determined by controlled freezing tests.

Cultivar (LSD <sub>0.05</sub> across dates)	LT <sub>50</sub> (°C) <sup>z</sup>		
	25 Nov. 2003	19 Jan. 2004	1 Mar. 2004
Patriot (ns) <sup>y</sup>	-24.7 a <sup>x</sup>	-21.1 bc	-23.9 a
Sierra (ns)	-23.2 ab	-24.3 a	-23.5 a
Elliott (ns)	nt <sup>w</sup>	-22.6 abc	-21.0 a
Brigitta (ns)	-19.6 bc	-23.5 ab	-20.1 a
Bluecrop (3.1)	-16.2 c	-20.8 c	-18.6 a
Legacy (4.6)	-14.9 c	-15.1 d	-8.1 b
LSD <sub>0.05</sub>	5.0	2.5	6.5

<sup>z</sup>Temperature resulting in 50% flower bud mortality<sup>y</sup>Means across columns not significantly different<sup>x</sup>Letters indicate significant differences between means in columns<sup>w</sup>Cultivar not tested

**Table 3.** Flower bud hardiness of blueberry cultivars at Grand Junction, Mich., as determined by controlled freezing tests.

Cultivar	LT <sub>50</sub> (°C) <sup>z</sup>		
	17 Nov. 2004	24 Jan. 2005	10 Mar. 2005
Sierra	-25.0 a <sup>y</sup>	-31.9 a	-27.4 a
Patriot	-24.6 ab	-32.0 a	-27.5 a
Elliott	-23.9 ab	-31.2 a	-22.9 bc
Ozarkblue	-23.8 ab	-26.2 bc	-21.4 c
Bluecrop	-23.7 ab	-29.4 ab	-26.1 ab
US 245	-22.7 ab	-24.0 c	-17.4 d
Brigitta	-21.2 b	-30.9 a	-26.7 a
Legacy	-15.7 c	-17.5 d	-13.4 e

<sup>z</sup>Temperature resulting in 50% flower bud mortality

<sup>y</sup>Letters indicate significant difference between means in columns (Tukey's HSD<sub>0.05</sub> = 3.6). The same HSD value can be used to compare means across columns

no significant change in LT<sub>50</sub> in the fall, winter and spring samples. 'Ozarkblue', 'Bluecrop', US 245 and 'Brigitta' were intermediate in hardiness. 'Brigitta' was significantly less hardy in the spring and fall than mid-winter. 'Ozarkblue' and US-245 were significantly less hardy in the spring, while 'Bluecrop' had a significantly lower LT<sub>50</sub> in the fall. The average daily temperatures for the 7 days prior to the Nov., Jan., and Mar. sampling dates were 5, -12, and -2 °C, respectively.

### Discussion

The new northern highbush releases 'Liberty' and 'Aurora' are as hardy as 'Elliott', the standard late-season cultivar in Michigan. 'Patriot' also proved quite hardy in all seasons, even though it is frequently reported to be damaged by cold. This may be due to its floral buds developing more rapidly than other cultivars once temperatures begin to rise in late winter, making it more likely to be frost damaged if temperatures drop suddenly (12).

'Bluecrop' and 'Brigitta' were intermediate in cold hardiness, and varied in their rates of acclimation and deacclimation. 'Bluecrop' was less acclimated in the late fall than winter in both years, while 'Brigitta' was less cold hardy in both the early spring and late fall of

2003-2004 but not in 2004-2005. These results suggest that 'Brigitta' may deacclimate more readily than 'Bluecrop' some years in the spring, but it does not explain why 'Brigitta' routinely suffers what has appeared to be cold damage. Perhaps 'Brigitta' is more sensitive to cold than the other northern highbush cultivars at periods earlier and later than those analyzed in this study.

Among the genotypes containing high proportions of genes from southern blueberry species, 'Legacy' was the least hardy at all sample dates. 'Legacy' has been observed to retain its leaves late into the fall like many southern highbush cultivars, suggesting that it might acclimate to cold slowly. However, 'Ozarkblue' and US 245 also retain their leaves until the very late fall and they proved to be just as hardy in the fall and winter as the mid-season standard 'Bluecrop'. This indicates that leaf retention is not a good indicator of potential winter hardiness. Bittenbender and Howell (2) also found no correlation between flower bud hardiness and fall leaf retention in seven northern highbush cultivars.

In summary, these data verify our informal observations that the ancestry of highbush blueberry cultivars can not be used to adequately predict season patterns of cold

hardiness. Hancock et al. (11), also found considerable levels of variation in cold hardiness in a highbush breeding population containing 12.5% *V. darrowii*, and in another study found several complex hybrids that were very winter hardy, even though a high percentage of their genes were from southern species (12). *V. constablaei*, which is distributed in higher elevation sites in the southern U.S., appears to provide consistently slower rates of deacclimation, which may be useful in developing genotypes with tolerance to late-winter cold (16). These observations suggest that a lack of cold hardiness should not impede the use of southern species in northern highbush breeding programs.

### Literature Cited

- Ballington, J. 1990. Germplasm resources available to meet future needs for blueberry cultivar improvement. *Fruit Var. J.* 44:54-63.
- Bittenbender, B.C. and G. S. Howell. 1975. Predictive environmental and phenological components of flower bud hardiness in highbush blueberry. *HortScience* 10:409-411.
- Bittenbender, B.C. and G.S. Howell. 1976. Cold hardiness of flower buds of selected highbush blueberry cultivars (*Vaccinium australe* Small). *J. Amer. Soc. Hort. Sci.* 101:135-139.
- Cain, J.C. and G.L. Slate. 1953. Blueberries in the home garden. *Cornell Agric. Exp. Sta. Bull.* 900.
- Constante, J. F. and B.R. Boyce. 1968. Low temperature injury of highbush blueberry shoots at various times of the year. *Proc. Amer. Soc. Hort. Sci.* 93:267-272.
- Draper, A. and J. Hancock. 2003. Florida 4B: Native blueberry with exceptional breeding value. *J. Amer. Pomol. Soc.* 57:138-141.
- Draper, A.D., G.J. Galletta and J.R. Ballington. 1982. Breeding methods for improving southern tetraploid blueberries. *J. Amer. Soc. Hort. Sci.* 107:106-109.
- Doughty, C.C. and G.A. Hemerick. 1975. Impedance as a measurement of blueberry bud hardiness. *J. Amer. Soc. Hort. Sci.* 100:115-118.
- Ehlenfeldt, M.K., L.J. Rowland, and R. Arora. 2003. Bud hardiness and deacclimation in blueberry cultivars with varying species ancestry: flowering time may not be a good indicator of deacclimation. *Acta Hort.* 626:39-44.
- Hancock, J.F. 1998. Using southern blueberry species in northern highbush breeding. *Proc. 8<sup>th</sup> N. Amer. Blueberry Res. and Exten. Workers Conf. Wilmington, North Carolina.* Pp. 91-94.
- Hancock, J.F., W.A. Erb, B.L. Goulart, and J.C. Scheerens. 1995. Utilization of wild blueberry germplasm: The legacy of Arlen Draper. In: *Blueberries, A Century of Research.* Eds. R.E. Gough and R. F. Korcak. Food Products Press, New York. Pp.1-16.
- Hancock, J.F., B.L. Goulart, W.E. Erb, and J.C. Scheerens. 1997. Blueberry hybrids with complex genetic backgrounds evaluated on mineral soils: cold hardiness as influenced by parental species and location. *Acta Hort.* 446:389-395.
- Howell, G.S. and C.J. Weiser. 1970. Fluctuations in the cold resistance of apple twigs during spring dehardening. *J. Amer. Soc. Hort. Sci.* 95:190-192.
- Lyrene, P.M. and J.R. Ballington. 1986. Wide hybridization in *Vaccinium*. *HortScience* 21:52-57.
- Quamme, H.A., C. Stushnoff, and C.J. Weiser. 1972. Winter hardiness of several blueberry species and cultivars in Minnesota. *HortScience* 7:500-502.
- Rowland, L., J., E. L. Ogden, M.K. Ehlenfeldt, and B. Vinyard. 2005. Cold hardiness, deacclimation kinetics, and bud development among 12 diverse blueberry genotypes under field conditions. *J. Amer. Soc. Hort. Sci.* 130:508-514.
- Stergios, B.G. and G.S. Howell. 1973. Evaluation of viability tests on cold stressed plants. *J. Amer. Soc. Hort. Sci.* 98:325-330.