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Freezing Survival of 'Illini Hardy' Blackberry Floral Tissues Before and After Budbreak

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Abstract

Dormant floral buds and inflorescences of 'Illini Hardy' blackberry (*Rubus* sp.) plants were subjected to controlled freezing tests. The susceptibility of dormant floral buds to low temperatures was determined by differential thermal analyses (DTA) and the hardiness of individual flowers at various stages of development was determined by viability testing. The mean temperature at which all low temperature exotherms (LTEs) were detected in primary buds was -15.0, -18.4, and -13.8°C in November, January, and March, respectively. The mean LTE temperature for secondary buds was -24.8°C in January and -18.9°C in March, indicating that secondary buds could provide a replacement crop when the primary bud was injured. After budbreak, inflorescences deacclimated as bloom progressed. When the flowers were at tight bud, the temperature range in which all flowers in the inflorescence were injured was 1.6°C. However, when the terminal flower was at full bloom or at a later stage of development, the critical temperature range was $\leq$ 0.3°C.

Introduction

'Illini Hardy' is an erect-growing hardy blackberry cultivar developed in the breeding program of the Illinois Agricultural Experiment Station (11). 'Illini Hardy' is a cross of 'Chester Thornless' and 'NY 95' made in 1980 (Fig. 1). It has been tested in several midwestern states, including Minnesota, Wisconsin, New York, and Mississippi. The most notable characteristic of 'Illini Hardy' is the capacity of its canes to survive temperatures $\geq$ -33°C (Gail Nonnecke, personal communication). In Geneva, N.Y., 'Illini Hardy' plants produced greater yield and fruit quality was better than that of the hardy blackberry cultivar, 'Darrow' (11).

'Illini Hardy' fruit is generally harvested from 4 July through 10 Aug. in Urbana. The glossy black fruit is elliptical in shape and averages 4.6 g in weight. 'Illini Hardy' yields range from 5.6 to 8.9 tons/ha (Otterbacher, unpublished data). Average seed size is 1.8 mm x 2.9 mm. Fruit flavor resembles that of wild blackberries and soluble solids average 10.1°Brix.

Canes of 'Illini Hardy' branch sparsely and are typically 1.5 to 2.0 m in length. Suckering is limited and plants tend to grow as a clump of 6 to 10 canes. Most root suckers are within 25 to 30 cm of the base of the plant. The straight to slightly curved thorns are 5 to 10 mm long and the average number of thorns is 1.7/cm on primocanes. Primary buds are conspicuous on the canes. Smaller secondary buds are often present below primary buds. The presence of secondary buds may also indicate that a replacement crop could be produced if primary buds are injured by low temperatures (6, 13, 16).

The purpose of this study was to determine the hardiness of overwintering floral buds of 'Illini Hardy' blackberry by DTA and to evaluate the susceptibility of individual flowers to low temperature injury at various stages of development after budbreak by viability testing.

Materials and Methods

DTA tests on overwintering buds. 'Illini Hardy' blackberry canes were collected from 2-year old plants at the University of Illinois Pomology Research Station, Urbana, IL. Two node samples were obtained at approxi-

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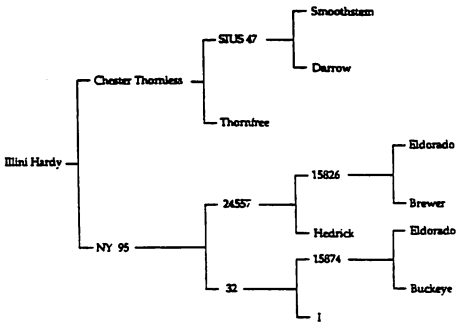

Figure 1. Pedigree of 'Illini Hardy' blackberry.

mately 1 m above the soil surface on 13 Nov. 1990, 8 Jan. and 4 Mar. 1991 for DTA tests. Tissue was sealed in polyethylene bags containing moist paper towels, packed on ice, and shipped to the University of Missouri by overnight mail. Samples were stored at 3°C until freezing tests were performed. Three to six primary buds were subjected to DTA at all three collection dates. Three secondary buds were tested in January and six were evaluated in March.

To prepare samples for DTA, individual buds with a small section of adjacent stem tissue were excised from canes. Each sample was placed in a small aluminum foil cup and attached to a thermistor sensor in a DTA system described by George (5). An empty foil cup was placed in contact with the reference chamber sensor to balance the heat capacity of the reference and sample chambers. The temperature of the DTA chamber was lowered rapidly to 0°C and cooling at 3°C/h was initiated when the bud temperature was within 1°C of the reference temperature. Data were collected at 40 sec intervals until the sample temperature reached -40°C.

After DTA was completed, buds were immediately removed from the freezing chamber and examined under a dissecting microscope to determine if cold injury had occurred prior to field collection. Floral primordia of buds injured in the field exhibited oxidative browning, whereas those injured during DTA appeared green, but water-soaked. Only data from buds that were uninjured in the field were recorded. The number of floral primordia per uninjured bud was also determined.

Viability testing after budbreak. 'Illini Hardy' inflorescences were collected from Urbana, IL at five different developmental stages (Table 1). Inflorescences at stage 1, 2, 3, 4, and 5 were collected on 1, 14, 16, 17, and 21 May 1991, respectively. Each sample consisted of an intact inflorescence, which was placed in an orchid tube containing water, and shipped to the University of Missouri by overnight mail.

Each sample was removed from the orchid tube and five intact inflorescences were placed in moist cheesecloth and wrapped in aluminum foil for each of five test temperatures. Tissue temperature was monitored with a 0.01 mm-diameter (30-gauge) copper-constantan thermocouple (Omega

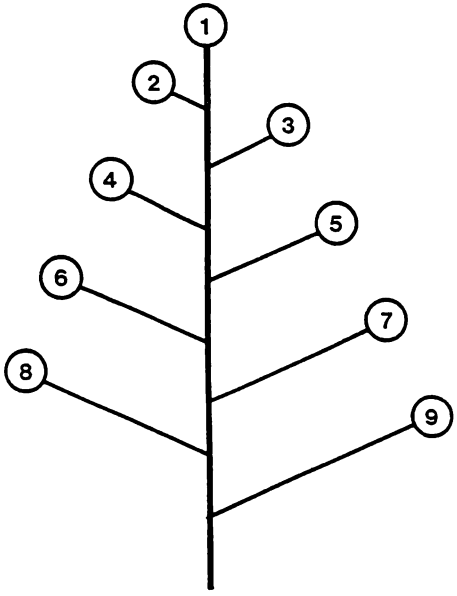

Figure 2. Numbering system of flowers within an 'Illini Hardy' inflorescence used to evaluate anther injury in viability tests.

Table 1. Stages of bud development after budbreak in 'Illini Hardy' blackberry.

Stage	Description
1	All flowers in tight bud.
2	Sepals separated on the first flower.
3	First flower at full bloom.
4	First, eighth, and ninth flowers at petal fall; second, third, and fourth flowers at first white; fifth, sixth, and seventh flowers at full bloom.
5	First fruit had substantial drupelet enlargement and the eighth and ninth fruits had less drupelet enlargement. All other flowers at first white or full bloom.

Engineering, Stamford, CT) attached to individual samples. Thermocouple output was recorded on a Honeywell Electronik 112 multipoint recorder (Honeywell, Fort Washington, PA). Samples were placed in a programmable freezer (Tenney Engineering, Union, NJ) and held at -1°C for 2 h. During this time, the cheesecloth froze and seeded the tissue with ice. Samples were then cooled at 1°C/h. Inflorescences at the first stage of development were removed from the freezing chamber at 2°C intervals from -2 to -10°C. Samples at all other floral stages were removed from the freezer at temperature intervals estimated to result in injury (between -1 and -4°C). After freezing, samples were slowly thawed at 3°C for 24 h. Unfrozen controls, maintained at 3°C during the freezing test, and samples that were subjected to freezing were then incubated at 23°C at 100% RH for 24 h. Preliminary tests revealed that anthers were the most susceptible floral organ to low temperature injury. Thus, the first temperature at which oxidative browning of anthers occurred (T_1 value) was recorded for individual flowers. The terminal flower on the inflorescence axis was considered the first flower, with the numbering proceeding basipetally (Fig. 2). At stage 5, anthers on the first, eighth and ninth flowers of unfrozen samples had already senesced, so data were not recorded for these flowers of samples subjected to sub-zero temperatures. T_1 values were subjected to an analysis of variance and means were separated by LSD.

Results

Temperatures throughout the 1990-1991 winter were generally mild (Fig. 3). The lowest temperature (-22°C) during the winter was recorded on 24 Dec. and 22 Jan. However, the average

Table 2. Temperatures at which LTEs were recorded in DTA tests of primary and secondary buds of 'Illini Hardy' blackberry collected from Urbana, Illinois.

Collection date	DTA test date	LTE temperature (°)		
		Bud 1	Bud 2	Bud 3
			Primary buds	
13 Nov. 1990	15 Nov.	-16.4	-15.8, -18.5	-15.8, -19.1
	19 Nov.	-16.4	-8.4	-14.0
7 Jan. 1991	8 Jan.	-12.0, -16.8	-14.0	-14.2, -21.4, -21.5
	10 Jan.	-24.6	no LTE	-20.0
4 Mar. 1991	5 Mar.	-9.0, -16.8	-7.8, -13.0, -17.2, -20.8	no LTE
			Secondary buds	
8 Jan. 1991	15 Jan.	-25.0	-24.8	-24.6
4 Mar. 1991	11 Mar.	no LTE	-18.4	-15.4
	14 Mar.	no LTE	-20.2, -24.4	-19.6

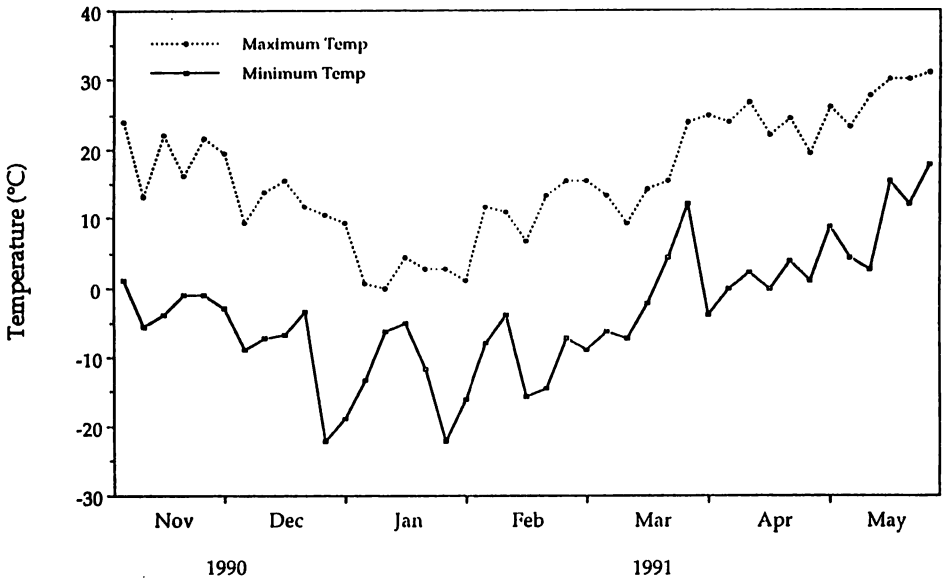

Figure 3. Maximum and minimum temperatures at five day intervals from November 1990 through May 1991 in Urbana, IL.

temperature during December and January was -1.4 and -4.7°C, respectively. The 5-day mean air temperature preceding the collection of floral buds has been correlated with the exotherm temperatures derived from DTA (10). The 5-day mean temperature preceding the sampling of 'Illini Hardy' buds on 13 Nov., 8 Jan. and 4 Mar. was 4.0, -5.6, and 4.1°C, respectively.

DTA on floral buds. 'Illini Hardy' primary floral buds displayed one or two LTEs per bud in the range of -8.4 to -19.1°C in November (Table 2).

Table 3. The first temperature at which anther injury was observed in flowers of 'Illini Hardy' blackberry after budbreak.

Flower no.	Stages of floral development				
	1	2	3	4	5
			Temperature (°C)		
1	-2.8	-1.7	-1.6	-1.7	---[z]
2	-4.4	-3.2	-1.6	-1.7	-1.2
3	-3.6	-3.2	-1.6	-1.8	-1.0
4	-3.6	-2.8	-1.6	-2.0	-1.2
5	-3.6	-2.1	-1.6	-1.8	-1.0
6	-4.0	-1.7	-1.6	-1.7	-1.0
7	-3.6	-1.7	-1.6	-1.8	-1.0
8	-3.6	-1.8	-1.7	-1.7	---
9	-3.6	-1.7	-1.7	-1.7	---
LSD 0.01	0.6	0.6	NS	NS	NS
ANOVA					
Stage		°°			
Flower		°°			
Stage x Flower		°°			

[z]Data not recorded since anthers of flowers of unfrozen controls had already senesced.
°°Significant at $P = 0.01$.

By January, all buds exhibited one to three LTEs per bud between -12.0 and -24.6°C, except one. By March, two to four LTEs per bud were detected in two samples ranging from -7.8 to -20.8°C. LTEs were not detected in one sample tested at this date.

One LTE was detected from each of the three secondary buds tested in January. Four of six secondary buds tested in March exhibited LTEs. One or two LTEs per bud were detected in the range of -15.4 to -24.4°C. One of the buds that did not display a LTE was vegetative.

When samples were examined under the microscope after the DTA test, nine to 12 differentiated floral primordia were evident in primary buds by November. In January, the number of floral primordia per bud was similar to that of November samples. However, stamen and carpel initiation was observed on the terminal flower and also on the tenth flower. The second flower was the least developed. In March, the number of floral primordia remained constant but floral enlargement was observed. Stamen and carpel development was observed on the first, ninth, and tenth floral primordia.

Secondary buds were present below the primary buds on 'Illini Hardy' canes at each collection date. All secondary buds examined in November had initiated up to nine floral primordia, which were at an early stage of development. By March, sepals, petals, carpels, and stamens had developed on the first, ninth, and tenth flowers.

Viability testing after budbreak. In general, the T_1 value of anthers increased as floral development progressed (Table 3). At the first stage of development after budbreak, anthers in the terminal flower were injured at a higher temperature than those in other flowers (Table 3). Anthers of the second flower were injured at a lower temperature than those of all other flowers except the sixth one. At the second stage of development, anthers in the second, third, and fourth flowers had a lower T_1 values than those in the other flowers of the inflorescence. T_1 values of anthers did not differ among flowers at the third, fourth, or fifth stage of development.

Discussion

Primary buds of 'Illini Hardy' exhibited up to four LTEs per bud when subjected to DTA. When the LTE temperatures were averaged for each bud, the mean temperature at which all LTEs were detected was -15.0, -18.4, and -13.8°C in November, January, and March, respectively. Although primary buds apparently acclimated to a lower temperature in January and deacclimated by March, the LTEs were detected at relatively high temperatures. The reason for this may be attributed to warm temperatures immediately preceding collection. Alternatively, buds may have deacclimated before the DTA test while in storage at 3°C. Other researchers (1, 8, 9) have demonstrated that peach and cherry buds deacclimate to a "minimum hardiness level" when exposed to temperatures above -2.2 and -1.1°C, respectively. As bud development progressed after rest completion, the minimum hardiness level rose. Both the mild temperatures preceding the collection of buds and exposure to 3°C prior to freezing may have contributed to the relatively warm temperatures at which LTEs were detected in 'Illini Hardy' buds.

DTA conducted on secondary buds revealed that the mean LTE temperature was -24.8°C in January and -18.9°C in March. Thus, the mean LTE temperature for secondary buds was lower than that of primary buds. These results concur with other reports in the literature where secondary buds were hardier than primary buds (7, 13, 14).

The number of LTEs detected by DTA did not correspond to the number

of floral primordia per bud. It is unclear if all the LTEs detected are related to freezing of the floral primordia. In 'Illini Hardy' buds, several primordia may have frozen simultaneously when a LTE was detected. 'Shawnee' blackberry buds also exhibited this type of freezing response when subjected to DTA (12).

Vegetative buds did not display LTEs when subjected to DTA were also absent in some floral buds tested in March. Supercooling of the floral primordium has been associated with vascular development in the bud axis of peach (2, 3). When vascular tissues of peach were procambial, the primordium supercooled. However, this capacity to avoid freezing was lost when vascular continuity was established between the bud axis and the floral primordium. In another study, differentiated vessel elements were present at the base of bud axes and near the pedicels of individual flowers in 'Illini Hardy' buds sampled in March (15). Thus, the absence of LTEs in 'Illini Hardy' blackberry buds tested in March may be attributed to vascular differentiation in the bud axis of samples that did not display LTEs.

Results from this study confirm that the floral differentiation in primary buds of 'Illini Hardy' is similar to that of other thorny, erect growing blackberry cultivars (12). However, the early differentiation of up to nine primordia with distinct floral organs in secondary buds prior to budbreak has not been previously reported. Warmund and George (13) found that by February, three floral primordia in secondary buds of 'Darrow' and up to seven floral primordia in secondary buds of 'Choctaw' and 'Navaho' had differentiated. Wood and Robertson (16) reported that primary buds of red raspberry *(Rubus idaeus)* suppressed development of secondary buds. However, when primary buds were injured, secondary buds provided a replacement crop.

Viability tests revealed that 'Illini Hardy' inflorescences were most tolerant of low temperature injury at the tight bud stage. Flowers became less resistant to low temperatures as bloom progressed. These results are consistent with those reported by Ballard et al. (4) on bloom stages of other fruits, including apple, pear, apricot, cherry, peach, and prune. At the second stage of development, the flowers that bloomed last (second, third, and fourth) were more resistant to low temperature injury than those that bloomed earlier. However, by the third stage of floral development, T_1 values of all flowers in the inflorescence were similar.

The temperature range of the T_1 values among all flowers in the inflorescence was 1.6 and 1.5°C at the first and second stage of floral development, respectively. However, at stages 3, 4, and 5, the temperature range for floral injury was very narrow ($\leq$0.3°C). Thus, much of the yield would be lost when the temperature dropped to the T_1 value of the terminal flower at these later stages.

In conclusion, 'Illini Hardy' plants often bear primary and secondary buds that are fruitful. Although these buds attained maximum hardiness in January, they had deacclimated by March. Secondary buds exhibited greater hardiness than primary buds and may provide a replacement crop if primary buds are injured by low temperatures. After budbreak, flowers became less resistant to low temperatures as bloom progressed. Before the terminal flower was at full bloom, the critical temperature range for floral injury within the inflorescence was $\geq$ 1.5°C. After this stage, flowers were injured in a narrow temperature range ($\leq$ 3°C).

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