

# Characterization of Southern Highbush Blueberry Floral Bud Cold Hardiness through Dormancy in a Sub-Tropical Climate

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## Abstract

Southern highbush blueberry (SHB; *Vaccinium corymbosum* L. interspecific hybrids) is highly susceptible to freeze damage in sub-tropical climates, but the process is poorly understood. To address the issue, freeze tolerance experiments were conducted on two common cultivars of SHB, Emerald and Farthing, during the winters of 2015-16 and 2016-17. Floral buds (attached and excised from stems) were preconditioned overnight at 4.0 °C or -2.0 °C and exposed to temperatures of -3.0 to -21.0 °C. The samples were then stored at 4.0 °C for a week and afterwards examined for bud damage. A lethal temperature threshold of 50% (LT<sub>50</sub>) was calculated on visual ratings for damage. Cold hardiness varied with preconditioning, bud type (attached or excised), and sampling date, and ranged from -6.8 °C to -20.2 °C in both cultivars. On average, LT<sub>50</sub> was lower in the attached than in the excised buds and when buds were preconditioned at -2.0 °C than at 4 °C. Attached buds displayed increasing hardiness as the chill hours accumulated, until the buds began to swell, at which time the hardiness decreased. Cold hardiness did not change in the excised floral bud in either cultivar or year. Preconditioning increased the hardiness of the attached buds at -2 °C, which may be artificially inflated considering sub-tropical climate. This work shows that attached SHB floral buds are sensitive to chill hour accumulation and deacclimation. Whereas, excised buds did not respond with similar sensitivity. For SHB, the best estimation for hardiness can be obtained through attached buds preconditioned at 4 °C.

Southern highbush blueberries were bred from northern highbush blueberry (NHB; *V. corymbosum* L.) and low chill blueberries (e.g. *V. darrowii* Camp) for cultivation in sub-tropical climates (Lyrene and Sherman, 2000; Lyrene, 2008a). In the southeastern U.S., the mean temperature for the coldest three months of winter may be as high as 15 °C, whereas, NHB may not be productive unless a mean temperature of 10 °C is attained due to insufficient chilling (Lyrene and Sherman, 2000; Retamales and Hancock, 2012).

Northern Florida and southern Georgia producers grow SHB cultivars that require an average winter chill of  $\leq 300$  h. Chill is commonly calculated as an accumulation of hours between 0 °C to 7 °C (Chandler et al.,

1937), which is the easiest and widely accepted method used by most producers to estimate chill hour accumulation. However, in sub-tropical climates there may be periods during winter months where temperatures are within the range for plant growth. For example, SHB cultivars with chill hour requirements of 300 h to 600 h were grown in a non-dormant production system in which only 69 and 118 chilling hours ( $<7$  °C) were accumulated over two seasons, respectively (Reeder et al., 1998). All of the cultivars tested had sufficient vegetative and reproductive growth suggesting the SHB cultivars tested were not regulated by endodormancy and may flower when conditions for physiological and morphological development are met

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(Reeder et al., 1998). 'Emerald' will flower with above normal temperatures ( $> 15^{\circ}\text{C}$ ) in late winter and early spring (Lyrene, 2008a) and in Georgia, 'Emerald' has been observed to flower and set fruit in the fall (E. Smith, personal communication). Concomitantly, *V. darrowii* and SHB are photoperiod sensitive and flowering was shown to be promoted by short day photoperiods (8 h) (Spann et al., 2003). These data show that SHB can flower without prolonged dormancy and with low to no accumulated chilling.

In Michigan, NHB harvest dates were observed to be a function of chill hour accumulation, low temperature threshold, and starting date for heat-unit accumulation (Carlson and Hancock, 1991). Their modeling suggested that blueberries tend to advance their harvest date with warm late winter temperatures. In sub-tropical climate, winter temperatures can be sufficiently warm to break dormancy of SHB, and without prolong periods of cold, may cause tight floral buds to have greater sensitivity to cold than blueberry grown in temperate climates.

Two important concerns in blueberry production are winter hardiness and susceptibility to spring frosts (Moore, 1993). Blueberry cold hardiness throughout dormancy has been reported on NHB and SHB cultivars grown in northern climes (Ehlenfeldt et al., 2009; Rowland et al., 2005; Rowland et al., 2008). However, low chill hour ( $< 300$  h) SHB cultivars sensitivity to freezing temperatures, while dormant, is not well understood under sub-tropical field conditions. In sub-tropical growing regions the chill is not steadily accumulated and periods of warmth between cold can trigger plant growth. The objective of this study was to investigate two SHB cultivars, Emerald and Farthing, sensitivity to cold using freeze tolerance tests over two winter seasons. Our expectation was that lethal freezing temperatures, where 50% of the florets are damaged ( $LT_{50}$ ), would be higher than what has been reported for SHB grown in northern climes (Ehlenfeldt et al., 2009).

## Materials and Methods

*Plant material.* 'Emerald' and 'Farthing' SHB terminal fruit bud bearing shoots were harvested periodically from contiguous rows at a commercial blueberry farm in Lakeland, GA ( $31^{\circ}06'28.00''$  N,  $83^{\circ}06'42.76''$  W) during the seasons 2015-16 (Nov. to Feb.) and 2016-17 (Nov. to Jan.). At the initiation of the experiment, the planting was in its fifth year of production under conventional farming practices for the southeastern U.S. At each sampling, shoots of 25 cm to 31 cm were collected from a minimum of 30 plants. In the field, stems were placed in sealable plastic bags wrapped in a moist paper towel and transported to the laboratory. At the lab, samples were prepared for shipment by removing leaves near the pruning cut and wrapping the pruned end of the stems in moist paper towels. Samples were shipped overnight to the University Georgia's Cold Hardiness Laboratory at the Griffin Campus, Griffin, GA. Samples were transported and shipped at ambient temperature to maintain field conditions. From previous blueberry floral bud hardiness studies, Ehlenfeldt et al. (2009) and Rowland et al. (2005) described transport using ice from samples collected on fields where temperatures ranged from  $13.4^{\circ}\text{C}$  to  $3.7^{\circ}\text{C}$  or above the snowline. Whereas, in south Georgia, winter season daily high temperatures regularly reach  $21^{\circ}\text{C}$  (GAEMN, 2017) and placing samples on ice would not reflect field conditions. Upon arrival in Griffin, samples were held at  $4^{\circ}\text{C}$  until preparation (within 48 h). The samples were prepared by removing remaining leaves; then stems with intact attached buds were trimmed to 5 cm segments with a minimum of 1.5 cm of stem remaining below the proximal bud. Excised buds were carefully cut to exclude stem tissue (Flinn and Ashworth, 1994).

*Freeze hardiness determinations.* For both 'Emerald' and 'Farthing' at each sampling date, eight sets of six randomly selected samples of attached (5 cm stems) or excised buds were placed into a moistened paper

towel (Kimwipe, Irving, TX) and enclosed in a sealable freezer bag. In season 2015-16, sample sets were treated to an overnight temperature of 4 °C (from 16 Nov. to 12 Jan.) and -2 °C (from 25 Jan. to 15 Feb.). During the 2016-17 season, a set of samples were each pre-treated at either 4 °C or -2 °C overnight before each freeze test. The bags were organized and placed on a rack in the ESPEC EY-101 (Tabai Espec Corp., Osaka, Japan) freeze chamber and set to a freezing rate of 4 °C h<sup>-1</sup>. Removal occurred every 3 °C, from -3 °C to -21 °C. A control bag of each tissue type (attached vs. excised) and cultivar was held at 4 °C. The freeze treated and control floral buds were stored in a refrigerator at 4 °C for a week. Samples were then brought to room temperature, dissected, and inspected for discolored floral tissue (e.g. browning) under a light microscope (Olympus BX51, Tokyo, Japan). The lethal temperature where 50% of the floral tissue was damaged, LT<sub>50</sub>, was rated as the percentage of injured florets compared to total florets within a floral bud (Arora et al., 2000; Flinn and Ashworth, 1994).

*Floral bud mass and size.* Water relations within the bud and tissue type can significantly alter the cold hardiness of a floral bud (Kader and Proebsting, 1992; Flinn and Ashworth, 1994). Floral buds of *Prunus* spp. that were dehydrated expressed lower hardiness temperatures than hydrated samples (Kader and Proebsting, 1992); while, blueberry with excised floral bud were less tolerant to freezing than attached buds (Flinn and Ashworth, 1994). However, both the *Prunus* spp. and blueberries were grown in temperate climates with longer duration of freeze through winter period, where blueberry grown in the subtropics may have interrupted periods of chill (> 7.2 °C). To identify subtropical cultivation on blueberry water relationship to freezing, fresh bud weight and length were measured using a scale (Model ML203E, Mettler Toledo, Columbus, OH) and a micrometer (Traceable, VWR International, Radnor, PA). Ten labeled excised buds from each cultivar were

measured on each sampling date. After fresh weight measurement, samples were dried in an oven (Thermo Electron Corporation, Beverly, MA) at 60 °C to a constant weight for about of 48 h and recorded.

*Weather Data.* All weather data was collected from the University of Georgia Automated Environmental Monitoring Network (GAEMN, 2017) accessing data from the Homerville, GA station (31°00'58" N, 82°39'02"W), which is 5° S and 44 km from the sample collection site. Chill hours were calculated as chill hours accumulated between 0 °C to 7 °C using the calculator provided by GAEMN. Daily minimum and maximum temperatures were downloaded from 14 Nov. 2015 to 15 Feb. 2016 and 27 Nov. 2016 to 31 Jan. 2017, which covers the sampling period for each respective season.

*Statistical Analysis.* The LT<sub>50</sub> curves were calculated as a nominal logistic model in JMP Pro (SAS, Cary, NC) version 13. All other analyses were evaluated using Proc GLM with SAS 9.4 (SAS Institute Inc., Cary, NC, U.S.) and means were separated at  $P < 0.05$  level using Tukey's honest significant difference (HSD) test.

## Results and Discussion

Mean daily maximum and minimum temperatures were erratic during the study and averaged 20.3 and 7.1 °C, respectively, in 2015-2016 and 21.3 and 7.7 °C, respectively, in 2016-2017 (Fig. 1). Both cultivars received sufficient chill hours for bloom, which is 100-400 h for 'Emerald' and 300 h for 'Farthing' (Lyrene, 2008 a, b) (Table 1, 2, 3, and 4).

Excised floral buds were generally less hardy than attached floral buds, regardless of cultivar or the preconditioning temperature (Table 1 and 2). Flinn and Ashworth (1994) also observed lower hardiness in excised buds in NHB and concluded that ice nucleators associated with woody tissue are removed when the stem is excised yielding more conservative results. In the present study, LT<sub>50</sub> of attached floral buds reached a

**Table 1.** Lethal temperature at which 50% of attached or excised buds were damaged by freezing ( $LT_{50}$ ) in ‘Emerald’ and ‘Farthing’ southern highbush blueberry. The buds were sampled during the winter months of 2015-2016.

Sample Date <sup>a</sup>	Chill Hours <sup>b</sup>	$LT_{50}$ (deg. C)							
		Emerald			Farthing				
		Attached Buds	Excised Buds		Attached Buds	Excised Buds			
16 Nov.	18				-9.8 a	A	-6.8 a	A	
7 Dec.	44	-11.7 ab	A <sup>c</sup>	-10.9 bc	A	-11.6 ab	A	-10.8 c	A
21 Dec.	96	-16.1 cd	B	-8.2 ab	A	-14.6 bc	B	-7.5 ab	A
12 Jan.	164	-16.4 cd	B	-13.0 c	A	-12.3 abc	A	-11.9 c	A
25 Jan.	266	-17.7 d	B	-10.9 bc	A	-17.7 d	B	-10.9 c	A
1 Feb.	314	-14.0 bc	B	-7.5 a	A	-16.7 d	B	-9.6 bc	A
8 Feb.	371	-16.7 d	B	-10.3 abc	A	-18.6 d	B	-11.7 c	A
15 Feb.	433	-10.4 a	A	-9.4 ab	A	-16.4 cd	B	-12.1 c	A

<sup>a</sup> Prior to freezing, bud samples were preconditioned overnight at 4 °C from 16 Nov. to 12 Jan. and at -2 °C from 25 Jan. to 15 Feb.

<sup>b</sup> Total hours at 0-7 °C.

<sup>c</sup> Means followed by the same lower-case letter within a column, or by the same upper-case letter within a row and cultivar, are not significantly different at  $P \leq 0.05$  according to Tukey HSD.

minimum of -17.7 and -18.6 °C in ‘Emerald’ and ‘Farthing’, respectively, in 2015-2016 and -20.2 and -20.2 °C, respectively, in 2016-2017. In each case, the minimum occurred in January or early February, and  $LT_{50}$  increased to -10.0 to -16.0 °C in the weeks afterwards. Excised floral buds, on the other hand, had only -7.0 to -10.0 °C over the entire sampling period, regardless of year, cultivar, or preconditioning. Clearly, excision reduced hardiness of the buds and is a poor method for determining freeze tolerance in SHB. Rowland et al. (2013) observed that when testing the freeze tolerance of blueberry flowers using stem tissue with buds attached responded similarly to whole plant freeze tolerance comparisons. This work suggests that attached floral buds reflect natural freezing in the field.

Preconditioning at or below freezing temperatures is often used in freeze tolerance tests to minimize ice nucleation and to allow intercellular water to supercool and better tolerate subfreezing conditions (Ashworth, 1991; Quamme, 1983). In our case, attached buds preconditioned at -2 °C were typically hardier than those preconditioned at 4 °C

(Table 2). Bittenbender and Howell (1975) also found that preconditioning increases hardiness in ‘Jersey’ NHB in Michigan. When attached buds were preconditioned at -2 °C, ‘Emerald’ reached a maximum  $LT_{50}$  of -10.4 and -13.6 °C on the last sampling date of each year, respectively (Table 1 and 2), while ‘Farthing’ reached a maximum  $LT_{50}$  of -16.4 and 13.4 °C on last sample date of each year, respectively (Table 1 and 2). In Georgia, a majority of the commercial SHB production occurs within USDA hardiness zone 8B, where the 30-year-average temperature is -6.7 to -9.4 °C (USDA, 2017). The lowest temperatures recorded at the Homerville weather station during the present study were -3.6 °C during the winter of 2015-2016 and -4.4 °C during the following winter. At no point did the samples of either cultivar show any browning florets in the control samples, which indicate critical temperatures had not been surpassed in the field. Because temperatures rarely fall below -10 °C or remain below 0 °C for 12 continuous hours (Fig. 1), preconditioning floral bud samples of SHB grown in the subtropics could be artificially increasing hardiness of field conditions.

**Table 2.** Lethal temperature at which 50% of attached or excised buds were damaged by freezing (LT<sub>50</sub>) in ‘Emerald’ and ‘Farthing’ southern highbush blueberry. The buds were sampled during the winter months of 2016-2017. Prior to freezing, bud samples were preconditioned overnight at 4 or -2 °C.

Sample Date	Chill Hours <sup>z</sup>	Emerald				Farthing			
		Attached buds		Excised buds		Attached buds		Excised buds	
		4 °C	-2 °C	4 °C	-2 °C	4 °C	-2 °C	4 °C	-2 °C
29 Nov.	118	-13.3 bc B <sup>y</sup>	-17.3 bc C	-10.5 ab A	-10.5 b A	-10.2 a A	-15.6 a B	-8.1 a A	-8.1 a A
13 Dec.	170	-14.0 c B	-18.1 bc C	-9.9 a A	-13.0 c B	-14.4 c B	-18.4 b C	-11 c A	-10.5 c A
5 Jan.	235	-14.5 c B	-19.0 c C	-10.3 ab A	-8.8 a A	-17.0 d B	-20.2 b C	-11 c A	-10.8 c A
17 Jan.	287	-13.8 bc B	-15.5 ab B	-10.5 ab A	-10.5 b A	-16.8 d B	-18.8 b B	-9.7 b A	-10.5 c A
24 Jan.	287	-9.8 a A	-13.7 a B	-11.2 b AB	-10.5 b AB	-11.5 ab A	-14.7 a B	-11 c A	-10.6 c A
31 Jan.	333	-11.5 ab B	-13.6 a C	-11.0 b AB	-9.9 b A	-12.6 bc B	-13.4 a B	-11 c AB	-9.3 b A

<sup>y</sup> Means followed by the same lower-case letter within a column, or by the same upper-case letter within a row and cultivar, are not significantly different at  $P \leq 0.05$  according to Tukey HSD.

Floral bud moisture content (%) in blueberry has been shown to decrease as water content increases during de-acclimation (Biermann et al., 1979; Bittenbender and Howell, 1975). At each sampling for both years and cultivars, bud water content, dry weights, and lengths are reported (Table 3 and 4). For ‘Emerald’, dry weights, water content and length increased from initial to final sampling in both years. In 2016, 8 Feb. to 15 Feb. showed a significant increase in fresh and dry weight (84% and 54%, respectively) (Table 3). For 2017, increase in fresh and dry weight was observed from 24 Jan. to 31 Jan. at 46% and 53%, respectively, though not significant

(Table 4). The lack of significance suggests that ‘Emerald’ had deacclimated and as was progressing towards bloom by 24 Jan. Similar trends were observed in bud length (Table 3 and 4) where increasing length of tight floral buds was associated with deacclimation. ‘Farthing’ demonstrated a similar pattern for both years for weight and length measurements of the last two sample dates (Table 3 and 4). Comparing years, ‘Farthing’ had 38% less weight at the end of sampling in 2016 than in 2017 (Table 1 and 2) and this could be attributed to higher temperatures in 2017. In 2016, the last 14 days of sampling had min/max temperatures of 18.1/4.2 °C with 3 days

**Table 3.** Dry weight, length, and water content of the floral buds collected from ‘Emerald’ and ‘Farthing’ southern highbush blueberry. The buds were sampled during the winter months of 2015-2016.

Date	Chill Hours <sup>z</sup>	Emerald			Farthing		
		Dry Bud	Bud Water	Bud Length	Dry Bud	Bud Water	Bud Length
		wt (mg)	content (%)	(mm)	wt (mg)	content (%)	(mm)
16 Nov.	18	10.8 b <sup>y</sup>	63.7 cd	4.6 b	10.0 e	62.2 a	5.7 c
7 Dec.	44	13.5 b	60.8 d	4.8 b	10.5 de	62.3 a	5.7 c
21 Dec.	96	15.8 b	63.6 cd	5.2 b	12.6 cde	65.0 a	6.2 bc
12 Jan.	164	14.2 b	64.4 bc	4.9 b	16.1 abc	64.8 a	6.8 ab
25 Jan.	266	13.2 b	66.0 bc	4.9 b	14.5 b-e	64.8 a	6.5 abc
1 Feb.	314	15.2 b	67.5 b	5.1 b	17.5 ab	64.4 a	6.8 ab
8 Feb.	371	14.4 b	66.2 bc	5.0 b	15.3 bcd	61.9 b	6.5 bc
15 Feb.	433	22.2 a	72.0 a	6.7 a	20.3 a	65.8 a	7.6 a

<sup>y</sup> Total hours at 0-7 °C.

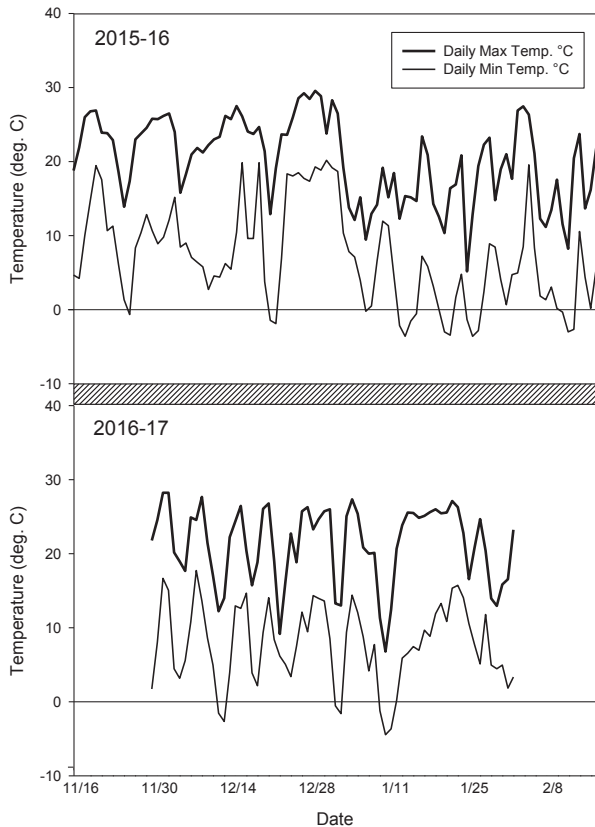
<sup>z</sup> Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$  according to Tukey HSD.

**Table 4.** Dry weight, length, and water content of the floral buds collected from ‘Emerald’ and ‘Farthing’ southern highbush blueberry. The buds were sampled during the winter months of 2016-2017.

Date	Chill Hours <sup>z</sup>	Emerald			Farthing		
		Dry Bud	Bud Water	Bud Length	Dry Bud	Bud Water	Bud Length
		wt (mg)	content (%)	(mm)	wt (mg)	content (%)	(mm)
29 Nov.	118	8.4 b <sup>y</sup>	56.1 c	4.2 b	5.4 d	59.0 bc	4.5 d
13 Dec.	170	12.3 b	57.5 c	4.5 b	8.6 cd	56.3 c	5.0 cd
5 Jan.	235	11.5 b	57.8 c	4.4 b	12.5 bc	59.5 abc	5.5 cd
17 Jan.	287	13.4 b	61.0 bc	4.8 b	12.6 bc	61.4 abc	5.9 bc
24 Jan.	287	18.0 ab	71.0 a	5.4 ab	18.0 b	66.2 ab	6.9 ab
31 Jan.	333	27.5 a	68.6 ab	7.0 a	26.2 a	68.3 a	7.9 a

<sup>y</sup> Total hours at 0-7 °C.

<sup>z</sup> Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$  according to Tukey HSD.



**Figure 1.** Daily minimum and maximum temperature measured over floral bud sampling period for 2015-16 (16 Nov. to 15 Feb.) and 2016-17 (29 Nov. to 31 Jan.). Data gathered from Georgia Automated Environmental Monitoring Network (<http://www.georgiaweather.net/>), Homerville, GA station.



below 0 °C (9, 10, 11 Feb. at -0.3 °C, -3.0 °C, and -2.7 °C, respectively); whereas, the last 14 days of sampling in 2017 had min/max of 21.2/9.1 °C with no freezing temperatures recorded (Fig 1). Water content in floral buds can fluctuate during dormancy (Quamme, 1983) and sensitivity is affected by the level of moisture within the bud (Bittenbender and Howell, 1975; Hewett et al., 1979). Our work suggests that increasing water content and length of tight floral buds are indicators of a loss of hardiness for subtropical field grown ‘Emerald’ and ‘Farthing’.

### Conclusions

The findings indicate that experimentally freezing excised SHB (‘Emerald’ and ‘Farthing’) floral buds tended to be more sensitive to freeze than attached floral buds. Pre-conditioned floral buds below freezing can significantly increase hardiness of the buds, which may be inflating hardiness of subtropical SHB. Attached floral buds for both cultivars with 4.0 °C preconditioning in both years were not > -9.8 °C, which is hardier than the 30 average for hardiness zone 8b. The least hardy buds were at the beginning of chill accumulation and when buds beginning to swell, which indicated deacclimation. This work should give subtropical SHB growers clear indication that tight SHB floral buds are not being damaged during average winters.

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