

# Cold Tolerance of Blueberry Genotypes Throughout the Dormant Period from Acclimation to Deacclimation

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*Additional index words.* cold acclimation, cold hardiness, dehardening, freezing tolerance, spring frost tolerance, *Vaccinium*

**Abstract.** Cold hardiness in woody perennials is determined by complex interacting factors: the timing and rate of cold acclimation; the maximum level of cold tolerance attained; the maintenance of cold tolerance during the winter; and the rate of loss of cold tolerance or deacclimation on resumption of spring growth. For highbush blueberry, the degree of winter freezing tolerance and susceptibility to spring frosts have been identified as the most important genetic limitations of current cultivars. Depending on the winter and the location, both winter freezes and spring frosts can cause damage to floral buds or flowers resulting in substantial losses in yield. To identify genotypes that are particularly slow or late to deacclimate and thus may be useful in breeding for spring frost-tolerant cultivars, we compared deacclimation kinetics under controlled laboratory and field conditions among several blueberry genotypes with diverse genetic backgrounds. Clear genotypic differences in timing and rate of deacclimation were found. In the field study, the species *Vaccinium constablaei* Gray was identified as particularly late to deacclimate, and 'Little Giant' (50:50 hybrid of *V. constablaei* and *V. ashei* Reade) was nearly as late to deacclimate as 100% *V. constablaei*. Recently, we extended our cold tolerance measurements from October through midwinter comparing acclimation kinetics and maximum cold tolerance levels among genotypes. Although all genotypes appeared to reach maximum cold tolerance about mid-December under the study conditions, genotypic differences were detected in other aspects, including initial cold tolerance, rate of acclimation, maximum cold tolerance, and length of the plateau. 'Little Giant' and 'Northsky' (75:25 hybrid of *V. corymbosum* L. and *V. angustifolium* Ait.) were very early to acclimate and were harder than the other genotypes both initially and when maximum cold tolerance was reached. Understanding how cold tolerance levels change throughout the dormant period should help us to develop cultivars better suited to their environments.

In preparation for winter, woody perennial plants of the temperate zone have evolved mechanisms to first enter a state of dormancy in late summer or early fall and then, as fall progresses, to develop cold hardiness. The development of cold hardiness, termed cold acclimation, is triggered by environmental stimuli of shortening day-length and declining temperatures (Powell, 1987; Sakai and Larcher, 1987; Weiser, 1970). A plant's ability to survive low temperatures is dependent on several complex interacting factors. They include the timing of dormancy induction in the summer, the timing and rate of cold acclimation in the fall, the level of freezing tolerance reached while plants are in the cold-acclimated state, the maintenance of freezing tolerance during the winter, and the timing and rate of deacclimation and budbreak in the early spring, among others (Wisniewski et al., 2003). How these factors are controlled physiologically and genetically in woody perennials is not well understood.

The United States is the world's leading producer of blueberries. In a survey of blueberry research and extension scientists in

the United States, lack of winter hardiness and susceptibility to spring frosts were identified as two of the most important genetic limitations of current cultivars (Moore, 1993). In nearly all blueberry growing areas in the United States, economic losses from early spring frosts can be significant. In the northern production regions, winter damage is considered the major factor limiting yields (Hanson and Hancock, 1990; Moore, 1994). In the Pacific Northwest, drastic drops in temperature in the fall to below freezing can cause flower bud damage in some crops, including blueberry (Doughty and Hemerick, 1975) and grape (Davenport and Keller, in press). As blueberry germplasm becomes more diverse, there is increased concern whether resulting selections and cultivars are sufficiently cold hardy for certain regions of the country, especially if southern-adapted germplasm comprises a significant part of the genetic background as it does for some of the newer releases (Ehlenfeldt et al., 2006).

To develop blueberry cultivars for specific regions of the country, a comprehensive understanding of blueberry cold hardiness is needed. Ideally, a blueberry cultivar should acclimate to cold quickly in the fall, have a high midwinter hardiness (actual level dependent on where the cultivar is to be grown), and deacclimate slowly during spring or during unseasonably warm spells in winter without adversely affecting the time of fruit-

ing (Arora et al., 2004). Therefore, in recent years, we have begun comparing patterns of acclimation, deacclimation, and maximum midwinter cold hardiness of flower buds for several different genotypes with diverse genetic backgrounds (Table 1). A summary of this work and our conclusions from it are described subsequently.

## MIDWINTER HARDINESS AND DEACCLIMATION UNDER CONSTANT, WARM TEMPERATURES

To determine if there are differences in the rate of deacclimation among genotypes that could be exploited for breeding more spring frost-tolerant cultivars, we initially examined the midwinter hardiness and rate of deacclimation among several blueberry cultivars under constant warm temperatures (Arora et al., 2004). Shoots were cut from field plants from a variety of genotypes mid-February and placed in flasks of water in the laboratory at  $\approx 20$  °C to trigger deacclimation. The first year (2000) cold-hardiness levels were determined every 3 d of deacclimation (DOD) over a 2-week period (0, 3, 6, 9, 12, 16 DOD). Because of the rapid rate of deacclimation, however, the second year (2001) cold-hardiness levels were determined every day for 6 d (0, 1, 2, 3, 4, 5, 6 DOD). Bud cold-hardiness levels (BCH)

Received for publication 10 Dec. 2007. Accepted for publication 10 Jan. 2008.

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were determined as we have described previously using our freeze-thaw protocol (Arora et al., 2004; Rowland et al., 2005). After freezing, buds were dissected and observed for injury (visual browning) of the ovaries in individual flowers. For example, Figure 1 shows a longitudinal section of a flower bud of highbush blueberry that was damaged by freezing. Buds were rated for browning and BCH defined as the temperature causing 50% injury (LT<sub>50</sub>).

Generally, the statistical analysis of BCH data were as follows. For each genotype by year combination, bootstrap estimates of LT<sub>50</sub> values and their 95% fiducial confidence intervals were calculated across the days of deacclimation using Proc Probit (SAS Institute, 1999). Nine observed data points (three proximal buds on each of three shoots) for each temperature were resampled (n = 9 with replacement) 30 times. A sigmoidal regression model was fit to percentage of injury (browning) versus treatment temperature for each of the 30 sets of resampled data. The 30 resulting values of LT<sub>50</sub> and lower and upper confidence limits were averaged to obtain the bootstrap estimates at each DOD. The LT<sub>50</sub> estimates were used to evaluate the deacclimation kinetics for each genotype by fitting a log-linear or asymmetrical sigmoidal (Gompertz) model, depending on the best fit for each genotype, to model the relationship between LT<sub>50</sub> and days of deacclimation. The LT<sub>50</sub> estimates from the models at each day of deacclimation and the differences in LT<sub>50</sub> values between subsequent days were compared among genotypes using analysis of covariance (Milliken and Johnson, 2002).

Log-linear deacclimation models, fitted to the BCH versus DOD data, for two genotypes, 'Bluecrop' and 'Tifblue', in the 2000 pilot study are shown in Figure 2A. The largest loss in BCH and, thus, the fastest rate of deacclimation occurred in the first three DOD for both genotypes. During this time interval, the *V. ashei* (southern species) genotype 'Tifblue' lost significantly more of its initial BCH, 11.2 °C or ≈51%, than did the *V. corymbosum* genotype 'Bluecrop', which lost ≈8.6 °C or ≈33% of its initial value. Therefore, the earliest and the faster deacclimator, during this first 3-d interval, was 'Tifblue'. 'Tifblue' was also the more cold-sensitive initially of the two genotypes with a midwinter cold-hardiness level of ≈ -20 °C as compared with -25.4 °C for 'Bluecrop' (Table 1). Deacclimation rates for both genotypes slowed as they reached a BCH level of ≈ -11 to -13 °C.

Overall, deacclimation occurred quickly at 20 °C and appeared to plateau for both genotypes by ≈6 DOD. Therefore, the deacclimation study undertaken in 2001, which included more genotypes, focused on this critical 6-d period. In Figure 2B, log-linear or Gompertz deacclimation models, depending on the best fit for each genotype, fitted to the BCH versus DOD data, for three genotypes, 'Bluecrop', 'Tifblue', and 'Legacy', are shown. By 1 DOD, 'Tifblue' had lost the most BCH, decreasing 5.7 °C, making it the

Table 1. Midwinter bud cold hardiness and germplasm composition of *Vaccinium* genotypes evaluated for timing and rate of deacclimation.<sup>z,y</sup>

Genotype	Bud cold hardiness (LT <sub>50</sub> ) <sup>x</sup>	Germplasm composition (%) <sup>w</sup>				
		Cold hardy species			Cold sensitive species	
		<i>V. cor.</i> <sup>y</sup>	<i>V. ang.</i>	<i>V. con.</i>	<i>V. dar.</i>	<i>V. ash.</i>
Bluecrop	-25.4 °C	94	6	—	—	—
Legacy	-16.0 °C	73	2	—	25	—
Little Giant	< -28.0 °C	—	—	50	—	50
Northsky	-28.4 °C	73	27	—	—	—
Tifblue	-20.0 °C	—	—	—	—	100
<i>V. constablaei</i>	< -28.0 °C	—	—	100	—	—

<sup>z</sup>Rowland et al., 2005.

<sup>y</sup>All but pure *V. constablaei* were also evaluated for timing and rate of cold acclimation.

<sup>x</sup>Bud cold hardiness defined as temperature causing 50% injury or lethal temperature<sub>50</sub> from our freeze-thaw protocol (Rowland et al., 2005).

<sup>w</sup>According to Clark et al. (1996), Ehlenfeldt (1994), and Hancock and Siefker (1982).

<sup>v</sup>*V. cor.* = *V. corymbosum*; *V. ang.* = *V. angustifolium*; *V. con.* = *V. constablaei*; *V. dar.* = *V. darrowi*; *V. ash.* = *V. ashei*.



Fig. 1. Longitudinal section of a flower bud of the *V. corymbosum* cultivar Duke that was frozen at -28 °C using our freeze-thaw protocol (Rowland et al., 2005). The black color indicates the flowers were killed.

earliest and fastest deacclimator of the three for the first time interval. By 2 DOD, its rate of deacclimation slowed considerably. By 1 DOD, 'Bluecrop' had changed the least, losing less than 1 °C of BCH, making it the slowest deacclimator. By 2 DOD, however, its deacclimation had begun, and it continued to lose 2.2 to 2.6 °C of BCH each day for the next 3 d, at which point its rate of deacclimation slowed. 'Legacy' was an intermediate deacclimator, losing 2.3 °C of BCH by 1 DOD.

#### COLD HARDINESS AND DEACCLIMATION UNDER FIELD CONDITIONS

From the previous studies, differences in deacclimation response were clearly evident among the cultivars, with 'Tifblue', the *V. ashei* genotype, being the earliest deacclimator under controlled laboratory conditions of constant 20 °C. Next, we wanted to try to compare deacclimation kinetics among more genotypes under natural field conditions of fluctuating temperatures. Although riskier than the controlled laboratory experiment, because flower buds can suffer freeze dam-

age in the field before shoots are cut and frozen in the laboratory, we were successful at comparing deacclimation kinetics of a variety of genotypes over 2 years (Rowland et al., 2005).

For these experiments, shoots were cut from genotypes at weekly intervals in the field. The time we began cutting shoots depended on the weather conditions. The first year (2002), because it was a mild winter, we thought buds might begin to deacclimate fairly early; hence, shoots were cut beginning at the end of January and ending in April. The second year (2003), because it was a much colder winter, shoots were not cut until the beginning of March. Although sampling was begun approximately a month earlier in 2002 than in 2003, a detectable loss in BCH (more positive LT<sub>50</sub>s) did not occur until sometime between Week 4 (19 Feb. 2002) and Week 5 (26 Feb. 2002). This was only slightly earlier than in 2003 when a detectable loss in BCH occurred sometime between Week 1 (4 Mar. 2003) and Week 2 (11 Mar. 2003). When the data from the 2 years were aligned and superimposed, beginning with the date before deacclimation began and continuing through

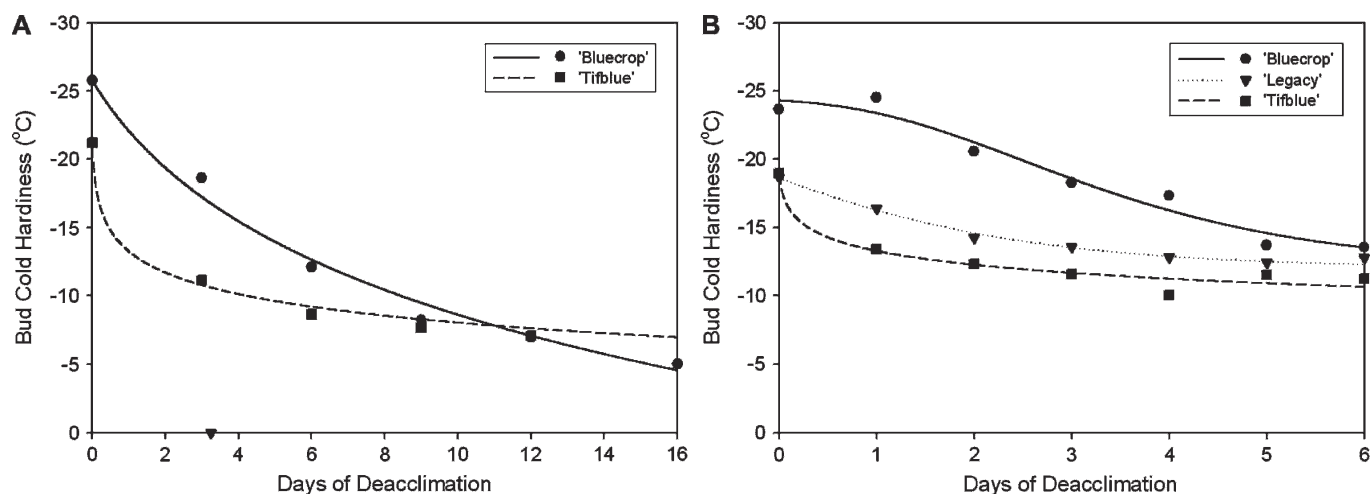


Fig. 2. (A) Deacclimation of two blueberry cultivars, 'Bluecrop' and 'Tifblue' from 0 DOD (days of deacclimation) through 16 DOD under controlled laboratory conditions in 2000 (Arora et al., 2004). Curves show the bud cold hardiness (BCH) values predicted from log-linear models. Points represent observed values. (B) Deacclimation of three blueberry cultivars (Bluecrop, Legacy, and Tifblue) from 0 DOD through 6 DOD under controlled laboratory conditions in 2001 (Arora et al., 2004). Curves show the BCH values predicted from log-linear ('Tifblue') or Gompertz ('Bluecrop' and 'Legacy') models. Points represent observed values.

to approximately mid-April (Weeks 4 to 10 in 2002/19 Feb. to 9 Apr. and Weeks 1 to 7 in 2003/4 Mar. to 15 Apr.), remarkably similar BCH levels were found between years. Consequently, BCH data from Weeks 4 to 10 of year 2002 and Weeks 1 to 7 of year 2003 were combined and averaged and are hereafter referred to as Weeks 1 to 7 of the combined-year study.

The statistical analysis of BCH data were carried out as described previously for the controlled laboratory experiments, except that BCH here was monitored at weekly intervals rather than every 3 d or daily. Gompertz deacclimation models, fitted to the BCH versus weeks of deacclimation data for the combined-year study for six of the 12 genotypes that were evaluated, are shown in Figure 3. There were clear genotypic differences in initial BCH levels. Of the genotypes shown, 'Legacy', which is three-fourths *V. corymbosum* and one-fourth the southern species *V. darrowi* Camp, was the least cold hardy at initial evaluation, even less so than 'Tifblue' (100% southern species *V. ashei*). 'Legacy' had an  $LT_{50}$  of  $-16^{\circ}\text{C}$ , whereas 'Tifblue' was hardy to  $-20^{\circ}\text{C}$  (Table 1; Fig. 3). 'Bluecrop' (predominantly *V. corymbosum*) was intermediate in BCH with an  $LT_{50}$  of  $-25.4^{\circ}\text{C}$ . Pure *V. constablaei*, 'Little Giant', which is half *V. constablaei* and half *V. ashei*, and 'Northsky', which is three-fourths *V. corymbosum* and one-fourth *V. angustifolium*, were the most cold hardy with BCH levels less than  $-28^{\circ}\text{C}$ , the lowest temperature that our glycol freezing bath would reach. *V. corymbosum* and *V. angustifolium* are northern highbush and lowbush species, respectively. *V. constablaei* is a highbush-like species that, although native to the southern United States, is found at high elevations in northern Georgia, western North Carolina, and eastern Tennessee.

Regarding deacclimation kinetics, the weekly intervals with the largest losses in

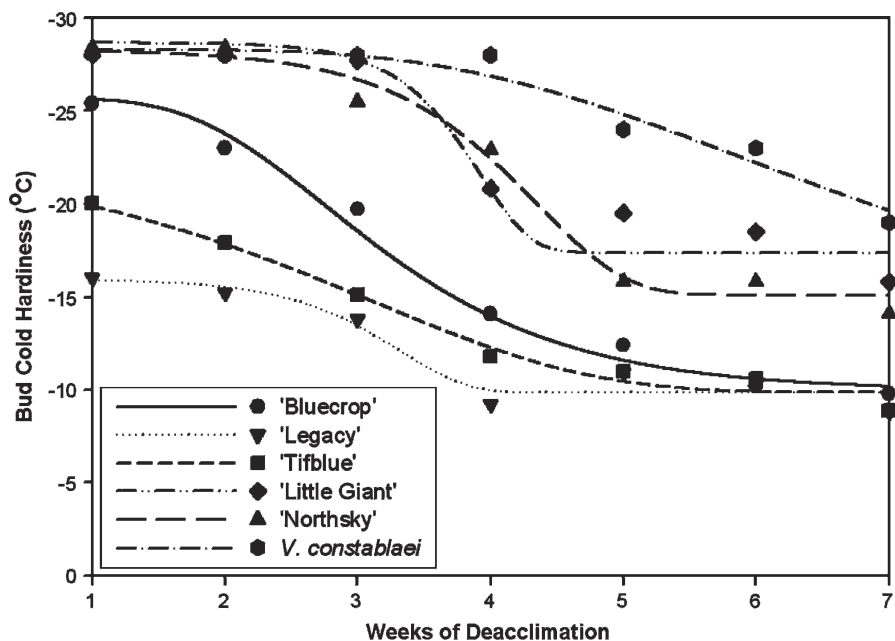


Fig. 3. Deacclimation of six blueberry genotypes ('Bluecrop', 'Legacy', 'Little Giant', 'Northsky', 'Tifblue', and *V. constablaei* selections) from 0 WOD (weeks of deacclimation) through 7 WOD under field conditions in the combined-year (2002 and 2003) study (Rowland et al., 2005). Curves show the BCH values predicted from Gompertz models. Points represent observed values.

BCH (i.e., highest rates of deacclimation) also varied among genotypes (Fig. 3). In the first 4 weeks, 'Tifblue' had a fairly constant rate of deacclimation, losing 2 to 3  $^{\circ}\text{C}$  of BCH per week, making it the earliest deacclimator of those shown. For 'Bluecrop' and 'Legacy', the greatest losses in BCH were observed at Weeks 3 and 4. For 'Little Giant' and 'Northsky', losses in BCH were greatest at Weeks 4 and 5, whereas for *V. constablaei*, losses were greatest at Weeks 6 and 7, making it the latest deacclimator. A comparison of timing of deacclimation with germplasm composition indicated that, like in the controlled laboratory study, *V. ashei* was

particularly early to deacclimate. *Vaccinium constablaei*, which was not studied previously, was particularly late to deacclimate, and 'Little Giant', a 50:50 hybrid of *V. constablaei* and *V. ashei*, was nearly as late to deacclimate as the 100% *V. constablaei* selections. From this work, we concluded that *V. constablaei* may be useful in breeding programs to contribute genes for late deacclimation, which should translate into greater spring frost tolerance in addition to contributing genes for midwinter hardiness.

To determine if the stage of bud opening could be used as an indicator of the level of BCH, we examined bud opening in the



progressively deacclimating flower buds of the various genotypes in the field study. At each weekly sampling for BCH, several shoots, with five to eight floral buds each, were evaluated for stage of bud opening. Stages were ranked on the scale of 1 to 7 as described by Spiers (1978), with 1 representing the stage with no visible swelling and 7 representing the stage after which corollas had completely expanded and dropped. Percentages of buds at stages 1 to 7 were recorded at each weekly sampling and from this, the average bud score for each week of deacclimation was calculated. Like BCH data, flower bud development scores from Weeks 4 to 10 of year 2002 were averaged with scores from Weeks 1 to 7 of year 2003. At Week 1 of the combined-year study, flower buds of all genotypes were fairly tightly closed (average bud stage of 1.0 to 1.5). They became progressively more developed during the deacclimation regime, reaching stages of 2.3 to 4.0 by Week 7 for all genotypes except *V. constablaei* and 'Little Giant', which remained in the 1.0 to 1.5 range. The correlation between bud opening (average bud score) and BCH values was determined using SAS Proc CORR (SAS Institute, 1999). A highly significant positive correlation was found between the stage of bud opening scores and BCH averaged over the 2 years ( $r = 0.84$ ,  $P < 0.0001$ ). Figure 4 shows a plot of BCH level versus stage of bud opening.

#### RATE OF COLD ACCLIMATION UNDER FIELD CONDITIONS

In a more recent study, we extended our cold tolerance measurements from October through midwinter comparing acclimation kinetics and maximum cold tolerance levels among several blueberry genotypes. For these experiments, shoots were cut from genotypes every 3 weeks in the field and BCH levels determined as described previously calculating  $LT_{50}$  values and their lower and upper confidence limits. We currently have only 1-year's data. The BCH results from this year, 17 Oct. 2006 to 10 Jan. 2007, are shown in Figure 5 for five of the genotypes that were analyzed.

All the genotypes appeared to reach maximum cold tolerance  $\approx 20$  Dec. 2006, but genotypic differences were detected in other aspects, including initial cold tolerance, rate of acclimation, maximum cold tolerance, and length of the plateau. From these preliminary results, it appears that 'Tifblue' was the least cold hardy initially followed by 'Legacy', 'Bluecrop', 'Little Giant', and 'Northsky'. 'Northsky' was already cold hardy to less than  $-22$  °C (the lowest temperature that was tested at the first time point) on 17 Oct., making it the earliest acclimator. Of the time points that were analyzed, however, 'Little Giant' appeared to acclimate the fastest (i.e., its line had the steepest slope at the early time points). By 20 Dec., when maximum cold tolerance was reached, the most hardy cultivars were Little Giant and Northsky ( $LT_{50}$ s less than  $-28$  °C) followed in descending

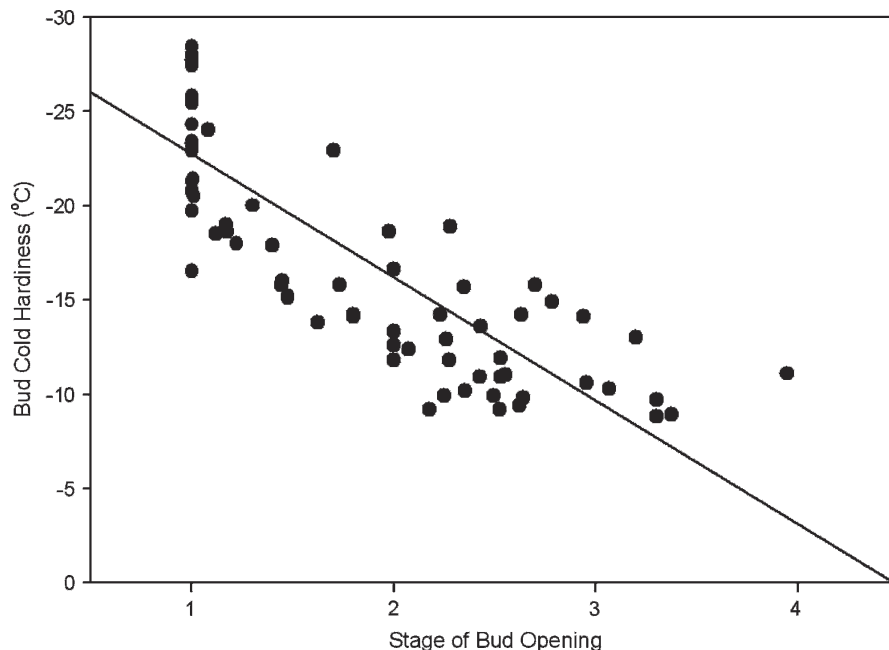


Fig. 4. Graph of bud cold hardiness levels versus average stage of bud opening for the blueberry genotypes in the combined-year (2002 and 2003) field study.

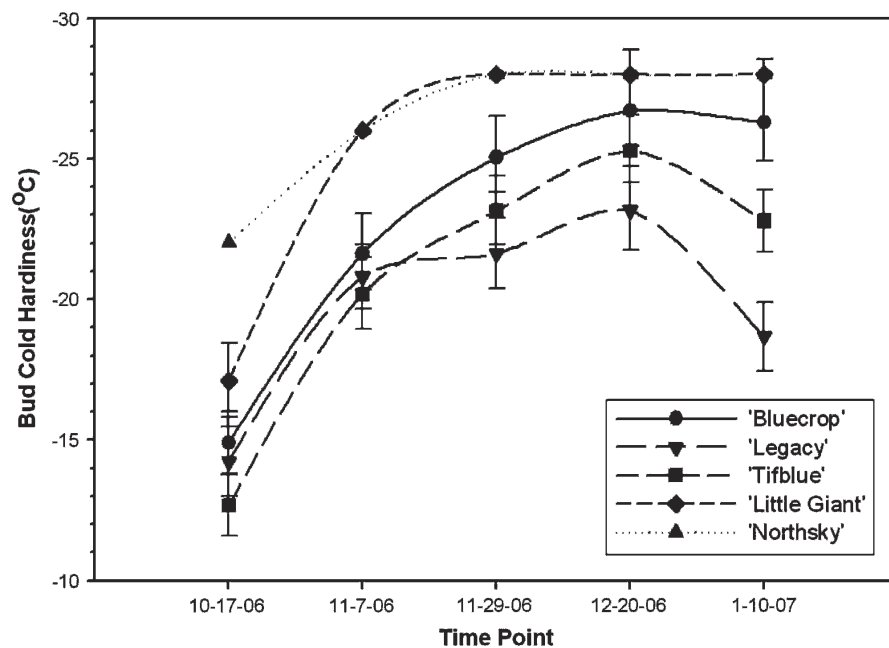


Fig. 5. Acclimation of five blueberry genotypes ('Bluecrop', 'Legacy', 'Little Giant', 'Northsky', and 'Tifblue') under field conditions. Lines show the bud cold hardiness values determined at 3-week intervals from 17 Oct. 2006 through 10 Jan. 2007. Points are the actual observed values and the bars represent the 95% fiducial confidence limits.

order by 'Bluecrop', 'Tifblue', and 'Legacy'. Surprisingly, by 10 Jan. 2007, the least hardy cultivars, Tifblue and Legacy, had already begun to deacclimate, having lost  $\approx 2.5$  and  $4.5$  °C of BCH, respectively, when compared with their maximum cold tolerance levels on 20 Dec. Deacclimation was not detected this early in our previous study (in 2002) when BCH levels were determined at weekly intervals beginning in January. It will be of interest to see when deacclimation begins for these two cultivars in 2008. An examina-

tion of the weather data revealed that November and December of 2006 were the warmest on record since 1895. The first 20 d of 2007 Jan. were also unusually warm, although temperatures cooled considerably by the end of January. Still, 2007 Jan. was  $6.4$  °F higher than average and the 11th warmest since 1895. Thus, the unusually warm early winter weather must have triggered deacclimation in the least hardy cultivars. BCH levels for the hardier 'Bluecrop', however, did not change significantly between the

December and January sampling dates. Because 'Little Giant' and 'Northsky' were both hardy to less than  $-28^{\circ}\text{C}$  (the limit of this method) at these sampling dates, it is impossible to determine whether there was a small drop in hardiness of either of these.

## CONCLUSIONS

Injury to blueberry buds can be caused by fall frosts as plants are cold acclimating or by spring frosts as plants are deacclimating. In the winter, fully acclimated buds can be injured by sustained freezing temperatures. To develop blueberry cultivars for specific regions of the country, a comprehensive understanding of cold hardiness is needed throughout the dormant period from acclimation to deacclimation. In earlier studies, to identify genotypes that are particularly slow or late to deacclimate and thus may be useful in breeding for spring frost-tolerant cultivars, we compared deacclimation kinetics under controlled laboratory (constant temperature) and field conditions among several blueberry genotypes with diverse genetic backgrounds. Clear genotypic differences were found. In the field study, pure *V. constablaei* selections and 'Little Giant', a 50:50 hybrid of *V. constablaei* and *V. ashei*, were particularly late to deacclimate compared with the other genotypes. In the laboratory and field study, 'Tifblue', a 100% *V. ashei* cultivar, was particularly early to deacclimate. In addition, we found a strong positive correlation between stage of bud opening and cold-hardiness level, suggesting that for many genotypes, stage of bud opening could be used as an indicator of cold hardiness in the progressively deacclimating buds. More recently, we have extended our cold tolerance measurements from October through

midwinter comparing acclimation kinetics and maximum cold tolerance levels among blueberry genotypes. Genotypic differences were detected in many aspects, including initial cold tolerance, rate of acclimation, maximum cold tolerance, and length of the plateau. 'Little Giant' and 'Northsky', a 75:25 hybrid of *V. corymbosum* and *V. angustifolium*, were very early to acclimate and were hardier than the other genotypes both initially and when maximum cold tolerance was reached. 'Bluecrop', the industry standard for many areas of the country, was intermediate in terms of its rate of acclimation and deacclimation and maximum cold tolerance level. For the most part, the rates of acclimation and deacclimation and midwinter cold tolerance levels of the genotypes were not surprising considering the natural habitat of the species comprising their genetic background, except perhaps for 'Little Giant' which, although a 50:50 hybrid of *V. constablaei* and *V. ashei*, was nearly as hardy and slow to deacclimate as pure *V. constablaei*.

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