Twig pre-harvest temperature significantly influences effective cryopreservation of *Vaccinium*

dormant buds

Maria M Jenderek¹, Justin D Tanner¹, Barbara D Ambruzs², Mark West³, Joseph D Postman⁴, Kim E

Hummer⁴

ABSTRACT

Cryopreservation of temperate woody-plant material by dormant buds is less expensive than using shoot tips isolated from tissue cultured plants; however currently, dormant buds are used only for preservation of selected temperate tree and shrub species. Using dormant buds could be an efficient strategy for long-term preservation of blueberry (*Vaccinium* L.) genetic resources. In this study, viability of *V.* hybrid 'Northsky' (PI 554943) dormant buds was evaluated at 30 harvest dates over

three consecutive fall/winter seasons to determine the optimal harvest time that promotes high post cryopreservation viability. Twigs with dormant buds were cut into 70 mm segments containing at least two nodes, desiccated, slowly cooled, stored in liquid nitrogen vapor and tested for post-cryopreservation regrowth. The highest regrowth of cryopreserved dormant buds was observed for buds harvested in mid-December and during the first half of January. Pearson's correlation coefficients were computed to evaluate the association between bud characteristics and viability at harvest date and logistic regression models were fit to test the ability of twig characteristics and temperatures to predict post cryopreservation bud viability. Post-cryopreservation viability was negatively correlated ($p < 0.05$) with average minimum, maximum and daily mean temperature preceding the bud harvest but was not correlated with the dormant bud initial and end moisture content, twig diameter, the number of dormant buds/cm of twig length and the number of days in desiccation. Regression tree analysis suggested post-cryopreservation viability to be between 52 and 80% for dormant buds harvested after a 10 day average maximum air temperature of $< 11.2^{\circ}\text{C}$. Pre-harvest air temperature was a significant indicator of optimal dormant bud harvest time to produce adequate viability for long term preservation of blueberry genetic resources.

Key words: gene banking, regression models, post cryopreservation regrowth, blueberry

32

33 Introduction

34 In cryopreservation, the use of dormant buds (DB) costs less, is simpler to accomplish and requires less
35 time than using shoot tips because it does not involve aseptic cultures and the time consuming excision
36 of shoots. The possibility of preserving plant dormant buds in liquid nitrogen has been known for years
37 [17] and was reported in *Malus* [3,18,21,27], *Fraxinus* [29], *Ulmus* [4], *Morus* [8,10], *Pyrus* [23], *Pinus*
38 *sylvestris* [6], *Betula pendula* [16], *Prunus cerasus* [25], and other tree species. Despite the successful
39 reports, the dormant bud method is not widely practiced in plant germplasm preservation. Some
40 possible reasons might be that the method is applicable only to shrubs and trees that undergo
41 dormancy and dormant buds of these species may not always result in satisfactory post-
42 cryopreservation viability.

43 In recent years, blueberry fruit consumption has increased due to its nutritional and potential medicinal
44 value [1,19,20,22] and in many countries, blueberries are an everyday food. The United States
45 contributes half of the world's commercial production [2]. Maintaining a diverse *Vaccinium* germplasm
46 collection is vital for selecting and breeding new cultivars that will support domestic blueberry
47 production. The U.S. Department of Agriculture-Agricultural Research Service, National Plant

48 Germplasm System (USDA-ARS, NPGS) currently houses >1,700 *Vaccinium* accessions ([http://www.ars-](http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?COR)
49 [grin.gov/cgi-bin/npgs/html/site_holding.pl?COR](http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?COR)). Only a small fraction (<3%) of the blueberry genetic
50 resources is backed up as cryopreserved shoot tips. The cryopreservation of *Vaccinium* by in vitro shoot
51 tips was described by Reed [12,13] and Uchendu and Reed [24], and was readily applicable to >40
52 accessions at our laboratory. Niino et al. [7] reported experimental cryopreservation of blueberry
53 dormant buds but their post-cryopreservation performance was tested only in vitro. Backing up the field
54 maintained blueberry collection by dormant buds would increase the number of cryopreserved
55 accessions in a shorter time period and with less cost than using shoot tips from in vitro cultures.
56 However, effective cryopreservation requires the identification of protocols that ensure satisfactory
57 post-cryopreservation viability that is measured by the ability of the stored material to grow a shoot or a
58 plant.

59 Several *Vaccinium* L. species (Ericaceae, section *Cyanococcus*) make ideal candidates for
60 cryopreservation by dormant buds. They are perennial shrubs and blueberry, cranberry and lingonberry
61 exhibit a distinct dormancy phase; hence, they might be amenable to conservation via dormant buds.
62 The objective of this study was to evaluate post-cryopreservation viability of blueberry dormant buds

harvested at various dates during three fall-winter seasons and to determine the relationship of pre-harvest temperature to viability after storage in liquid nitrogen.

MATERIAL AND METHODS

Terminal twigs (last year growth) of blueberry cv. Northsky (*Vaccinium* hybr. PI 554943) were collected during three fall/winter seasons in 2011-2012 (12 harvest dates), 2012-2013 (14 harvest dates) and 2013-2014 (4 harvest dates; a total of 30 harvests; Table 1) at the National Clonal Germplasm Repository, Corvallis, OR. In the 2013/2014 season, the selection of the four harvest dates was based on the collection dates of the previous two seasons that resulted in the highest post storage viability. At the day of harvest, the twigs were sealed in plastic bags and shipped overnight to the Plant and Animal Germplasm Preservation Unit (PAGRPU) in Fort Collins, CO. Upon arrival, the top and bottom diameter and the twig length was measured using a digital caliper and the number of nodes on six twigs counted. Fresh weight of 30 dormant buds excised from six twigs (5 DB/twig) was recorded. After, the twigs with dormant buds were processed for storage in liquid nitrogen vapor (LNV). The processing followed the procedure for cryopreservation of *Malus* dormant buds outlined by Forsline et al. [3] with the following modifications: blueberry twigs were cut into 70 mm segments on a band saw and the bottom and the top 25-30 mm of twigs were not used. Each segment contained at least two dormant nodes and the

79 segments were kept on ice in an ice chest (ca. 0° C) until all material was cut. The same day, the
80 segments were randomized, placed in mesh bags and exposed to desiccation at -5°C. The initial moisture
81 content (MC %) of the twig segments varied from 32.9 to 51.4 % across harvest dates while the final MC
82 observed before the buds were placed into the slow cooling step ranged from 25.2 to 30.7% (Table 1).
83 After desiccation, twig segments were sealed in two replicate polyolefin tubes (10 DB/tube), slow cooled
84 in a programmable freezer (from -5°C to -30°C; at 1°C/h; Sigma Systems, M256) and after a 24 h hold at -
85 30°C, the DB were stored in LNV (-190°C). For some harvest dates, the number of DB/replication was
86 <10. After 4-5 weeks of cryostorage, the dormant buds (on the twig segments) were rehydrated in
87 moist, sterile peat moss at 2°C for 12 days in darkness. Viability of the cryopreserved and the control
88 (not stored in LNV) DB was tested by assessing the ability of the dormant buds to grow leaves and a
89 shoot described as a regrowth. The twig segments were planted in Oasis Horticubes (Hummert, cat. No.
90 142790) and placed under plastic tunnels, at temperature range of 23-26°C at 65-75 % relative humidity
91 (RH) in a greenhouse. Dormant buds that developed green leaves and a shoot were considered viable.
92 The leaves and shoot development occurred within 5-12 days for the control (DB not exposed to LN) and
93 between 7 to 17 days for DB stored in liquid nitrogen. The viability was tested in two replications, 10
94 DB/replication (or less as mentioned above).

95 At each harvest date, twigs with the DB were evaluated for initial and end MC (20 twig segments),
96 number of days in desiccation (number of days between the beginning and the end of the process), DB
97 fresh weight (excised from the twig segment; 30 DB from 6 twigs), twig bottom and top diameter (6
98 twigs), number of DB per twig length (on 6 twigs; done for two fall/winter seasons). These
99 characteristics were evaluated on twigs that would typically be used for cryopreservation (terminal, last
100 year growth, free of pathogens and mechanical injury). Control (not exposed to storage in LNV) and
101 post-LNV storage DB viability) was evaluated on 20 DB for each treatment. The control DB viability was
102 tested to assure that material used for cryopreservation was viable prior to LNV exposure.

103 Temperature data for the Corvallis, OR location were retrieved from
104 <http://www.usbr.gov/pn/agrimet/webarcread.html> and the average of minimum, mean and maximum
105 air temperatures for 90, 30, 14, 10, 7, 5, 3, 2 and 1 day preceding the harvest dates were acquired and
106 used in statistical computations.

107 For each dormant bud collection date, twig characteristics and weather histories were used to evaluate
108 their predictive abilities for post cryopreservation regrowth using correlation and regression methods
109 for binomial data. Pearson's r correlation was computed to evaluate the association between
110 temperature and the DB post-cryopreservation regrowth. Regression approaches including logit models

and regression trees were used to test the predictive ability of twig characteristics and pre-harvest temperatures. To test binominal proportions (percent viability) likelihood-ratio chi-square (χ^2) was used to compare full models (with date as a predictor) with reduced models (without the date predictors). The data analysis was done using SAS JMP, Version 11.1 [5] and R [11].

RESULTS AND DISCUSSION

Twig characteristics

The twigs harvested across the 30 dates, were 11 to 23 cm long. The bottom twig diameter varied significantly from 1.9 to 3.6 mm and so did the diameter of the twig top 1.3 to 2.7 mm (Table 1); hence the individual twig segments had a different average diameter (not measured individually). Nevertheless, no significant correlation was observed between these characteristics and the viability in the control and LNV exposed dormant buds. Testing of combined characteristics, i.e., the twig segment diameter (either top or bottom) and the average maximum 10 day temperature preceding the harvest, for their ability to forecast the post cryopreservation viability of the DB, did not increase the predictive capacity. Characteristics of field harvested twigs varied and will vary in the future; the lack of their predictive ability is a practical attribute in routine cryopreservation. The dormant bud density on twigs (number of DB/cm of twig) ranged from 0.4 to 0.8. The fresh weight of the buds varied among harvest

dates and ranged from 26.2 (harvest 12/16/13) to 181.6 mg (harvest 2/13/12). The time needed to reach the end MC (between 25.2 and 30.7%) was 14 (1/23/12 harvest) to 38 days (2/3/14 harvest; Table 1).

The correlations between DB viability and the twig characteristics, when computed across all fall/winter seasons, were weak and not statistically significant (Table 2). However, when evaluated over similar harvest dates for each of the three seasons, fresh weight of the dormant buds was negatively correlated with the DB post-cryopreservation viability but the correlation was significant only in the 2013 fall/winter season ($r=-0.636$, $P=0.0003$, $n=28$; Table 3). Perhaps in that season, the dormant buds were more uniform than in the other two seasons (2012 and 2014). The twigs with the DB were harvested from field grown *Vaccinium* shrubs which growth and development was influenced by annual environmental factors that are not easy to control and were not measured. This is a biological material and year-to-year variations in the dormant bud fresh weight may be expected. Further research may be warranted to determine if the DB fresh weight does influence post-cryopreservation viability.

Dormant bud viability

The viability of the control (not exposed to LNV) dormant buds was between 65 and 100% for all harvest dates except 10/1/12 where the viability was 57% and 10/29/12 – 25% (Table 1). Such variation is not

143 unusual in living plant material; however, these observed differences were not significant ($\chi^2 =$
144 9.43, DF = 29, P = 0.9998) and might reflect the differences in DB viability due to changing
145 environmental factors before the specific harvest dates. Post cryostorage viability varied significantly
146 among the harvest dates ($\chi^2 = 191.7$, DF = 29, P < 0.0001) and ranged from 100% to zero. The
147 highest post-cryopreservation viability (84-100%) was observed for DB harvested at 1/9/12, 1/16/12,
148 12/10/12, 12/17/12, 1/7/13 and 1/6/14 (six dates; Table 1). DB harvested on 2/13/12 and on 10/1/12,
149 10/15/12, 10/29/12 and 11/12/12 (five harvest dates) were not viable after exposure to LNV. Most likely
150 buds on twigs harvested during the fall dates were not dormant enough to withstand cryopreservation,
151 while buds harvested in mid-February of 2012 were already out of the dormant phase and hence could
152 not survive in the ultra-low temperature of LNV. The post cryostorage viability of DB harvested at the
153 remaining 19 dates (out of the 30 harvest dates evaluated) ranged from 25 to 79% (Table 1).

154 To eliminate data from harvest dates that were not repeated in either evaluation season, viability of the
155 control and cryopreserved material and its characteristics were compared among similar harvest dates
156 (within 2 to 5 days, with similar average air temperature) (Table 4). All control DB were viable (83 to
157 100%) and no significant differences were observed in viability between the harvest dates (Table 4); this
158 indicated that the experimental material used in the study was uniform in its ability to grow shoots and

leaves. However, the viability of the cryopreserved DB was significantly different ($\chi^2 = 50.1, DF = 9, P < 0.0001$); the highest viability was observed for DB harvested in the first ten days of January (1/9/12, 1/7/13 and 1/6/14; 90%) and the lowest viability was observed for DB harvested in November (29-38%; Table 4). When mean viabilities for harvests made in January, February, November and December for the four calendar years (2011-2014) were compared no statistical differences for the control or the cryopreserved dormant buds viability were found (Table 5). Significant differences were observed for DB moisture content at the end of desiccation, with the highest in 2013 (30.2%) and in the number of days needed to desiccate the buds, with the lowest in 2012 (21 days). The average fresh bud weight was the highest in 2012 (120.8 mg) but the number of days in desiccation was the lowest in that year; hence, the fresh weight of the DB seems to be irrelevant to the length (number of days) of the desiccation process (Table 5). Only in the 2012/13 season, the fresh DB weight was negatively correlated with post-cryopreservation viability (Table 3) implying smaller dormant buds survive LNV exposure better than larger buds. Perhaps using twigs with smaller buds would support higher post cryopreservation viability than larger buds; however, the exact weight for considering a bud small or large would vary for different accessions. The number of buds on twigs was significantly lower in 2014 (0.4 buds/cm; Table 5) but this did not seem to influence the viability of the control or LNV-stored DB.

This is understandable because development of one DB on a twig segment qualified the segment as viable and the 70 mm long twig segments had more than one DB.

The lack of correlation (Table 2) between the twig top and bottom diameter, the number of DB/cm and the post cryostorage viability of dormant buds observed in this study, might have a practical advantage when cryopreserving DB on twigs of different width. In applied cryopreservation of dormant buds, homogeneity of material for processing is often unattainable due to limitations in material availability (i.e., it's not possible to select enough DB with the same characteristics) and biological attributes will change with seasons.

Temperature and viability of cryopreserved dormant buds

All average minimum, mean and maximum twig pre-harvest temperatures analyzed in the study were negatively correlated to cryopreserved blueberry dormant bud viability ($P < 0.0001$); the higher the average temperature preceding the harvest dates the lower the viability of cryopreserved blueberry buds. In an attempt to identify pre-harvest temperature ranges that were associated with high post LNV storage viability, a regression tree partition method was used [5]. The algorithm recursively split the range of values of the predictors (in this case the temperature variables) by finding cut-points (temperature values) that provided ranges of values of the predictors that could be used to forecast the

191 dependent variable (viability). The recursive process of splitting ranges of the predictors' ended when
192 further splitting no longer improved the predictability of the dependent variable. An increase of R^2 of
193 0.05 was used as the criterion for stopping the recursive process. If further splitting the ranges of the
194 predictors could not improve prediction with an increase of R^2 by 0.05 then the partitioning process was
195 stopped. Applying this process resulted in using only the maximum temperature for 10 days prior to the
196 twig harvest as a predictor. This is not surprising as the temperature variables were highly collinear. The
197 regression trees (not shown) suggested that if the average maximum temperature 10 days prior to the
198 dormant bud harvest is less than 11.2°C the predicted post cryopreservation viability would be 66% (52
199 to 80 % at a 95% confidence level) (Fig. 1). Fig. 1 shows the plot of post cryopreservation viability (%)
200 against the average maximum temperature 10 days before harvests. For the same time period
201 preceding the harvests, the mean and minimum temperature averages had similar patterns but they
202 were not any more useful for predicting the viability. It seems that monitoring the average maximum
203 temperature before the twig harvest would be the most practical to use.

204 The temperature (<11.2°C) identified in the regression trees can be helpful in estimating a twig harvest
205 time that would support a high post cryopreservation viability of blueberry dormant buds. Monitoring
206 the daily temperature until the 10 day average maximum falls below 11.2°C and planning the twig

207 harvest date accordingly should be easy and effective. This prediction was based on data collected for
208 the Northsky cultivar which is a hybrid between *V. corymbosum* and *V. angustifolium*. Further work is
209 needed to test if this temperature range is applicable to other blueberry genotypes of the highbush (*V.*
210 *corymbosum*) and lowbush (*V. angustifolium*) blueberries or their other hybrids. Dormancy and cold
211 hardiness influence post cryopreservation viability of *Malus* dormant buds [3,26,28]. Rowland et al.
212 [14,15] reported distinct differences in cold hardiness and deacclimation time for the Northsky cv. and
213 several other blueberry accessions with diverse genetic backgrounds. Most likely, the dormant buds that
214 resulted in high post LNV viability were harvested at the peak of their cold hardiness. Measuring the
215 number of days leading to cold hardiness would be a good indicator for the twig harvest date; however,
216 from a practical standpoint, it is difficult to do. Monitoring daily maximum temperatures would require
217 fewer efforts. Further research is warranted to understand other conditions that contribute to effective
218 plant cryopreservation; although, tracking pre-harvest maximum temperature is at least one of the
219 factors aiding high post storage viability of dormant buds. Our minimum laboratory standard for plant
220 germplasm storage in liquid nitrogen or its vapor is 40% viability. Several viabilities of the blueberry
221 dormant buds harvested at the 30 different harvest dates met the standard. The objective of the PAGRP
222 preservation efforts is to obtain the highest possible viability at one targeted twig harvest date. This
223 would lower costs connected with twig collection and eliminate multiple processing; often the twig

224 availability is limited and not easy to obtain. Harvesting material for processing at the right time is
225 critical for assuring high viability of long-term stored buds. For cryopreservation, dormant buds should
226 be harvested during the tree/shrub dormancy phase which typically occurs in the Northern hemisphere
227 during late November to the end of January, sometimes extending to the beginning of February.
228 Describing the temperature range that contributes to high viability of cryopreserved buds can assist with
229 logistics of cryopreserving blueberry genetic resources. Data obtained from the three season study
230 emphasize the importance of harvesting twigs with dormant buds at an appropriate time and suggest a
231 10 day average maximum temperature of $<11.2^{\circ}\text{C}$ for successful post cryopreservation viability of
232 blueberry dormant buds. While other unidentified environmental factors might influence the viability,
233 our results suggest the identified temperature provides a robust predictor.

234

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240

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Fig. 1. Average viability (%) of ‘Northsky’ blueberry (*Vaccinium* hybr.) dormant buds cryopreserved in three fall/winter seasons (2011/2012, 2012/2013, 2013/2014)

The vertical line shows the average maximum temperature 10 days before harvest of 11.2°C. The lower horizontal line represents the bottom limit for the mean (%) of the predicted dormant bud viability (52%), the upper horizontal line marks the top mean (%) limit of the predicted dormant bud viability (80%) for the 95% confidence interval.

Table 1. Selected twig characteristics and viability (%) of dormant buds of 'Northsky' blueberry (*Vaccinium hybr.*) cryopreserved in three fall/winter seasons (2011/2012, 2012/2013 and 2013/2014)

Harvest season	Harvest date	Initial MC (%)	End MC (%)	No. of days in desiccation to reach 25 to 30% of MC	DB fresh weight (mg)	Twig top diameter (mm)	Twig bottom diameter (mm)	No. of DB/cm of twig length	Average viability of control DB (%)	Average viability of cryopreserved DB (%)
2011/2012	11/14/11	48.5	28.4	16	31.5	1.8	2.7		100	57
	11/21/11	44.6	25.2	20	77.7	2.0	2.8		100	70
	11/28/11	44.7	27.3	20	31.0	1.6	2.0		100	50
	12/5/11	49.0	28.8	36	111.4	1.4	2.1		100	67
	12/12/11	46.7	26.1	20	107.4	1.3	1.9		100	59
	12/19/11	45.3	29.0	28	59.9	1.4	3.6		100	75
	1/3/12	32.9	27.6	23	128.2	1.6	2.6		100	45
	1/9/12	48.6	30.6	24	155.7	2.1	2.5		94	100
	1/16/12	49.5	26.9	20	126.1	1.6	2.2		100	100
	1/23/12	49.8	27.5	14	124.8	1.7	2.5		100	70
	1/30/12	47.9	29.8	23	56.0	2.1	2.5		100	50
	2/13/12	48.1	29.6	16	181.6	1.7	2.8		100	0
2012/2013	10/1/12	48.8	29.2	36	165.7	1.6	2.9	0.6	57	0
	10/15/12	49.8	29.2	23	93.1	1.6	2.3	0.6	80	0
	10/29/12	43.0	29.4	19	163.8	1.5	2.3	0.6	25	0
	11/12/12	48.4	29.7	20	141.7	1.6	2.7	0.6	67	0
	11/26/12	48.3	30.3	20	107.6	1.8	2.7	0.6	67	25
	12/3/12	48.3	30.7	17	142.4	1.6	2.5	0.6	65	30
	12/10/12	46.6	30.4	24	61.4	1.7	3.3	0.6	100	90
	12/17/12	46.4	29.8	17	88.4	2.1	2.8	0.5	100	88
	12/31/12	46.5	28.1	36	75.7	1.7	2.8	0.7	100	45
	1/7/13	48.6	29.4	29	85.7	1.7	2.3	0.7	100	84
	1/14/13	46.4	30.2	22	115.4	1.6	2.2	0.7	92	67
	1/21/13	49.3	30.6	25	87.0	1.5	2.0	0.8	100	79
2013/2014	1/28/13	47.2	30.7	24	40.2	1.5	2.3	0.7	92	75
	2/4/13	51.4	30.4	23	86.2	1.6	2.2	0.7	100	58
	12/16/13	47.0	30.2	29	26.2	2.7	3.2	0.5	100	35
	1/6/14	46.4	29.8	29	27.0	1.9	2.6	0.5	100	85
	1/21/14	48.4	30.6	28	43.2	1.8	2.3	0.4	95	45
	2/3/14	47.7	28.9	38	28.8	1.8	2.9	0.4	100	65
	Mean value	47.1	29.1	24	92.4	1.7	2.6	0.6	94	54
	SE±	0.41	0.21	0.9	8.4	0.03	0.02	0.02	2.3	4.4
n		60	60	60	60	180	180	108	60	60

hybr.-hybrid; No.-number; MC-moisture content; DB-dormant bud; %-percent; g-gram; mm-millimeter.

Table 2. Pearson’s correlation between characteristics of ‘Northsky’ blueberry (*Vaccinium* hybr.) branches and viability of dormant buds cryopreserved and averaged over all harvest dates in three fall/winter seasons (2011/2012, 2012/2013 and 2013/2014)

Characteristic	Correlation coefficient	Sample size	p-value
Initial moisture content	0.043	60	0.7453
End moisture content	-0.087	60	0.5069
No. of days in desiccation	0.114	60	0.3850
DB fresh weight	-0.037	60	0.7773
Twig top diameter	-0.103	60	0.4329
Twig bottom diameter	0.039	60	0.7654
No. of DB/cm of twig length	0.026	34	0.8852

Table 3. Pearson’s correlation between dormant bud fresh weight and post cryopreservation viability for ‘Northsky’ blueberry (*Vaccinium* hybr.) averaged across similar harvest dates during three fall/winter seasons (2011/2012, 2012/2013 and 2013/2014)

Twig harvest season	Correlation coefficient	Sample size	p-value
2011/2012	0.0692	24	0.7479
2012/2013	-0.6360	28	0.0003
2013/2014	-0.4644	8	0.2464

Table 4. Viability of control and cryopreserved ‘Northsky’ blueberry (*Vaccinium* hybr.) dormant buds harvested at similar harvest dates in three fall/winter seasons (2011/2012, 2012/2013 and 2013/2014)

Harvest date at the three fall/winter seasons			Initial MC in dormant buds (%)	End MC in dormant buds (%)	No. of days in desiccation (days needed to reach 25 to 30 % MC)	Fresh weight of dormant buds (mg)	Top twig diameter (mm)	Bottom twig diameter (mm)	No. of dormant buds/cm of twig	Average control dormant bud viability (%)	Average viability of cryopreserved dormant buds (%)
2011/12	2012/13	2013/14									
11/14/11	11/12/12		48.5	29.1	18	86.6	1.7	2.6	0.6	84	29
11/28/11	11/26/12		46.5	28.8	20	69.3	1.7	2.3	0.6	84	38
12/5/11	12/3/12		48.7	29.8	27	126.9	1.5	2.3	0.6	83	48
12/12/11	12/10/12		46.6	28.3	22	84.4	1.5	2.6	0.5	100	74
12/19/11	12/17/12	12/16/13	46.3	29.6	25	58.2	2.1	3.2	0.5	100	66
1/3/12	12/31/12		39.7	27.8	30	102.0	1.6	2.7	0.6	100	45
1/9/12	1/7/13	1/6/14	47.9	29.9	27	89.5	1.9	2.5	0.6	98	90
1/16/12	1/14/13		47.9	28.5	21	120.8	1.6	2.2	0.7	96	83
1/23/12	1/21/13	1/21/14	49.2	29.5	22	85.0	1.7	2.2	0.6	98	65
1/30/12	2/4/13	2/3/14	49.0	29.7	28	57.0	1.8	2.5	0.5	100	58
Mean value			47.0	29.1	24	88.0	1.7	2.5	0.6	94	60
SE±			0.49	0.23	0.9	6.4	0.05	0.06	0.03	1.7	4.2

control-not exposed to liquid nitrogen vapor

Table 5. Viability of control and cryopreserved ‘Northsky’ blueberry (*Vaccinium* hybr.) dormant buds at four calendar years
(2011, 2012, 2013 and 2014)

Year	Initial MC in dormant buds (%)	End MC in dormant buds (%)	No. of days in desiccation	Fresh weight of dormant buds (mg)	Top twig diameter (mm)	Bottom twig diameter (mm)	No. of dormant buds/cm of branch	Average viability of control dormant buds (%)	Average viability of cryopreserved dormant buds (%)
2011	48.8	27.5	23	69.8	1.6	2.5	nc	100	63
2012	46.9	29.2	21	120.8	1.7	2.6	0.6	84	45
2013	48.3	30.2	25	73.5	1.8	2.3	0.6	89	66
2014	47.5	29.7	25	33.0	1.8	2.6	0.4	98	65
Mean value	47.9	29.2	24	74.3	1.7	2.5	0.5	93	60
SE±	0.41	0.21	0.9	17.2	0.04	0.06	0.03	2.4	4.3

Control - not exposed to liquid nitrogen vapor; hybr.-hybrid; MC-moisture content;%-percent; nc-not counted; mg-milligram; mm-millimeter