

Crop load and harvest date have minimal impact on bud cold hardiness and cane carbohydrate levels of four grapevine cultivars

R. LEFEBVRE^{1,2)}, A. REYNOLDS¹⁾, and F. DIPROFIO^{1),3)}

¹⁾Cool Climate Oenology and Viticulture Institute, Brock University, St. Catharines, Ontario, Canada

²⁾Current address: Pillitteri Estate Winery, Virgil, Ontario, Canada

³⁾Current address: Pondview Estate Winery, Virgil, Ontario, Canada

Summary

Four grapevine cultivars ('Pinot gris', 'Riesling', 'Cabernet franc', 'Cabernet Sauvignon') were subjected to six different field treatments in 2011 [two crop loads (full, half) X three harvest dates [normal (T₀), 3 weeks after T₀, 6 weeks after T₀] in a randomized block design with a factorialized treatment arrangement. All treatments were sampled four times over the 2012 dormant season from January to March. Bud cold hardiness was evaluated for all four cultivars by measuring low temperature exotherms (LTEs) of dormant buds using differential thermal analysis. Cane carbohydrates (CHOs) were likewise analyzed in 'Pinot gris' and 'Riesling'. CHO analysis was done using an 80 % ethanol extraction and HPLC. Neither CHO levels nor cold hardiness were substantially affected by either crop level or harvest date. Consistent patterns of CHO changes and LTE values in each cultivar indicated that deacclimation was unaffected by treatment. Cold hardiness may be influenced more by cultivar specificity based on rates of maturation than by treatment.

Key words: differential thermal analysis; oligosaccharides; winter hardiness.

Introduction

Ontario winter temperatures can fall below -20 °C, which can compromise vine hardiness and survival. Overcropping has been identified as a contributing factor to depletion of sugar reserves that could otherwise be used for winter survival. Additionally, delayed harvest of grapes to produce late harvest style wines is commonly employed; however, this may affect carbohydrate (CHO) levels in buds and canes necessary for winter survival.

Grapevine overwintering survivability is based upon relationships between cane and bud CHOs and grapevine bud winter hardiness (WAMPLE and BARY 1992). Non-structural sugars linked to woody plant acclimation include oligosaccharides (stachyose, raffinose), glucose, fructose, and sucrose (HAMMAN *et al.* 1996). Higher sucrose vs. glucose and fructose occurs in grapevine buds at onset of winter

(GRANT *et al.* 2009). Oligosaccharides accumulate with acclimation and decrease with deacclimation, whereas glucose and fructose remain stable until first frost, rapidly increase, and reach maxima during lowest temperatures (HAMMAN *et al.* 1996). Oligosaccharides are implicated in cellular cryoprotection (GRANT *et al.* 2009); however, fructose and glucose also correlate with bud survival at low temperatures (MOHAMED *et al.* 2010). These inverse correlations with temperature do not necessarily guarantee direct cryoprotective roles. During acclimation, as winter temperatures decrease, bud hardiness increases, and inverse relationships occur between grapevine bud and cane non-structural sugars vs. bud low temperature exotherms (LTE) (WAMPLE and BARY 1992).

Cultural practices e.g. crop control and canopy management, affect CHO distribution and vine hardiness (HOWELL 2000). Understanding relationships between cane CHOs, harvest date, and crop size vs. winter survival contribute to avoiding grapevine winter injury (HOWELL 2000). Excess crop size reduces CHOs that may be required for winter survival (HOWELL 2000). High crop levels increase fruit sinks and CHO demand, leading to delayed fruit and wood maturity and reduced cold hardiness (EDSON *et al.* 1995). Inadequate CHO production leads to reduced stored energy required for sustaining winter hardiness (HOWELL 2000). Overcropping delays fruit maturity, shortens the post harvest period, and inhibits maximum potential to accumulate CHOs. Well-managed vines produce sufficient viable buds for balanced crop loads and can withstand 40 % injury without economic loss (HOWELL 2000). Vine balance is defined as the ratio of yield vs. cane pruning weights (crop load, Ravaz Index). Balanced vines have crop loads of 10-12 (BRAVDO *et al.* 1985). Several have likewise investigated relationships between delays in harvest and winter hardiness. Bud and cane sugars and winter hardiness were uninfluenced by delayed harvest ['normal' (22 °Brix) vs. 'late' (28 °Brix)] in 'Cabernet Sauvignon' (WAMPLE and BARY 1992), and were not influenced in late harvested 'Chardonnay' or 'Riesling' (HAMMAN *et al.* 1996).

Study objectives were to determine existence of relationships between harvest date (HD) and crop level vs. grapevine cane CHOs and bud winter survival in four *V. vinifera* cvs. in Ontario. It was expected that the lethal temperature (LT₅₀; temperature at which 50 % of buds die)

Correspondence to: Prof. A. REYNOLDS, Cool Climate Oenology and Viticulture Institute, Inniskillin Hall, room 311, Brock University, 500 Glenridge Ave., St. Catharines, Ontario L2S 3A1, Canada. E-mail: areynolds@brocku.ca

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and cane CHO levels would not be impacted by crop level. It was anticipated that in situations where vines were balanced with respect to yield and vine size, no impact of crop level would occur in terms of bud hardiness or CHO accumulation. Moreover, it was hypothesized that LT_{50} and cane CHOs would not be impacted by HD. It was not foreseen how delayed HD could physiologically compromise hardiness or cane CHOs.

Material and Methods

The study vineyard was located in Virgil, Ontario. Four cvs. ('Pinot gris', 'Riesling', 'Cabernet franc', 'Cabernet Sauvignon') on 3309C were selected. Vines were head trained, cane pruned, vertically shoot positioned, with a 2.1 m canopy height, and 1.2 x 2.7 m vine x row spacing. The experiment was a randomized block with two crop levels (full, half; FC, HC) imposed at veraison and three HDs (Tab. 1; normal, 3 weeks later, 6 weeks later; T0, T1, T2) in a factorialized arrangement with six replicates. Each block consisted of a part-row and treatment replicates comprised two six-vine post-lengths. Yields (kg, $p \leq 0.0001$) and respective vine size (kg, NS) of FC vs. HC were: 'Pinot gris': 2.8 vs. 2.0, 0.53 vs. 0.51; 'Riesling': 3.0 vs. 1.7, 0.44 vs. 0.41; 'Cabernet franc': 2.5 vs. 1.8, 0.72 vs. 0.71; 'Cabernet Sauvignon': 2.5 vs. 1.8, 0.66 vs. 0.51. °Brix increased in 2011 (FC vs. HC) for Riesling: 19.9 vs. 20.3 ($p \leq 0.01$) and Cabernet franc: 25.1 vs. 25.5 ($p \leq 0.05$).

Bud LTEs were measured by differential thermal analysis (DTA) (MILLS *et al.* 2006) using identical hardware. DTA determines lethal temperatures of grape buds by measuring intracellular LTE that occur when tissues with supercooled cells are exposed to destructive low temperatures. The primary (1°) bud LTE is largest, followed by 2° and 3° buds (QUAMME 1986). Vines were sampled four times throughout the season (Tab. 1). Two cane samples per

treatment replicate 8-10 mm diameter with fully-formed periderm were collected randomly on each sampling date. Buds 2-7 (from the base) were excised, loaded into a thermoelectric module (cell) and used for DTA. Trays were loaded into a programmable freezer and voltage output was collected by a data acquisition system, which provided a report showing bud LT_{50} values.

A method based on REED (2004) for extracting sugars was used. Cane samples were stored in plastic bags and frozen at -25 °C until ready for preparation. All prepared samples were kept in a desiccated environment until analysis. Samples consisted of two canes cut between buds 2 and 7 (from the base) into eight-internode pieces. Each internode was 2 to 10 cm long. The canes were cut into thinly sliced (< 0.3 cm) pieces, with the periderm left on, and placed in a 15-mL test tube. Samples were plunged into liquid N_2 and freeze dried for 48 hr. The sample was ground in a 6750 Fischer mill grinder for 2 min at level 15. After grinding, 1 g dry weight was measured out in replicate for sugar extraction analysis. Replicates were washed three times using 5 mL of 80 % ethanol. Each wash was homogenized using a vortex and sonicated for 10 min in a Fisher Scientific FS20H sonicator before being centrifuged (IEC Centra CL2) for 10 min at 1975 g. The three washes were combined and placed in Buchi Multivapor P-12 rotoevaporator for a 2 hr cycle. The Buchi vacuum system consisted of a vacuum controller V-700, vacuum pump V-855, and an R-200 condenser. The chiller used with the Buchi system was Masterline Forma Scientific Model 70 set to 4 °C. The rotovaporation cycle began at 175 mbar, reduced to 40 mbar within the first hour and maintained at 40 mbar for the last hour using a continual temperature of 60 °C. The sugars were reconstituted with 2.25 mL of water for 1 h using the rotoevaporator. A solid phase extraction was completed using a CH Agilent Bond Elut cartridge conditioned with 1 unit of methanol followed by 2 units of water. The sample was filtered with a 0.45 µm Whatman disk

Table 1

Comparison of two crop levels and three harvest dates (2011) with respect to LT_{50} values (°C) of buds in 'Pinot gris', 'Riesling', 'Cabernet franc', and 'Cabernet Sauvignon' over four different sampling periods, Pondview Estate Winery, Virgil, ON

Sampling date ^a	Pinot gris				Riesling				Cabernet franc				Cabernet Sauvignon			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Crop level																
Full	-24.1	-23.7	-19.9	-10.0	-23.3	-24.0	-22.9	-10.8	-23.6	-22.8	-19.6	-10.4	-22.4	-23.5	-21.6	-14.3
Half	-24.0	-23.4	-19.9	-10.2	-23.6	-23.9	-23.2	-10.6	-23.5	-22.9	-19.6	-10.2	-22.9	-22.9	-21.5	-15.1
Significance ^b	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Harvest date ^c																
T0	-23.9	-23.2	-19.8	-10.2	-23.3	-24.0	-23.0	-10.5	-23.4	-22.9	-19.6	-10.3	-22.6	-23.2	-21.9	-14.8
T1	-24.1	-23.6	-19.9	-9.8	-23.7	-24.0	-23.2	-10.9	-23.5	-22.8	-19.2	-10.1	-22.7	-22.9	-21.2	-14.7
T2	-24.2	-23.9	-19.9	-10.2	-23.5	-23.9	-22.9	-10.7	-23.7	-22.7	-20.0	-10.5	-22.8	-23.3	-21.5	-14.6
Significance ^b	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction ^b	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^a Sampling dates (2012): 'Pinot gris': 18 Jan., 16 Feb., 8 Mar., 23 Mar.; 'Riesling': 17 Jan., 15 Feb., 7 Mar., 27 Mar.; 'Cabernet franc': 21 Jan., 18 Feb., 10 Mar., 24 Mar.; 'Cabernet Sauvignon': 22 Jan., 19 Feb., 11 Mar., 26 Mar.

^b NS: not significant.

^c Harvest date (2011): T0: Normal harvest; T1: T0 + 3 wk; T2: T0 + 6 wk; 'Pinot gris': 22 Sept., 13 Oct., 3 Nov.; 'Riesling': 11 Oct., 1 Nov., 22 Nov.; 'Cabernet franc': 22 Oct., 5 Nov., 19 Nov.; 'Cabernet Sauvignon': 22 Oct., 5 Nov., 19 Nov.

filter prior to extraction. The extract was filtered using a 0.45- μm syringe filter into a 1.5-mL HPLC vial.

Analysis of 10- μL samples was performed on an Agilent 1100 Series HPLC, consisting of a binary pump, autoinjector, Aminex HPX-42C column heated to 80 °C, DAD, and RID (for sugar detection). Data for sugars were analyzed with a manually adjusted baseline and peak separation. Treatment combinations were analyzed in duplicate for all replicates (144 samples per cv. per sampling date).

ANOVA of LT_{50} and CHO data was carried out by PROC GLM (SAS Institute, Cary, NC). Duncan's Multiple Range Test was used post-hoc at $p \leq 0.05$.

Results and Discussion

No treatment differences in LT_{50} occurred for all cultivars (Tab. 1; sampling dates (SD) 1-4). Bud LT_{50} were consistently lowest on SD1 in 'Pinot gris' and 'Cabernet franc' in all treatments and on SD2 for 'Riesling' and 'Cabernet Sauvignon' and increased thereafter. Treatment did not affect LT_{50} , suggesting no impact on acclimation/ deacclimation. Hypotheses that bud cold hardiness would not be impacted by treatment were therefore proven. This is noteworthy as it eliminates crop level and HD as concerns for cold susceptible areas provided that vines are balanced in terms of crop load. FC vs. HC had no impact on bud LT_{50} perhaps because FC vines were in balance; crop loads

(FC, HC) were: 'Pinot gris' (6.4, 4.7), 'Riesling' (8.0, 4.9), 'Cabernet franc' (4.4, 3.1), 'Cabernet Sauvignon' (4.9, 5.5). Since crop loads were near/ below optimal, it is not surprising that CHOs were unaffected and crop stress impacting bud hardiness was minimal. Delayed HDs increased °Brix in 2011 for all cvs. (T0-T2; $p \leq 0.0001$): 'Pinot gris': 23.2-25.2; 'Riesling': 19.7-21.3; 'Cabernet franc': 23.6-27.4; 'Cabernet Sauvignon': 22.6-26.5. However, delayed HDs had no impact on bud LT_{50} , likely because vines accumulated enough CHOs by normal fruit maturity. Photosynthesis slows by harvest, and CHO accumulation thereafter is minimal, having little influence on cold hardiness (EDSON *et al.* 1995). As translocation to fruit slows at harvest, maximum CHOs accumulate in woody tissues (MOHAMED *et al.* 2010).

No crop level or HD differences occurred for stachyose, glucose, and fructose ($\text{mg}\cdot\text{g}^{-1}$ dry wt) in 'Pinot gris' (Figure A, B). CHOs increased from SD1 to SD2 (maximum), and decreased thereafter, reaching lowest levels on SD4 (both cvs.). Sucrose was most responsive, and was affected twice by HD, decreasing in T1 (SD2) and increasing in T2 (SD4), and decreasing in HC vines (SD4). Interactions (SD3) indicated increased sucrose and raffinose in HC/T1 and a decrease in FC/T1. Crop level had no impact on sugars except sucrose in 'Riesling', which increased in HC (SD2) (Figure C, D). HD had greater influence: sucrose and stachyose increased in T0 (SD1) and raffinose decreased in T2 (SD2). The hypotheses that cane soluble

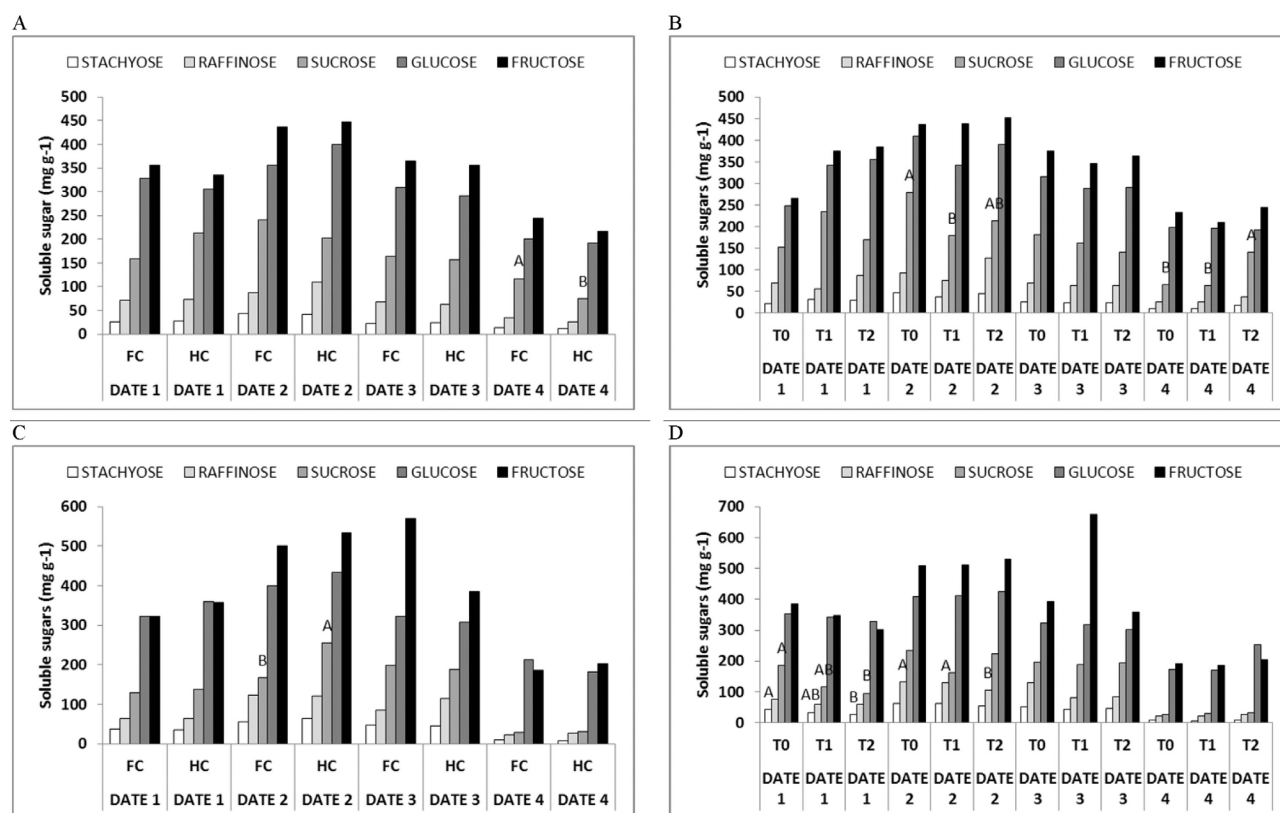


Figure: Mean values for stachyose, raffinose, sucrose, glucose, and fructose concentration ($\text{mg}\cdot\text{g}^{-1}$ dry wt.) in canes of 'Pinot gris' grapevines (A,B) and 'Riesling' (C,D) treated with different crop levels (full and half crop; FC, HC) and harvest dates (T0: Normal harvest; T1: T0 + 3 weeks; T2: T0 + 6 weeks), Pondview Estate Winery, Virgil, ON, 2011. Means between crop levels or harvest dates within dates for individual sugars are significantly different ($p < 0.05$) if labelled by different letters, Duncan's Multiple Range Test. Sampling dates (dates 1-4) were 18 Jan., 16 Feb., 8 Mar., 23 Mar. 2012.

sugars would not be impacted by crop levels or HDs were proven in this study. Sugars in both cvs. differed little between crop levels or HDs. Excess crop levels normally delay fruit and vine maturation and decrease winter hardiness (EDSON *et al.* 1995). HD had little impact on cane CHOs because sufficient CHOs accumulated by normal harvest. This lack of difference between CHOs and HD concur with others (HAMMAN *et al.* 1996). Understanding treatment impact on CHO accumulation is critical for Ontario grape growers to assess management strategies for vine survivability.

Temperature data were collected for Virgil Niagara from Vine and Tree Fruit Innovations website (www.vineinnovations.com; Tab. 2) for the 2011-2012 sampling period. The 2012 winter season was warmer than the historical 30-yr average. Monthly temperature trends over the 2012 sampling period showed temperature averages of -1.9 °C (15 d > 0 °C) in January, 0.9 °C (18 d > 0 °C) in February, and 7.0 °C (26 d > 0 °C) in March (Tab. 2). CHOs are influenced by temperature. CHO levels increase gradually when ambient temperatures decline at steady rate; however, following sudden cold episodes, sharp increases in CHOs will occur allowing for higher total CHO content (WAMPLE and BARY 1992). Mean temperatures for January, February, and March were -4.7, -3.6, and 0.9 °C, respectively (Vineland Research Station 1971-2000; data not shown). Atypical and early long warming periods were experienced. In March 2012 there was a 15-d span where temperatures reached > 13 °C vs. the 29 year average with 4.9 d reaching > 10 °C. In early March, large temperature fluctuations occurred with rapid chilling drops below 0 °C followed by > 10 °C warming. Data collection four times throughout January to the end of March did not correspond to specific warming or chilling events so no pattern could be identified. Also, data were collected for one season only so changes in CHOs or LTEs may not compare to previous years.

Buds used for cold hardiness analysis were excised from the same canes used for the sugar analysis, therefore, bud cold hardiness and total cane CHO levels for each cv. could be compared. Since both cold hardiness and CHOs were not different in either case, it can be implied that sufficient CHOs accumulated to provide maximum cold winter hardiness regardless of treatment. In some cases, total CHOs were higher in later harvest times (Tab. 3). For instance, in 'Pinot gris' on SD1, the T1 harvest had higher total CHOs than T0, but no differences were measured on subsequent sampling dates. In 'Riesling', highest CHOs occurred in the T0 harvest, with no differences measured thereafter.

CHO changes and fluctuations have been well documented (GRANT 2009). Temporal patterns in starch levels can almost entirely account for increases in stachyose, raffinose, sucrose, glucose, and fructose (MOHAMED *et al.* 2010). Starch hydrolysis is implicated as the main source for sucrose via amylase activity (MOHAMED *et al.* 2010). Amylase activity is initiated during cooling ambient temperatures but quickly slows after 200 chilling units (PCU) as sucrose levels reach a maximum (MOHAMED *et al.* 2010). Sucrose degradation to glucose and fructose gradually increases at 100-500 PCU. Invertase activity, which is responsible for sucrose conversion to glucose and fructose, is at its peak at this time, and inversely proportional to sucrose levels up to 300 PCU. In late endodormancy (500 PCU), invertase activity slows and sucrose begins to accumulate, while amylase activity increases once again for starch conversion to other sugars (MOHAMED *et al.* 2010). CHO patterns in this study concurred with documented patterns for stachyose, raffinose, glucose, and fructose showing increasing concentrations until mid-winter where a subsequent decrease occurred, rapidly dropping in March. Sucrose reached maxima between January and February in 'Pinot gris', while 'Riesling' had maximum sucrose in February and early March. Both cvs. had subsequent decreases in su-

Table 2

Temperature, precipitation, and sampling dates in 2012 for 'Pinot gris', 'Riesling', 'Cabernet franc', and 'Cabernet Sauvignon'. Data are for the Virgil area in Niagara Region, Ontario, Canada^a

Date	Cultivar	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Rain (mm)
01/17/2012	Riesling	11.0	-0.6	5.6	11.0
01/18/2012	Pinot gris	-0.6	-7.5	-4.5	0
01/21/2012	Cabernet franc	-2.3	-8.2	-5.5	0
01/22/2012	Cabernet Sauvignon	1.4	-7.3	-3.0	0
02/15/2012	Riesling	3.6	-3.9	1.0	0
02/16/2012	Pinot gris	6.2	-1.8	3.0	0.4
02/18/2012	Cabernet franc	4.2	-1.0	1.3	1.2
02/19/2012	Cabernet Sauvignon	-0.3	-4.1	-1.8	0
03/07/2012	Riesling	17.8	3.9	12.9	0
03/08/2012	Pinot gris	15.7	0.9	9.5	5.6
03/10/2012	Cabernet franc	1.8	-6.4	-2.2	0
03/11/2012	Cabernet Sauvignon	15.2	-2.5	8.4	0
03/23/2012	Pinot gris	19.9	9.9	14.7	0
03/24/2012	Cabernet franc	14.4	5.5	9.4	24.4
03/26/2012	Cabernet Sauvignon	6.2	-0.5	2.0	0
03/27/2012	Riesling	8.0	-5.7	1.5	0

^a Vine and Tree Fruit Innovations (WIN Weather Innovations Incorporated, www.vineinnovations.com).

Table 3

Total carbohydrate concentrations (mg·g⁻¹ dry wt.) in 'Pinot gris' and 'Riesling' canes during sampling periods from January to March 2012, Puglisi Vineyard, Virgil, ON

Sampling date ^a	Pinot gris (mg·g ⁻¹ dry wt.)				Riesling (mg·g ⁻¹ dry wt.)			
	Date 1	Date 2	Date 3	Date 4	Date 1	Date 2	Date 3	Date 4
Crop level								
Full	925.9	1174.6	929.7	609.4	907.5	1246.9	1226.9	468.0
Half	958.6	1200.5	890.3	519.2	972.9	1406.5	1023.8	452.1
Significance ^b	NS	NS	NS	NS	NS	NS	NS	NS
Harvest date ^c								
T0	726.1 b	1263.7	965.2	531.3	1126.2 a	1346.3	1095.3	436.8
T1	1041.2 a	1072.8	884.6	503.0	936.0 ab	1276.9	1293.5	416.9
T2	1002.9 ab	1247.3	887.9	628.8	798.8 b	1340.8	988.4	526.1
Significance ^b	*	NS	NS	NS	**	NS	NS	NS
Interaction ^b	NS	NS	*	NS	NS	NS	NS	NS

^a Sampling dates were January 18, February 16, March 8, March 23, 2012.

^b *, **, NS: Significant at $p \leq 0.05$, 0.01, or not significant, respectively. Means between harvest dates are separated by Duncan's Multiple Range Test, $p < 0.05$.

^c Harvest date: T0: Normal harvest; T1: T0 + 3 weeks; T2: T0 + 6 weeks.

crose with rapid declines in late March. During dormancy only starch and sucrose produce monosaccharides. Starch produces equal amounts of sucrose and monosaccharides; however, sucrose conversion to monosaccharides has not been proven to have a definitive relationship (MOHAMED *et al.* 2010). It may be mandatory to assess starch levels as well as individual sugars to fully understand sucrose changes observed in this study.

Conclusion

Altering crop level and HD in balanced vines did not impact minimum LT_{50} or accumulation of cane soluble sugars. Crop level and HD had no impact on deacclimation rates. Crop level and HD effects on soluble sugars were similar for all cvs. These results may be useful for vine growers in regions with cold winters, as it suggests that high crop level and delayed HD may not pose risks for winter injury provided that vines are balanced with respect to crop loads.

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