# Morphological, Physiological, and Biochemical Changes in *Vitis* Genotypes in Response to Photoperiod Regimes

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**Abstract:** The purpose of this study was to identify morphological, physiological, and biochemical changes in *Vitis* genotypes in response to photoperiod regimes. Experiments were conducted under greenhouse conditions using cold-sensitive Cabernet franc (*Vitis vinifera*) and cold-tolerant Couderc 3309 (3309C, *V. riparia* x *V. rupestris*) and Concord (*V. labruscana*). Potted vines were exposed to short day (SD) (8 hr) or long day (LD) (16 hr) for 4, 6, and 8 weeks. Shoot growth, periderm formation, dormancy, freezing tolerance (lethal temperature that kills 50% of primary buds: LT50), and soluble sugar concentrations in leaf and bud tissues were examined. Shoot growth slowed in all cultivars under SD accompanied with increased periderm formation and dormancy depth. Concord initiated these changes first, followed by 3309C, then Cabernet franc. The three cultivars did not show differences in freezing tolerance under LD, with LT50 ranging between -6.1 and -8.1°C. However, freezing tolerance increased by 0.7, 2.0, and 2.7°C after 4, 6, and 8 weeks under SD, respectively. Freezing tolerance of Concord increased after 4 weeks of SD treatment, whereas that of 3309C and Cabernet franc did not increase until after 6 weeks of SD treatment. Among all sugars, raffinose had distinctive responses associated with photoperiod, remaining low and similar (0.5 to 2.3 mg/g dry weight) under LD. Under SD, raffinose concentration was generally higher, ranging from 2.2 to 5.7 mg/g dry weight in leaves and 1.6 to 3.7 mg/g dry weight in buds, with cold-tolerant 3309C and Concord accumulating higher concentrations compared to cold-sensitive Cabernet franc. These results suggest that raffinose accumulation might be an early step in response to photoperiod coinciding with slowed shoot growth, the induction of endodormancy, and the initial acquisition of freezing tolerance.

**Key words:** cold acclimation, freezing tolerance, dormancy, raffinose, leaves, buds

Woody plants including *Vitis* species go through an annual cycle of growth and dormancy related to seasonal changes of the critical environmental cues: photoperiod and temperature. The first stages of acclimation relate to decreasing photoperiod and the later stages to low but above freezing temperatures, both of which function in preparing plants for freezing temperatures (Sakai and Larcher 1987). This early acclimation has distinct events that occur, including physiological changes such as growth cessation, dormancy induction in buds, partial development of freezing tolerance (FT), and the synthesis of metabolites that aid in promoting these changes (Guy 1990, Sakai and Larcher 1987). These environmental stimuli (i.e., photoperiod and temperature) then become important factors in winter survival. Short-day (SD) photoperiod influences the timing of growth cessation and dormancy at the end of the growing season (Sakai and Larcher 1987). Plant leaves receive the photoperiod stimulus, and then stems and buds initiate the changes in the plant. The changes in photoperiodic conditions perceived by the leaves probably induce the synthesis of hormones such as abscisic acid and/or facilitate the release of abscisic acid, which is then translocated to the various plant tissues inducing temporary suspension of metabolic activities and totally inhibiting mitotic activity (Chao et al. 2007).

Dormancy, generally defined as the temporary suspension of visible growth of any plant structure including buds, is further categorized into paradormancy, where growth is inhibited by physiological factors outside the bud; endodormancy, where growth is inhibited by physiological factors inside the bud; and ecodormancy, where growth is inhibited by environmental factors (Lang et al. 1987). Some species of grapes begin to acclimate in response to SD prior to the low temperature stimulus, allowing the grapevines to initiate the changes involved in cold acclimation and a state of endodormancy (Fennell and Hoover 1991, Garris et al. 2009, Wake and Fennell 2000). While grapevines do not set terminal buds, they exhibit other hallmark phenotypes such as periderm development, growth cessation, shoot tip abscission, and induction of endodormancy in axillary buds (Fennell and Hoover 1991, Wake and Fennell 2000). There are also photoperiod-sensitive ecotypes such as *V. riparia* and *V. labruscana* that will shed their leaves much earlier, even before low temperature stimulus is present. The understanding of why these grapevines respond differentially to photoperiod is not known, but genetic marker studies have revealed some candidate genes for further study, such as the phytochrome

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genes *PHYA* and *PHYB* and the Flowering Locus T gene family *FT/TFL1* (Garris et al. 2009).

External signals such as light and internal signals such as hormones and sugars act through specific overlapping signal transduction pathways to regulate endo-, eco- and paradormancy (Sakai and Larcher 1987). The physiological changes that occur in response to the external signal SD photoperiod have been documented for some *Vitis* species (Fennell and Hoover 1991, Garris et al. 2009, Wake and Fennell 2000). The role of sugars in the development of cold acclimation has been well documented in grapes. Total soluble sugars in grapevines generally increase during the initial stages of cold acclimation, and among sugars the raffinose family of oligosaccharide (RFO) is speculated as the most important since these sugars have been frequently observed to change exclusively with cold acclimation (Grant et al. 2009, Hamman et al. 1996, Stushnoff et al. 1993, Wample and Bary 1992). There is also differential accumulation of raffinose in cold-sensitive and cold-tolerant grape cultivars in side-by-side controlled-environment experiments. Cold-tolerant cultivars such as Frontenac (*Vitis* spp.), 3309C, and Concord accumulated the highest amount of raffinose in both bud and leaf tissues under cold treatment conditions, while cold-sensitive Cabernet franc accumulated the least (Grant et al. 2009). However, internal responses of sugars to SD have not been documented in grapes.

Separating the effects of photoperiod and temperature on cold acclimation and dormancy may be challenging, as a plant may need both external signals to fully implement biophysical changes needed for survival (Garris et al. 2009, Li et al. 2002). It is important to understand any interplay between these environmental cues that will help determine possible downstream targets of the environmental signals, the effects of the timing of the growth cessation, and the depth of dormancy and cold acclimation. The differential expression of the development of dormancy, growth cessation, and initial changes in cold acclimation and the biochemical changes may explain why some grapevines are more cold-tolerant than others. The purpose of this study was to characterize the morphological, physiological, and biochemical changes that occur in cold-sensitive and cold-tolerant grape cultivars in response to SD and to determine how SD may influence their dormancy, freezing tolerance, and raffinose accumulation.

## Materials and Methods

**Plant materials and treatments.** Grape cultivars of contrasting freezing tolerance (FT), as rated previously (Dami et al. 2005, Zabadal et al. 2007), were used in this study: Cabernet franc (*V. vinifera*, cold sensitive), Couderc 3309 (3309C; *Vitis riparia* x *V. rupestris*, rootstock, cold tolerant), and Concord (*V. labruscana,* cold tolerant). In the first year, dormant vines were planted in 7.6 L pots in a 25:75 mix of steam-sterilized soil and potting mix (Promix BX Micorrhiza, Premier Horticulture, Quaker Town, PA) and placed on benches in the greenhouse. Plants were dormant-pruned to retain three to four buds. After budbreak, two of the strongest shoots were retained and trained vertically on bamboo stakes. All flower clusters were removed. The plants were grown under a climate-controlled unshaded glass greenhouse with 25/20°C temperature and 40/70% relative humidity (day/night). Supplemental light was provided automatically when photosynthetic photon flux density dropped below 600 µmol/$m^2$/sec using 1000 W metal halide and 1000 W high pressure sodium lights (Sunlight Supply, Woodland, WA) and to maintain a 16-hr photoperiod. All experimental plants were watered daily and fertilized every other day with 100 mg/L 20-20-20 (N-P-K) fertilizer (Peter's Professional, Marysville, OH). Vines having uniform growth with 12 to 15 leaves at EL stage 17 or 18 (Eichhorn and Lorenz 1977) were selected and randomly assigned to each photoperiod treatment. The experiment was set up using a split-plot design with three blocks: photoperiod as the main plot and cultivar as the subplot. Four replications with two vines per plot were used.

Photoperiod experiments were conducted for 4, 6, and 8 weeks. Plants were grown under long day (LD; 16/8 hr day/night) or short day (SD; 8/16 hr day/night) in a climate-controlled unshaded greenhouse. To maintain darkness, black plastic sheeting (Sunbelt Plastic, Minneapolis, MN) was draped over all plants and opened and closed at time points to maintain the specified length of time for SD (8 hr) or LD (16 hr). Air circulation under the tents was maintained using electric fans. The experiments were conducted twice using the same vines. In the second year, the same potted vines were used after being pruned back to two nodes and stored in a 4°C cooler to satisfy their chilling requirements.

**Physiological and morphological assessment.** Leaf and node numbers and shoot length were measured on one vine in each pot prior to the exposure to the photoperiod treatment and every week thereafter. Data were recorded as a change in growth (measurement of the initial growth subtracted from the final growth) at 4, 6, and 8 weeks of treatment. Periderm development was also assessed at the end of each time period by counting shoot internodes that changed color from green to tan or brown. Periderm formation was expressed as the percentage of brown internodes to total number of internodes per shoot.

Dormancy induction was determined at the end of 4, 6, and 8 weeks. Each shoot was cut back to two basal buds (node positions one and two) and the plants placed under LD conditions (16/8 hr day/night). Budburst was recorded at EL stage 5 (Eichhorn and Lorenz 1977) and monitored every two days for 32 days. Dormancy was estimated as the number of days until 50% budburst (D50BB) and the percent of dormancy at 32 days.

Freezing tolerance (FT) was determined using thermal analysis in year two only. Thermal analysis measures the low temperature exotherm (LTE) detected at the ice nucleation temperature in primary buds. Basal buds, node positions three to seven, were collected from one vine, the leaves removed, and then wrapped in moistened paper towels placed in plastic bags to prevent moisture loss. To determine changes in FT, the five buds from each replication were excised from the collected vines in the laboratory and loaded onto thermoelectric modules (Melcor Corp., Trenton, NJ). The loaded modules were placed in a programmable freezer (Tenny Inc.,

New Columbia, PA) and subjected to a controlled freezing rate of 4°C/hr by lowering the temperature from -2 to -45°C. The FT of each treatment-replication is the mean or median LTE, which corresponds to 50% primary bud kill, or LT50 in degrees Celsius.

**Analyses of soluble sugars.** Analyses of soluble sugars were conducted on leaves and buds at the end of 4, 6, and 8 weeks of photoperiod treatment. Leaves and buds (node positions three to seven) were collected from one vine and were immediately plunged in liquid nitrogen then stored at -80°C until further analysis. Frozen leaves were lyophilized (VirTis Freezemobile, New York, NY) and ground to pass through a 20-mesh (0.85 mm) screen. Buds were excised, plunged in liquid nitrogen, and pulverized with mortar and pestle, and then lyophilized. The dry bud tissue was mixed with 75% ethanol in a tube, vortexed, and allowed to stand at room temperature for 2 to 4 hr, vortexing occasionally. The tubes were centrifuged for 5 min at 10,000 rpm. The supernatant was transferred to a vial then dried under air at 45°C. The extraction was repeated twice. The extracted bud metabolites and ~5 mg of ground leaf tissue were derivitized using the method by Streeter and Strimbu (1998), which also allows simultaneous extraction and derivitization of the leaf tissues. This involved mixing the ground leaf tissue or extracted bud metabolites with pyridine containing hydroxylamine and an internal standard, phenyl ß-D-glucopyranoside (Sigma-Aldrich, St. Louis, MO). After incubation at 70°C, the oxime derivatives were reacted with hexamethyldisilazane and trifluoroacetic acid (Sigma-Aldrich) to form the tri-methylsilyl derivatives of the oximes. The derivatives were injected into a gas chromatograph (HP 5890 Series II, Hewlett Packard, Boulder, CO) with a 30 m capillary column (HP 5-MS, 250 µm i.d. and 0.25 µm thickness). Injection temperature was 280°C and oven ramp was 180°C held for 2 min, 6°C/min ramp to 215°C, held 1 min, 40°C/min ramp to 320°C, held for 22 min. Helium, the carrier gas, was at a constant flow rate of 1.0 mL/min. Soluble sugars were identified and peaks quantified (ChemStation Quantitation Process Program, Agilent Technologies, Wilmington, DE) by comparison to standard sugars and the internal standard, phenyl ß-D-glucopyranoside.

**Statistical analysis.** Statistical analyses of morphological and physiological characteristics and soluble sugar concentrations were conducted using SAS software (SAS Institute, Cary, NC). Fisher's least significant difference test at $p \leq 0.05$ was used to compare means. Regression analyses between LT50 and raffinose concentrations at 4, 6, and 8 weeks in leaves and buds were also conducted. (To avoid redundancy and due to similar findings when repeated, only one year is presented unless otherwise indicated.)

## Results

**Shoot length.** Grapevines grown under SD photoperiod had slowed growth compared to grapevines grown under LD for all time periods and both years (Figure 1; data not shown for 2009). LD plants showed a 20% difference in the change in shoot length after 4 weeks of photoperiod treatment and a 33% difference after 6 and 8 weeks compared to SD. Comparisons among cultivars in 2009 revealed significant differences in the change in shoot length for all time periods, with Cabernet franc growing the fastest and Concord the slowest, but no differences were seen among cultivars in 2010. When comparing LD to SD among cultivars, all three cultivars showed reduced shoot growth beginning after 2-week exposure to the SD treatment with little change in shoot elongation after week 4. These differences were seen in both years. There were no interactions between cultivar and photoperiod except at 4 weeks in 2009.

**Node number.** Similar to the changes seen in shoot length, there was a reduction in the number of nodes produced in SD grapevines compared to LD for both 2009 and 2010 (Figure 1). The number of nodes formed per vine increased under LD conditions, with vines producing 23 and 32 nodes after week 4 and up to 29 and 41 nodes by week 8 in 2009 and 2010, respectively. Under SD conditions, however, the increase over time was very minimal, with the grapevines gaining only one node between 4 and 6 weeks. The difference in the change in node number is comparable to that seen for shoot growth, with both morphological traits showing approximately 23% and 38% less growth at weeks

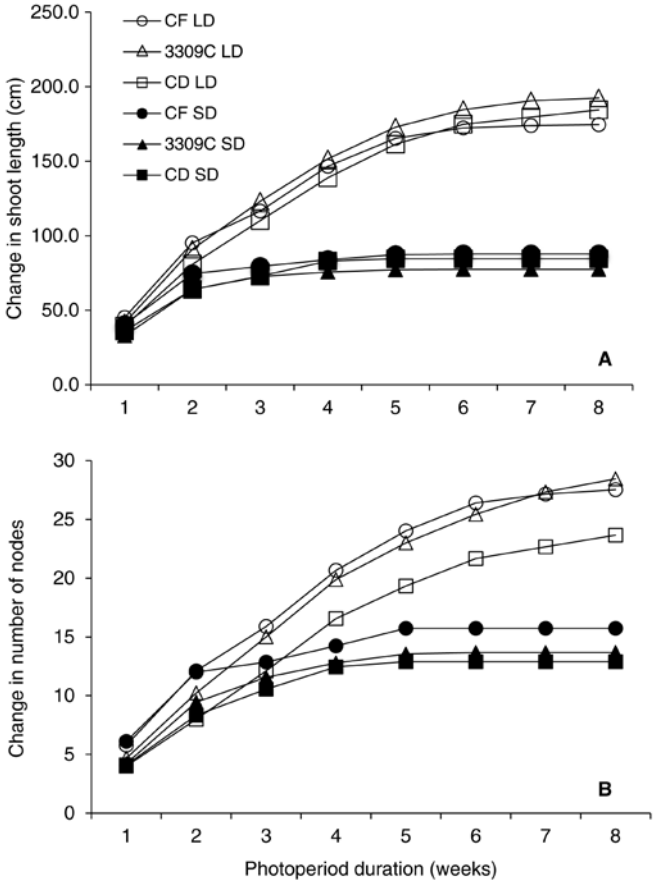


**Figure 1** Change in shoot length progression (**A**) and number of nodes (**B**) for Cabernet franc (CF), Couderc 3309 (3309C), and Concord (CD) grapevines exposed to long-day (LD) and short-day (SD) photoperiod regimes during an 8-week period in 2010. Measurements are means (n = 12).

6 and 8 for SD vines, respectively. There were also cultivar-related differences; however, no interactions between cultivar and photoperiod were found.

**Periderm development.** There was an increase of periderm formation on SD vines beginning 4 to 6 weeks into the photoperiod treatment compared to LD vines (Figure 2). No periderm was seen at week 4 in 2009, but there was some periderm development in 2010. At week 6 there were marked increases in the periderm development, with SD vines having an average of 37% of their internodes turning brown for both 2009 and 2010, a 95% difference compared to LD vines. The trend continued after 8 weeks under SD: vines developed periderm on more than 49% to 61% of the vine internodes compared to only 0% to 30% under LD (Figure 2).

**Dormancy.** For vines exposed to photoperiod treatment for 4 weeks, LD vines needed 12 to 20 D50BB (Table 1) and they had 100% budburst within 2 to 3 weeks after 32 days under forcing conditions for both 2009 and 2010 (Figure 3; data not shown for 2009). SD vines needed up to 32 D50BB (Table 1). Concord had the highest number of days for D50BB under SD treatment, needing more than 30 days after just 4 weeks. For vines exposed to 6 and 8 weeks of SD treatment, the trend was the same; even after 32 days under forcing conditions, there was minimal or no budburst, with levels ranging from to 0% to 11% after 32 days under forcing conditions (Figure 3). The delay of budburst indicated that the SD vines entered endodormancy after 4 weeks of exposure and reached full endodormancy after 8 weeks (Figure 4). There were also differences among cultivars in response to SD. Concord had increased response to the SD photoperiod, with the highest percentage of dormancy after 4 weeks compared to Cabernet franc and 3309C (Figure 4). This trend continued for vines exposed to 6 weeks SD; after 8 weeks SD treatment, there were no differences among cultivars, which all had ~100% dormancy.

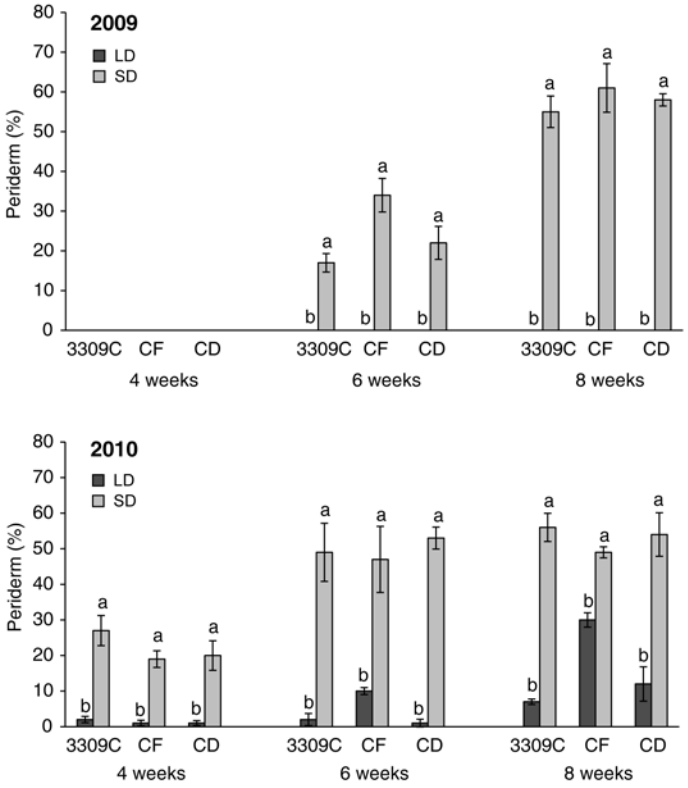


**Figure 2** Periderm formation recorded as percent (%) of shoot internodes that changed color from green to brown for Couderc 3309 (3309C), Cabernet franc (CF), and Concord (CD) grapevines grown under long-day (LD) and short-day (SD) photoperiod regimes for 2009 and 2010. Means (± standard error) with different letters for each photoperiod duration are significantly different by Fisher's LSD test at $p \leq 0.05$ (n = 12).

**Table 1** Number of days to 50% budburst (D50BB) for Cabernet franc (CF), Couderc 3309 (3309C), and Concord grapevines grown under long-day (LD) and short-day (SD) photoperiod regimes in 2009 and 2010.

| Photoperiod duration | Cultivar | D50BB | |
|---|---|---|---|
| | | LD | SD |
| **2009** | | | |
| 4 weeks | CF | 15 c[a] | 29 a |
| | 3309C | 14 c | 23 b |
| | Concord | 19 b | 32 a |
| 6 weeks | CF | 15 c | 30 a |
| | 3309C | 14 c | 22 b |
| | Concord | 19 b | >32 a |
| 8 weeks | CF | 19 b | >32 a |
| | 3309C | 12 c | >32 a |
| | Concord | 29 a | >32 a |
| **2010** | | | |
| 4 weeks | CF | 12 c | 12 c |
| | 3309C | 12 c | 15 c |
| | Concord | 20 b | 30 a |
| 6 weeks | CF | 13 bc | 18 b |
| | 3309C | 11 c | 26 a |
| | Concord | 19 b | 27 a |
| 8 weeks | CF | 14 c | >32 a |
| | 3309C | 12 d | >32 a |
| | Concord | 16 b | >32 a |

[a]Means with different letters in columns for each photoperiod duration are significantly different by Fisher's LSD at $p \leq 0.05$ (n = 12).

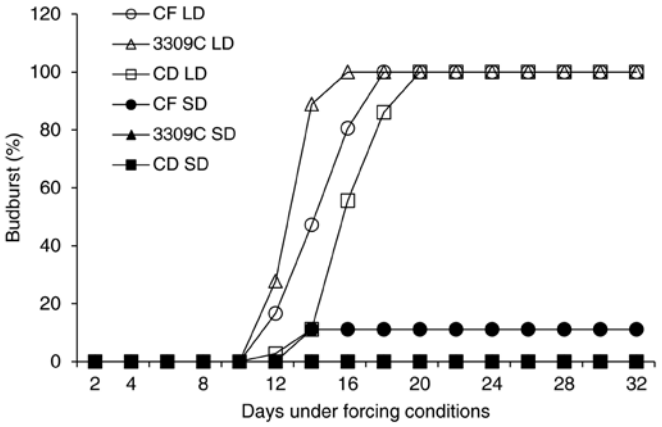


**Figure 3** Percent budburst for Cabernet franc (CF), Couderc 3309 (3309C), and Concord (CD) grapevines grown for 8 weeks under long-day (LD) and short-day (SD) photoperiod regimes in 2010. Measurements are means (n = 12).

**Freezing tolerance.** The LT50s were the same among all cultivars under the LD treatment for 4, 6, and 8 weeks and ranging between -6.1 and -8.1°C (Table 2). However, under the SD treatment, FT increased (LT50 decreased). LT50 in buds was different between LD (averaging -7.7°C) and SD (-8.4°C) even after 4 weeks of treatment. After 6 weeks, LT50 also decreased, with a 2°C difference between LD and SD. After 8 weeks of SD treatment, the average LT50 decreased to approximately -10.5°C. There were significant interactions between cultivars that were photoperiod dependent (Table 2). There were no differences among cultivars after 4 weeks of treatment except for Concord, which had the lowest killing temperature of -9.5°C (Table 2). After six weeks, the LT50 of 3309C and Concord was 2 to 3°C lower than that of Cabernet franc, which showed no difference between photoperiod treatments. After 8 weeks Cabernet franc began to show significant differences between photoperiod treatments, with a difference in LT50 of -2.4°C for SD vines. The LT50 of Concord and 3309C also decreased, reaching -10.5°C and -12.5°C, respectively.

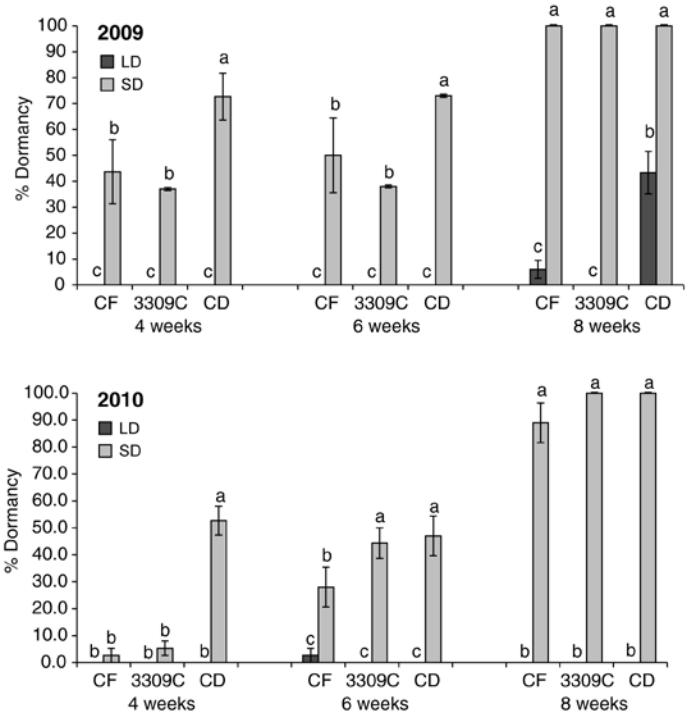


**Figure 4** Percent dormancy for Cabernet franc (CF), Couderc 3309 (3309C) and Concord (CD) grapevines grown under long-day (LD) and short-day (SD) photoperiod regimes in 2009 and 2010. Means (± standard error) with different letters for each photoperiod duration are significantly different by Fisher's LSD test at $p \leq 0.05$ (n = 12).

**Table 2** Freezing tolerance (LT50 in °C) of Cabernet franc (CF), Couderc 3309C (3309C), and Concord basal buds after exposure to long-day (LD) and short-day (SD) photoperiod at 4, 6, and 8 weeks in 2010.

| Photoperiod duration | Cultivar | Freezing tolerance (LT50 °C) | |
|---|---|---|---|
| | | LD | SD |
| 4 weeks | CF | -7.6 a[a] | -7.5 a |
| | 3309C | -8.1 a | -8.4 ab |
| | Concord | -7.3 a | -9.5 b |
| 6 weeks | CF | -8.1 ab | -8.3 b |
| | 3309C | -8.0 ab | -10.3 c |
| | Concord | -7.0 a | -10.5 c |
| 8 weeks | CF | -6.1 a | -8.5 b |
| | 3309C | -6.9 a | -12.5 c |
| | Concord | -7.4 ab | -10.5 c |

[a]Means with different letters for each photoperiod duration are significantly different by Fisher's LSD at $p \leq 0.05$ (n = 15).

**Qualitative and quantitative analyses of soluble sugars.** The sugars detected in both leaves and buds for all cultivars were fructose, glucose, myo-inositol, sucrose, raffinose, and galactinol. Sucrose was the predominant sugar in leaf tissues with 34% to 44% (dw) of the total soluble sugar concentrations, ranging between 25 and 52 mg/g for LD and between 33 and 60 mg/g for SD (data not shown). In bud tissues, sugar concentrations were generally much lower (43% less) than in leaves, and fructose, glucose, and sucrose were the predominant sugars, each one ranging between 25 and 35% of the total soluble sugar concentration (Figure 5).

Most sugars detected increased in response to photoperiod treatment; these changes, however, were not consistent with the photoperiod treatment or the length of the treatment. In both leaf and bud tissues, myo-inositol and galactinol had little to no variation in concentration (data not shown). In leaf tissues, there was variation in fructose, glucose, and sucrose concentrations for both years and all photoperiod durations; however, there were no consistent interactions or trends (data not shown). In bud tissues, fructose, glucose, and sucrose showed consistent trends, all increasing under SD compared to LD (Figure 5). These increases were mainly seen after 6 and 8 weeks of SD treatment, and the cold-tolerant cultivar, 3309C, accumulated the highest concentration of these sugars.

Raffinose was the only sugar that consistently changed in response to the photoperiod treatment for both leaf and bud tissues. In both years, raffinose concentrations were consistently higher in SD vines than in LD vines and also had consistently significant interaction between photoperiod and cultivar (Figure 6). After just 4 weeks of treatment, raffinose concentrations were at least two times higher in both leaf and bud tissues for both years and the concentrations were consistently higher in SD than in LD after 6 and 8 weeks. All cultivars generally had higher concentrations under the SD regime for leaves and buds, and the results were consistent in both years (Figure 6). Raffinose concentrations in leaves showed on average an eight-fold increase in year 1 and a 12-fold increase in year 2 for the 4- to 8-week time period. In bud tissues, there was a single-fold increase in year 1 and five-fold increase in year 2.

Cultivar variation in the raffinose concentrations was also photoperiod dependent, as indicated by significant interaction ($p \leq 0.05$ for leaves and $p \leq 0.01$ for buds). After 4 weeks of SD treatment, Cabernet franc had the lowest concentrations, ranging from 0.5 to 1.0 mg/g in leaves and buds with minimal to no difference between photoperiod treatments, whereas 3309C and Concord had much higher concentrations of up to

1.7 and 3.4 mg/g, respectively (Figure 6). Analyses of the 6 and 8 wk photoperiod treatments also showed that raffinose concentrations were generally higher in the cold-tolerant cultivars than in the cold-sensitive Cabernet franc.

Regression analyses between leaf and bud raffinose concentrations and LT50 showed different results between plant tissues. There was a significant linear regression ($p < 0.0001$, $R^2 = 0.43$) between leaf raffinose and LT50 for all three photoperiod durations (Figure 7). However, the linear regression between bud raffinose concentration and LT50 was not significant.

## Discussion

Short-day treatment consistently inhibited the shoot growth of the grapevines starting two weeks into the experiment for all three cultivars tested, resulting not only in shorter internodes but also in reduced number of nodes grown. The responses to SD were cultivar and time dependent. Concord was the first to slow in shoot growth and it completed growth cessation by week 3. Cabernet franc and 3309C also had reduced shoot growth but vines did not cease growth until after 4 weeks of SD treatment. Growth cessation is considered an early step in the process of dormancy development and has been widely documented as an early response for FT development (Sakai and Larcher 1987). It involves the termination of cell division in apical and axillary meristematic tissues. The suppression of internode elongation and its occurrence before leaf fall allows the plant to begin the process of FT development through the redirection of resources into overwintering plant tissues (Kalcsits et al. 2009, Sakai and Larcher 1987).

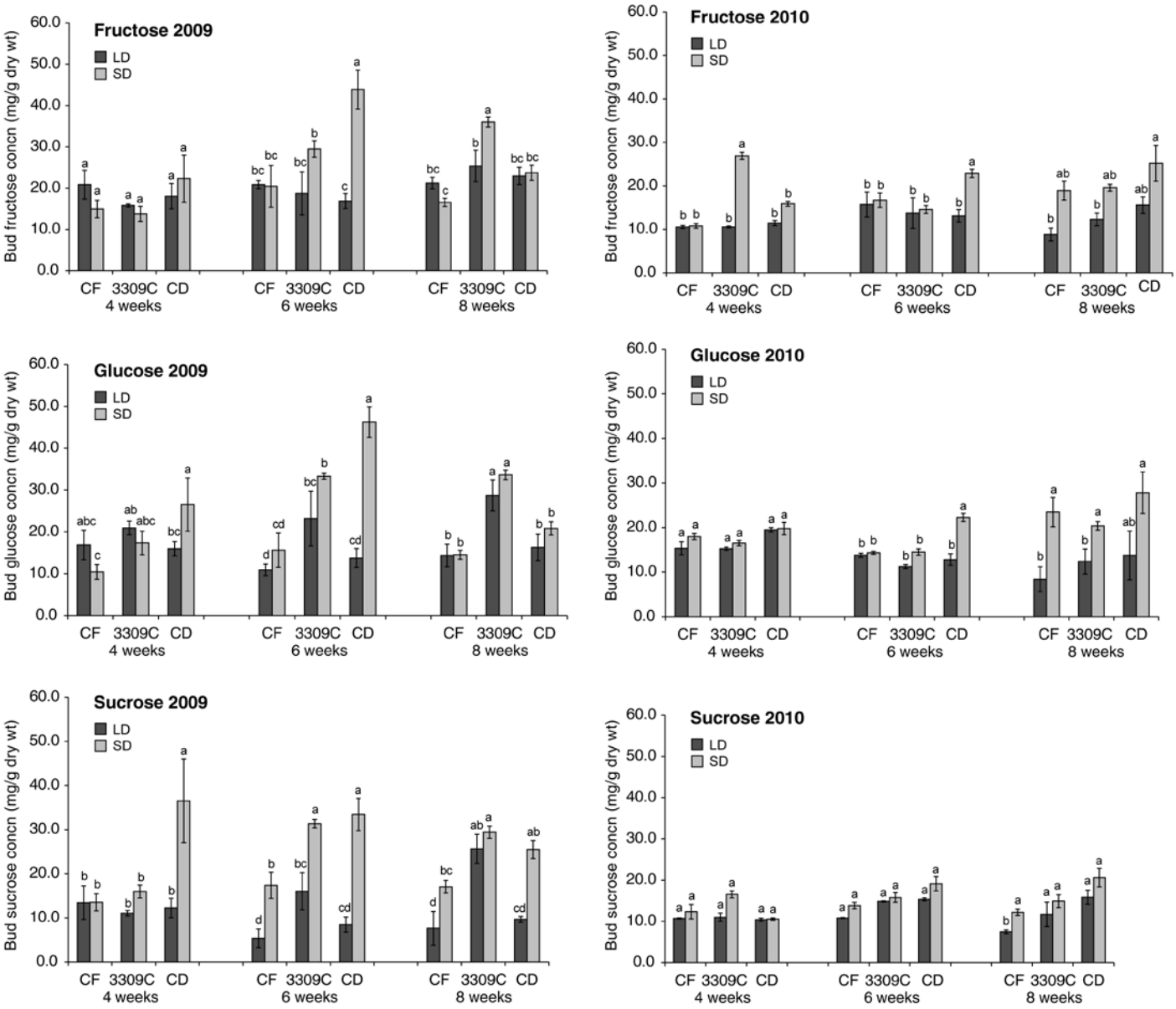


**Figure 5** Soluble sugar concentrations for fructose, glucose, and sucrose in basal buds of Cabernet franc (CF), Couderc 3309 (3309C), and Concord (CD) grapevines after exposure to long-day (LD) and short-day (SD) photoperiod at 4, 6, and 8 weeks in 2009 and 2010. Means (± standard error) with different letters for each photoperiod duration are significantly different by Fisher's LSD test at $p \leq 0.05$ (n = 4).

Fennell and Hoover (1991) demonstrated that *V. labruscana* and *V. riparia* responded to SD and initiated growth cessation. While several woody plants cease growth in response to both temperature and photoperiod, it has been demonstrated that grapevines are able to initiate growth cessation in response to SD only (Fennell and Hoover 1991, Salzman et al. 1996, Schnabel and Wample 1987, Wake and Fennell 2000). Other woody plants that also show growth cessation in response to SD only include cottonwood (*Populus trichocarpa*) (Howe et al. 1995) and birch (*Betula pubescens*) (Juntilla et al. 2003). The mechanisms relating to growth cessation and onset of dormancy of these woody species include setting terminal apical buds, a feature not expressed by grapevines, which generally have apical tip abscission.

The browning of the green stem upon periderm formation is an indicator of acclimation and dormancy development in grapes (Fennell and Hoover 1991, Salzman et al. 1996). This so-called wood maturation is evidenced by the progression of periderm from the base of the shoots in grapevine. As the green stem matures, the cortex senesces and many compounds are translocated to interior tissues that are acclimating (Zabadal et al. 2007). In this study, periderm development on grapevines occurred in both photoperiod treatments, but SD vines had a significantly higher percentage of internodes with periderm. The development of periderm was observed in all three cultivars, with its progression increasing throughout the experiment. This is consistent with previous experiments and confirms that the vines are initiating early responses to the SD photoperiod stimulus. Periderm formation from the base of the shoot of vines to the tip has been reported to be related to a development program that divides the vine into distinct zones (Salzman et al. 1996). Tissues that develop periderm are programmed to begin endodormancy and will begin developing FT, but those tissues that are without periderm are not programmed to do so, indicating that the induction of endodormancy and FT occur in separate pathways (Fennell and Hoover 1991, Salzman et al. 1996). The results of this study agree with previous reports. SD-treated grapevines displayed significant increases in periderm formation and entered endodormancy, which then resulted in the acquisition of some FT. Even though LD plants developed some periderm, it was at a significantly reduced rate and these grapevines did not enter endodormancy and did not increase in FT. Wake and Fennell (2000) had similar observations and proposed that periderm may instead be more related to grape tissue development and is enhanced by SD, but it cannot only be used as an indicator of dormancy or FT.

Short-day photoperiod is the main environmental factor that induces dormancy development (Lang et al. 1987). Concord grapevines responded to the SD treatment and had the highest percentage of dormancy after 4 weeks as compared to the other two cultivars, indicating it had achieved endodormancy earlier. Cabernet franc and 3309C also responded to the SD treatment by entering endodormancy, but both cultivars

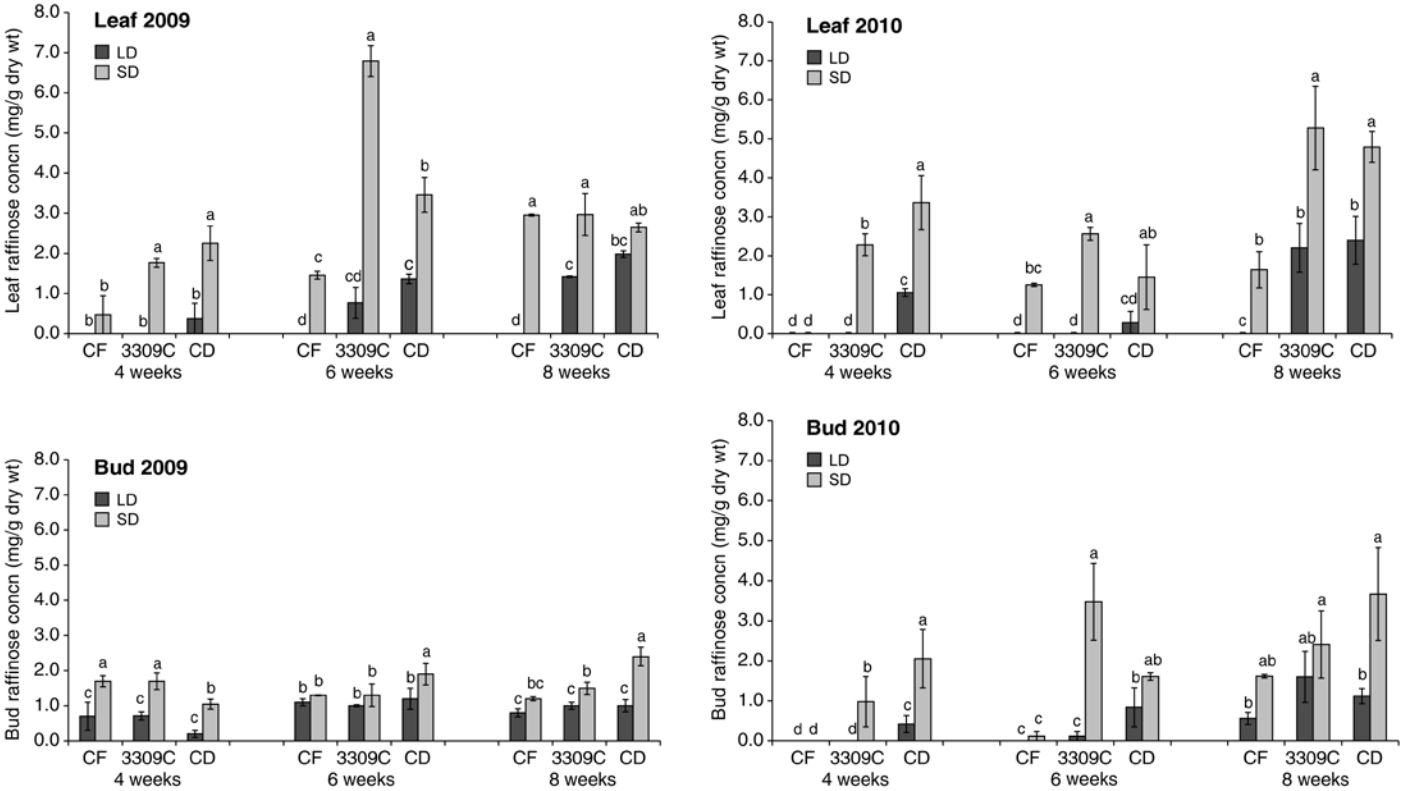


**Figure 6** Raffinose concentrations in basal leaves and buds of Cabernet franc (CF), Couderc 3309 (3309C), and Concord (CD) grapevines after exposure to long-day (LD) and short-day (SD) photoperiod at 4, 6, and 8 weeks in 2009 and 2010. Means (± standard error) with different letters for each photoperiod duration are significantly different by Fisher's LSD test at $p \leq 0.05$ (n = 4).

needed at least 6 weeks of SD. This study confirms the observation that variation in response to SD is cultivar dependent in grapevines. Fennell and Hoover (1991) showed that SD induced dormancy in both *V. labruscana* and *V. riparia*. Wake and Fennell (2000), however, demonstrated that Seyval blanc (*V.* spp.) did not go dormant when treated with SD and suggested that the cultivar might need low temperature treatment for dormancy induction. Schnabel and Wample (1987) demonstrated this synergistic effect in Reisling (*V. vinifera*). Short day or low temperature promoted some dormancy and FT, but the combination produced an additive effect much more than a combination of warm temperature and SD. In this study, all three cultivars initiated dormancy development and reached a state of deep endodormancy after 8 weeks of SD without cold temperature treatment. The differences seen here indicate that cultivar responses to photoperiod have an effect on dormancy induction, which may relate to the differences in FT observed for these cultivars. Overall, bud tissues were able to withstand freezing stress at significantly lower temperatures for SD buds compared to LD buds. Concord was the first cultivar to initiate this change, followed by 3309C at 6 weeks and Cabernet franc at 8 weeks. Fennell and Hoover (1991) also demonstrated a change in the LT50 of grapevine buds after SD treatment and concluded that the change corresponded with the timing of dormancy induction. In this study, Concord plants initiated dormancy after 4 weeks of SD treatment and also had an increase in FT for the same time period. The timing of the development of dormancy and then FT development are therefore linked to the responses of plants to the environmental cue of photoperiod.

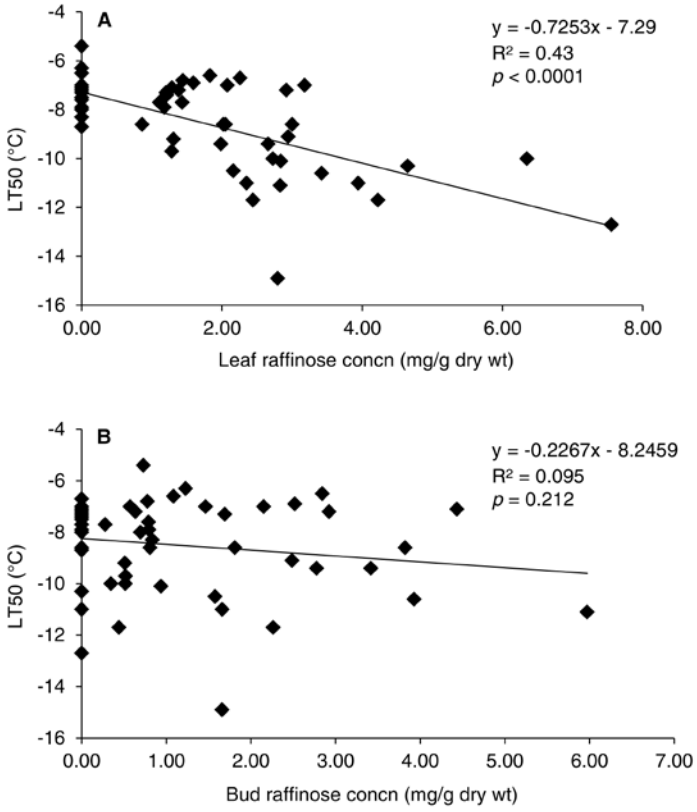


**Figure 7** Regression analysis of bud freezing tolerance (FT) with leaf (**A**) and bud (**B**) raffinose concentrations for Cabernet franc (CF), Couderc 3309 (3309C), and Concord (CD) after exposure to long-day (LD) and short-day (SD) photoperiod in 2010.

Short days initiate reduced growth, endodormancy, and FT (Kalcsits et al. 2009). In this study, grapevines had growth cessation from as early as 3 weeks into the photoperiod treatment, long before the beginning of deep endodormancy. The changes related to increased FT coincided with endodormancy development. Concord grapevines that had ceased growing 3 weeks into the SD treatment began to show changes in FT at week 4 and were the only vines to also have some dormancy initiation at 4 weeks, indicating that the cold acclimation process has begun. Previous research has demonstrated that signal transduction pathways starting from the perception of SD and leading to initial changes in dormancy and FT are operational in leaves of birch (*Betula pendula*); this early development is a possible protective mechanism during autumn, enabling prolonged periods for photosynthesis (Li et al. 2002).

Changes in soluble carbohydrates in buds are correlated with the subsequent increased FT in grapevines beginning with exposure to low nonfreezing temperatures and through the dormant season (Hamman et al. 1996, Wample and Bary 1992). Changes in soluble carbohydrates in buds have also been documented in response to low nonfreezing temperatures (Grant et al. 2009). In this study, in response to photoperiod, myo-inositol and galactinol concentrations had little to no variation, whereas fructose, glucose, sucrose, and raffinose concentrations did vary. However, the variation among fructose, glucose, and sucrose was not consistent in leaves, indicating that photoperiod treatment may have little to no control over the changes observed in these vines, and the accumulation of these sugars in leaf tissues and the changes observed may be related to other physiological changes in the vine. In buds, however, these sugars did show a slight increasing trend in response to SD. Raffinose concentration also did change consistently in response to the photoperiod treatment in both leaf and bud tissues. FT was closely associated with leaf raffinose concentrations but not bud raffinose, confirming the inconsistent relationship between raffinose in dormant bud tissues and LT50 reported in the literature.

The smaller mono- and disaccharide sugars have been shown to begin accumulation in buds during the fall and later play an important role in FT and the lowering of supercooling temperature. This research has shown that their accumulation is also photoperiod-related, an indication of the early changes related to FT development. The larger raffinose family of oligosaccharides (RFO) may play an important role in desiccation tolerance in addition to maintaining FT. Raffinose leaf concentration could indicate that the early changes seen in LT50 may be more related to an early response to desiccation stress in the leaves, which had much higher concentrations than buds. This relationship could be used as an indicator of FT, and raffinose in leaves could predict LT50, as it explained 43% of the variability in FT. In earlier studies, similar results were also obtained with leaf raffinose as an indicator of FT when cold treatment was applied (Grant

et al. 2009). Both cold and photoperiod treatments resulted in increased leaf raffinose concentration, with the highest concentrations in cold-tolerant cultivars and the lowest in cold-sensitive cultivars.

The accumulation of RFO during cold acclimation has been documented in a wide variety of plants, including leaves of *Ajuga reptans* L. (Bachmann et al. 1994) and alfalfa (*Medicago sativa* L.) (Castonguay et al. 1995) and in dormant tissues of woody plants including grape (*Vitis* sp.) (Hamman et al. 1996, Jones et al. 1999, Grant et al. 2009), crabapple (*Malus* sp.), apple (*Malus sylvestris*), dogwood (*Cornus* sp.) (Stushnoff et al. 1993), red raspberry (*Rubus idaeus* L.) (Palonen and Juntilla 2002), and aspen (*Populus tremuloides* Michx.) (Cox and Stushnoff 2001). The response in grapes has also been cultivar-related, with differential accumulation of RFO in cold-sensitive and cold-tolerant grape cultivars. Cold-tolerant cultivars such as Frontenac (*Vitis* spp.), 3309C, and Concord accumulated the highest raffinose concentrations in both bud and leaf tissues and cold-sensitive cultivars such as Cabernet franc accumulated the least (Grant et al. 2009). This pattern of accumulation is consistent with this study, with Concord and 3309C also accumulating higher concentrations of raffinose than Cabernet franc. To our knowledge, this is the first report demonstrating the accumulation of raffinose response to SD in leaves and buds of grapevines and a comparison of grapevines with contrasting FT.

The beginning of these changes in response to SD without any temperature stimulus may indicate a role outside of cryoprotection. Water content decreases with induction of both dormancy and FT (Salzman et al. 1996, Wolpert and Howell 1986), which would result in desiccation stress. Raffinose may be important in dormancy and FT because it aids in protecting the tissues from desiccation stress. Raffinose accumulation has been shown to occur concurrently with the reduction in water content as occurs in seed maturation and desiccation (Hannah et al. 2006, Peterbauer and Richter 2001). Many overwintering plant parts have to survive desiccation stress, so it may be that the factors that are activated by changes in shortening photoperiod are the same factors that up-regulate changes in raffinose concentration.

Raffinose has special properties other than storage. At low temperatures, raffinose delays the crystallization of sucrose (Caffrey et al. 1988, Koster and Leopold 1988) and raffinose does not change its configuration with decreasing temperatures (Jeffrey and Huang 1990), allowing it to have structure-preserving effect upon binding to proteins and membranes (Lineberger and Steponkus 1980, Santarius 1973). The sugar molecules may function by forming hydrogen bonds with macromolecules and may substitute for water during desiccation stress, thus allowing the macromolecules to maintain their hydrated orientation (Crowe et al. 1988). Solute accumulation may also decrease the osmotic potential, which depresses the freezing point of cell water. This is possible through a colligative effect where the sugars change the bulk properties of the solution (Burke et al. 1976). Soluble sugars may also protect cells by forming intracellular glass, an undercooled liquid with the viscosity of a solid, and its formation would ensure stability during periods of dormancy by preventing further desiccation and stabilizing cell structures (Burke 1986). Glass forms at the glass transition temperature. Raffinose is a trisaccharide and has a higher molecular weight than monosaccharides and disaccharides; therefore, it is more effective because it has a higher glass transition temperature and will form glass more readily (Franks 1985). In other words, raffinose is more protective than the disaccharide sucrose or the monosaccharides glucose and fructose. It is possible that the characteristics of raffinose allow it to function by any or all of the above mechanisms.

## Conclusion

In an earlier study, exposure to low temperature increased raffinose concentration in leaves and buds that led to changes during the first stages of the acclimation process coinciding with slowed shoot growth, but preceding periderm formation and subsequent acquisition of freezing tolerance. In this study, we demonstrated that short day also influences many of these same factors and that the responses are also genotype dependent, which may explain changes that make one grape cultivar more freezing tolerant than another. Grapevines responded similarly when the environmental cues (short day and low temperature) were administered separately. Although responses such as slowed shoot growth, periderm formation, dormancy induction, and the acquisition of freezing tolerance have been previously reported, to our knowledge this is the first report on the increased accumulation of raffinose in grapevine leaves and buds and a comparison of grapevines with contrasting freezing tolerance in response to short day or prior to any exposure to subfreezing temperatures. Cold-tolerant cultivars 3309C and Concord accumulated the highest raffinose in both bud and leaf tissues and cold-sensitive cultivar Cabernet franc accumulated the least. The finding of differential raffinose accumulation could be used as a predictor of freezing tolerance differences among grape genotypes.

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