Fruit Ripening Has Little Influence on Grapevine Cold Acclimation

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Abstract: This four-year study tested whether the physiological demand of fruit ripening may interfere with grapevine cold acclimation in autumn or with midwinter hardiness. Three harvest time treatments were established in a mature vineyard of own-rooted Cabernet Sauvignon vines: clusters were removed after fruit set, at veraison, or after the first fall frost. Average yield of the late harvested vines varied from 4.2 to 5.1 kg/vine (7.5 to 9.2 t/ha) among years, and soluble solids varied from 23.4 to 25.6 Brix. The presence of fruit during ripening delayed leaf senescence, measured as chlorophyll decline. The fruit also tended to delay the senescence-associated decrease in photosynthesis. All vines showed typical patterns of autumn cold acclimation, midwinter hardiness, and spring deacclimation. Cold hardiness of buds, cane phloem, and cane xylem varied during winter depending on prevailing temperature, and during the coldest winter reached levels of -27°C for 50% bud damage and -28°C for the onset of xylem injury. Early fruit removal had no effect on cane nonstructural carbohydrates and, with few exceptions, failed to enhance cold hardiness. Depending on the year, early fruit removal improved bud hardiness on 5 to 15% and xylem hardiness on 8 to 38% of all measurement dates. On those dates, the early harvested vines tended to be 1 to 2ºC hardier than the late harvested vines, irrespective of the time of crop removal. No trend was found for phloem hardiness. These results indicate that cropping, at least within commercially acceptable limits in regions with sufficiently long or warm growing seasons, rarely impacts cold acclimation and maximum hardiness. Grapevines appear to adjust seasonal leaf physiology to meet their carbon demand for both fruit ripening and cold acclimation.

Key words: carbohydrate reserves, cold hardiness, harvest time, photosynthesis, senescence, *Vitis vinifera*

Fruit growth and ripening in grapevines is thought to compete for resources with shoot growth, and the presence of fruit may slow down vegetative growth (Pallas et al. 2008, Winkler et al. 1974). Although the developing berries typically dominate the sink hierarchy of a vine, the perennial structure may become a high-priority sink late in the growing season (Candolfi-Vasconcelos et al. 1994, Loescher et al. 1990). Once rapid fruit sugar accumulation slows, the plant may change its focus from fruit ripening to replenishment of storage reserves and cold acclimation. Cold acclimation in grapevines is triggered by decreasing photoperiod, which induces bud dormancy, followed by cool temperatures (Fennell and Hoover 1991, Ferguson et al. 2014, Schnabel and Wample 1987). This implies that the genetic programs for fruit ripening and cold acclimation must be superimposed and are likely to be regulated independently.

Nonetheless, many growers and crop insurers at higher latitudes fear that delaying harvest to improve fruit quality or to produce icewine may compromise cold hardiness in winter, especially when adverse environmental conditions limit the availability of resources. No such effect could, however, be identified in a study that compared cold hardiness of Cabernet Sauvignon with fruit harvested at 18 Brix, 22 Brix, or not at all (Wample and Bary 1992). Another study found few and unpredictable effects of harvest date on cold hardiness of Chardonnay and Riesling (Hamman et al. 1996). In a third study, higher crop levels slightly reduced fall acclimation rates of Cabernet Sauvignon in three of the six years studied, while midwinter hardiness of buds and canes was unaffected by crop level (Wolf 2004). Nevertheless, it remains unknown whether the need to develop and ripen a crop adversely influences a vine's cold acclimation rate or the ability to acquire adequate cold hardiness.

The present study was designed to extend those earlier findings (Hamman et al. 1996, Wample and Bary 1992, Wolf 2004) by investigating whether the presence of fruit during all or a portion of the growing season may alter cold acclimation in autumn and/or cold hardiness throughout winter via changes in leaf physiology. Cabernet Sauvignon grapevines (*Vitis vinifera* L.) were used because the generally late ripening of this cultivar makes it a good candidate to study interactions between fruit ripening and cold acclimation under the conditions of short growing seasons (<160 days) and cold winters (plant hardiness zones 7a–6b; Widrlechner et al. 2012) in the region of this study.

Materials and Methods

The study was conducted from 2005 through 2008, using own-rooted Cabernet Sauvignon vines planted in 1983 in a

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vineyard at the Irrigated Agriculture Research and Extension Center (lat. 46°17'49"N; long. 119°44'07"W; 265 m asl) in the Yakima Valley of arid southeastern Washington state. The vines were planted at 3.1 m between rows and 1.8 m within rows in north–south oriented rows, trained to bilateral cordons with a single pair of foliage wires. Vines were spur-pruned in winter to 35 to 40 buds per vine. The vineyard was drip-irrigated, using regulated deficit irrigation (RDI) after fruit set to control shoot growth as described earlier for this site (Keller et al. 2005). No other canopy manipulations were carried out during the growing season. Temperature data were obtained from the on-site Washington State University AgWeatherNet weather station (http://weather.wsu.edu). Growing degree days (GDD) for the period 1 Apr to 31 Oct were estimated from daily mean temperatures, using a base temperature of 10ºC.

Crop load can be manipulated during the growing season by either leaf (source) removal or crop (sink) removal. The former leads to an increase in crop load, while the latter leads to a decrease in crop load. Because we wanted to test whether the need to develop and ripen the fruit might alter cold acclimation and midwinter hardiness, three severe crop-removal treatments were established: (1) all clusters were removed after fruit set; (2) all clusters were removed at veraison; and (3) all clusters were harvested after the first killing frost in fall. Fruit harvested from the latter treatment was used to estimate yield and fruit maturity in terms of soluble solids. Each treatment was applied to 10 individual vines in a completely randomized design across five adjacent rows.

Following the observation in 2005 that crop removal seemed to be associated with earlier leaf yellowing, a SPAD-502 chlorophyll meter (Minolta, Osaka, Japan) was used from 2006 through 2008 to estimate leaf chlorophyll repeatedly after veraison. Loss of chlorophyll is a marker of leaf senescence, serving as a visible indicator of mesophyll chloroplast disassembly (Fracheboud et al. 2009, Lim et al. 2007). In addition, gas exchange was measured on the same leaves in 2007 and 2008, using a CIRAS-2 system (PP Systems, Haverhill, MA). The external CO_2 concentration was set to 375 µmol/mol. Measurements were taken between 1000 and 1200 local standard time under clear skies with photosynthetic photon flux (PPF) >1200 µmol/m^{2}s. Only chlorophyll was measured when weather conditions did not permit gas exchange measurements. All measurements were taken on the leaf above the upper cluster on a two-cluster shoot of each vine.

After leaf fall in 2008, nonstructural carbohydrates (NSC) were determined in the same canes that had been used for physiological measurements. Basal internodes were sampled on 17 Nov, dried at 70ºC to constant weight, and ground to pass a 0.8 mm sieve in a ZM100 ultra-centrifugal mill (Retsch, Haan, Germany). Soluble sugars were extracted with 80% (v/v) ethanol and analyzed using commercially available enzymatic assay kits for sucrose, glucose, and fructose (Megazyme International, Bray, Ireland), following the manufacturer's instructions. Starch was further digested with α-amylase and analyzed using a glucose oxidase-peroxidase method (Smith and Holzapfel 2009). All NSC assays were conducted using a SpectraMax Plus^{384} microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Pruning weights and cane numbers per vine were determined before budbreak the following spring. Canes removed for cold-hardiness measurements throughout winter accounted for 24% of the total cane number. They were added to the pruning weights, using the average cane weight determined at pruning.

Each year, cane sections spanning nodes three to five were sampled approximately weekly (sometimes biweekly) throughout the dormant season (late September through early April). These were used to measure cold hardiness of buds, cane phloem, and cane xylem by differential thermal analysis (Mills et al. 2006). Measurements were taken on four replicates of five buds each or two 3 cm internode sections each. Each replicate was from two canes per vine; thus, four vines per date were sampled, cycling through the 10 treatment vines as sampling progressed. Cold hardiness was expressed as lethal temperature for 50% of the buds (bud LT_{50}), 10% phloem injury (phloem LT_{10}), or 10% xylem injury (xylem LT_{10}).

Data were analyzed using Statistica (ver. 10.0; StatSoft Inc., Tulsa, OK). Measurements taken on a single date were analyzed using one-way analysis of variance (ANOVA). Two-way ANOVA, applying a repeated-measures design, was used to test crop removal treatment effects within each year. Because significant time × treatment interactions were very common for the frequent cold-hardiness measurements, those data were also analyzed using one-way ANOVA within dates. Duncan's new multiple range test was used for post-hoc means comparisons of significant treatment differences. Associations between key response variables were tested using Pearson product moment correlation analysis.

Results

The 2005 (1500 GDD) and 2006 (1499 GDD) growing seasons were warmer than average (1444 GDD); both years accumulated higher than average heat units in the summer and early fall. The 2007 season (1422 GDD) was close to average with a warm summer and cool fall, while 2008 (1344 GDD) was cooler than average with a cool summer and average fall. The first fall frost occurred on 8 Nov 2005, 30 Oct 2006, 27 Oct 2007, and 5 Nov 2008. Implementation of RDI stopped shoot growth soon after fruit set, thus preventing the vines from growing more vigorously following early crop removal. Yet in the fourth year of the trial, the vines that had been defruited after fruit set grew ~30% more shoots than the other vines (Table 1), suggesting that some compensatory budbreak may have occurred in response to early fruit removal. The average yield of fruit-bearing vines varied from 4.2 to 5.1 kg per vine (7.5 to 9.2 t/ha) between years, and soluble solids at harvest varied from 23.4 to 25.6 Brix. In 2008, the yield:pruning-weight ratio was 7.0 ± 0.5 (mean ± SE), which is considered within the optimal crop load window for premium-quality winegrape production (Bravdo et al. 1985, Smart et al. 1990). Because harvest was delayed until after the first fall frost, it is likely that the berries lost some weight due to dehydration prior to harvest. Therefore, these vines were certainly not undercropped for the amount of vigor on this site.

Chlorophyll measurements showed that leaf senescence always started earlier on vines without fruit than on fruit-bearing vines (Figure 1). Data are not shown for 2006 when measurements were taken on two dates only. These were, however, consistent with the 2007 and 2008 data: immediately prior to the first fall frost, leaf chlorophyll of fruit-bearing vines was still more than double that of defruited vines ($p < 0.001$), regardless of whether the clusters had been removed after fruit set or at veraison. The decline in chlorophyll at the end of the growing season was associated with a decrease in photosynthesis in both 2007 ($r = 0.72$, $p < 0.001$, $n = 118$) and 2008 ($r = 0.62$, $p < 0.001$, $n = 90$). As the chlorophyll content decreased, the substomatal CO_2 concentration increased (2007: $r = -0.58$, $p < 0.001$; 2008: $r = -0.47$, $p < 0.001$), indicating that the senescing leaves were increasingly unable to assimilate the incoming CO_2. In 2008, but not in 2007, the presence of ripening fruit delayed the late-season decrease in photosynthesis (Figure 2). Stomatal conductance did not show a consistent response to fruit removal (data not shown).

Table 1 Cane nonstructural carbohydrates (NSC, DW = dry weight) after leaf fall and winter pruning weight (wt) of field-grown Cabernet Sauvignon vines following three different times of harvest (complete crop removal) in 2008. Values are means ± SE (n = 10).

	Time of crop removal		
	Fruit set	**Veraison**	**Leaf fall**
Soluble sugars (% DW)	9.1 ± 0.44	9.9 ± 0.26	10.1 ± 0.53
Starch (% DW)	5.3 ± 0.32 a[a]	4.6 ± 0.26 ab	4.2 ± 0.26 b
Total NSC (% DW)	14.3 ± 0.55	14.5 ± 0.24	14.4 ± 0.66
Pruning wt (kg/m)	0.50 ± 0.04 a	0.31 ± 0.02 b	0.40 ± 0.04 ab
Canes per m	27 ± 1.4 a	20 ± 1.3 b	22 ± 1.9 b
Cane wt (g)	18 ± 1.0 a	15 ± 0.7 b	18 ± 1.1 a

[a]Means followed by different letters differ significantly at $p < 0.05$ by Duncan's new multiple range test.

The time of crop removal did not impact total cane NSC at the beginning of the 2008 dormant season (Table 1). Soluble sugars comprised 67% of the total NSC. Of the measured sugars, sucrose accounted for approximately 50%, and glucose and fructose for 25% each. The starch concentration was slightly lower in canes from vines that were not harvested until after the first fall frost than in canes that had their crop removed after fruit set ($p = 0.049$). However, this was balanced by an inverse trend in sugar concentration, so that total NSC were identical in all treatments (Table 1). Similarly, there was no consistent impact of the time of cluster removal on winter pruning weight and its components.

No cold damage was observed in these vines in any year. Each year all vines showed typical patterns of autumn cold acclimation, midwinter hardiness, and spring deacclimation

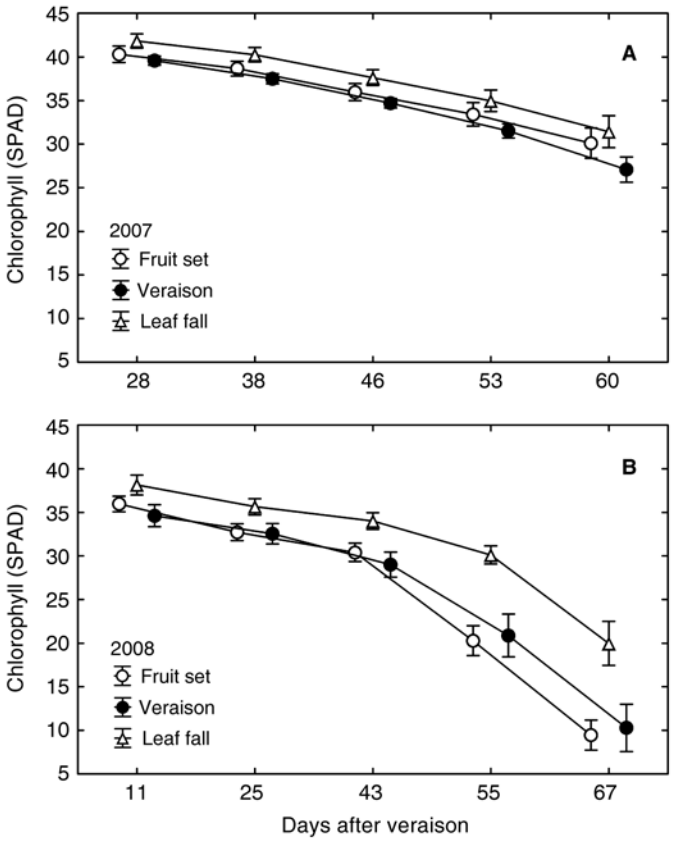

Figure 1 Leaf chlorophyll of field-grown Cabernet Sauvignon vines harvested at fruit set, veraison, or leaf fall in 2007 (**A**) and 2008 (**B**). Values are means ± SE (n = 10).

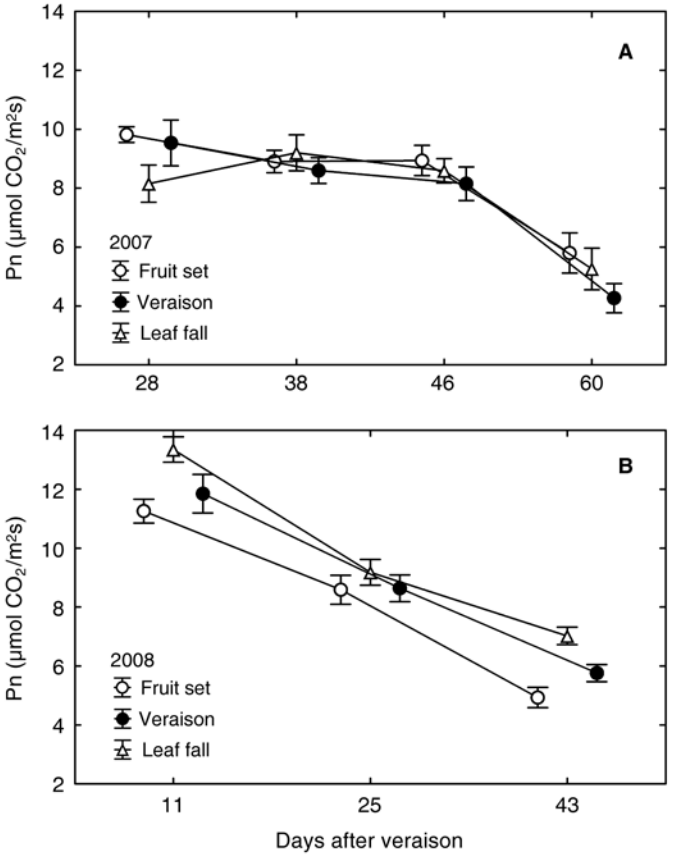

Figure 2 Leaf photosynthesis (Pn) of field-grown Cabernet Sauvignon vines with three different times of complete crop removal in 2007 (**A**) and 2008 (**B**). Values are means ± SE (n = 10).

(Figures 3 and 4). Bud, phloem, and xylem hardiness generally increased (i.e., *LT* decreased) from mid-October to reach maximum levels of hardiness by early December. For example, bud LT_{50} changed from approximately -10°C to -25°C during this acclimation phase. Cold acclimation of the xylem was less affected than that of the buds and the phloem by annual and daily temperature variation; xylem LT_{10} consistently declined at a rate of -0.15°C per day between early October and early December. Thereafter, the hardiness of both buds and vascular tissues varied depending on prevailing temperature; warming was associated with rapid partial deacclimation and cooling was associated with rapid reacclimation. Spring deacclimation usually began in late February. The lowest absolute values were attained in January 2008, when bud LT_{50} reached -27°C, phloem LT_{10} reached -21ºC, and xylem LT_{10} decreased below -28°C (Figures 3C and 4C).

Early fruit removal, irrespective of whether it was done after fruit set or at veraison, only infrequently improved cold hardiness during the dormant season. On one to two sampling dates (out of 21 to 26) each winter, the bud LT_{50} was 1 to 2ºC higher ($p < 0.05$) when the fruit was left on the vines until leaf fall (Figure 3). On several other dates only one of the two early fruit removal dates (and not consistently the same one) improved bud hardiness. In some cases in which the treatment effect was significant, however, it was in the wrong direction: the late-harvest vines had the *lowest* bud LT_{50}. Moreover, significant differences in bud LT_{50} were rarely observed after midwinter. Phloem hardiness remained largely unaffected by the crop-removal treatments (Figure 4). The most consistent, yet still minor and infrequent, trend was found for xylem hardiness (Figure 4). On two to eight sampling dates each winter, xylem LT_{10} was approximately 1°C higher ($p < 0.05$) for vines whose fruit was not harvested until leaf fall, compared with vines whose crop was removed early. This effect on xylem hardiness was more common during the fall acclimation phase than in midwinter or during the deacclimation phase in early spring.

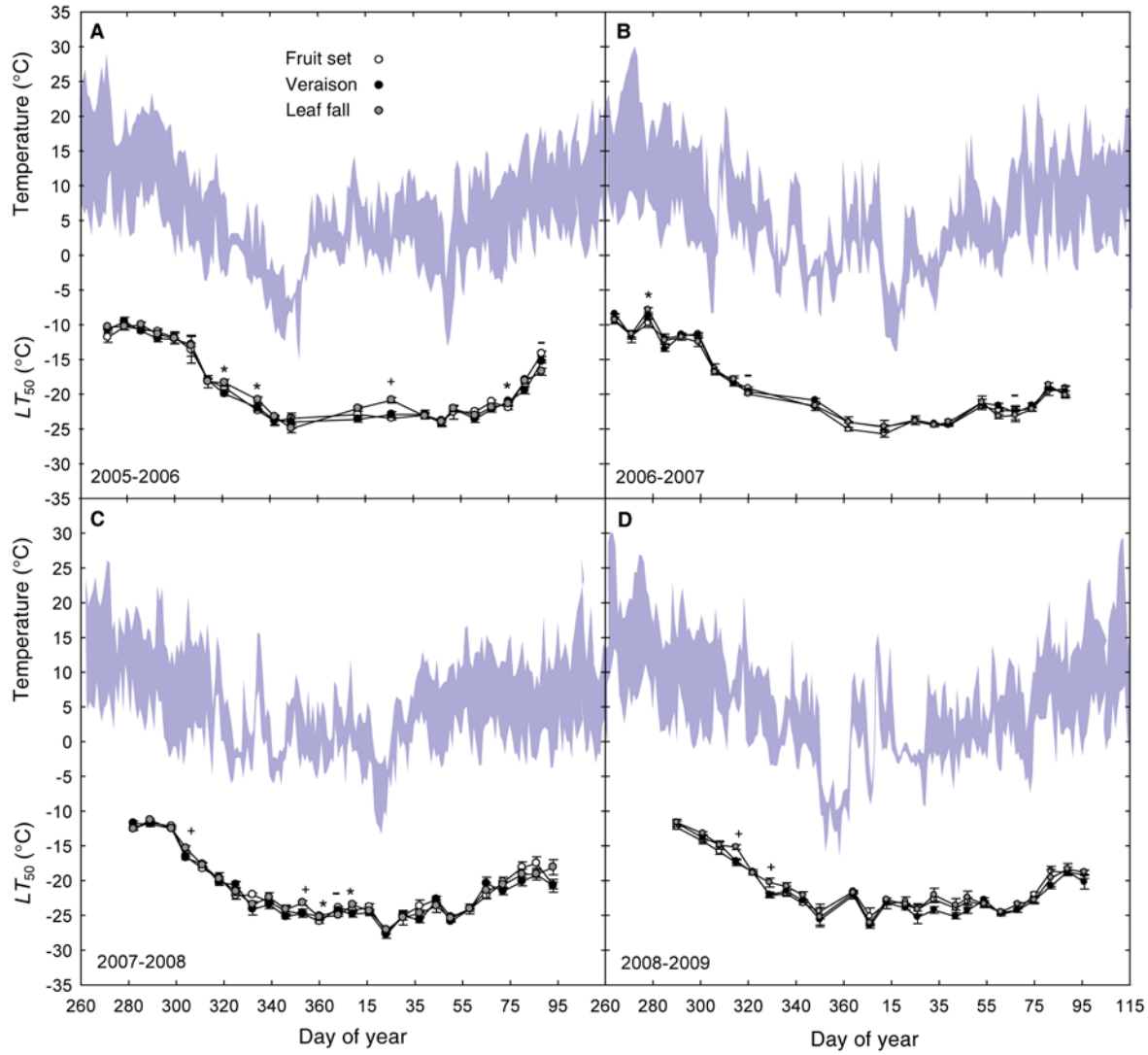

Figure 3 Dormant-season changes in minimum/maximum temperatures and bud cold hardiness (LT_{50}) of field-grown Cabernet Sauvignon harvested at fruit set, veraison, or leaf fall in 2005 (**A**), 2006 (**B**), 2007 (**C**), and 2008 (**D**). Values are means ± SE (n = 4). Symbols above *LT* values indicate significant treatment effect at $p < 0.05$: * = any treatment difference; + = late harvest least hardy; – = late harvest most hardy.

Discussion

The present study demonstrated that fruit ripening itself or extended periods of ripening (i.e., hang time) do not normally interfere with cold acclimation in grapevines with well-balanced reproductive growth and vegetative growth. Results also confirmed earlier findings that the crop load (i.e., source-sink balance) of vines may alter leaf senescence (Petrie et al. 2000). Early fruit removal, regardless of whether it occurred after fruit set or near veraison, was associated with an advancement of leaf senescence and a decline in photosynthesis after veraison. This indicates that the low sink demand of vines without ripening fruit led to downregulation of source activity toward the end of the growing season. These results support the idea that grape clusters reach their greatest sink strength at, and shortly after, veraison (Candolfi-Vasconcelos et al. 1994). Earlier studies also found a tendency for grape leaf photosynthesis to decrease following fruit removal or girdling (Downton et al. 1987, Harrell and Williams 1987, Hofäcker 1978, Roper and Williams 1989). Early sink removal in this study may have led to carbohydrate accumulation in the leaves, which eventually would have resulted in feedback inhibition of photosynthesis (Downton et al. 1987, Quereix et al. 2001, Roper and Williams 1989). As a result, there may have been an imbalance between light absorption by chlorophyll and photon use for photosynthesis. Therefore, it is conceivable that high leaf sugar content triggered chlorophyll degradation and premature senescence to avoid or respond to oxidative stress (Duan et al. 2008, Wingler et al. 2009). Clear skies with high solar radiation (PPF = 1000 to 2000 µmol/m^{2}s) are common in the summer and fall in southeastern Washington; during the ripening period such days are often coupled with low nighttime temperatures (0 to 15°C). While low temperatures typically accelerate the rate of leaf senescence (Fracheboud et al. 2009), they also favor cold acclimation in grapevines (Fennell and Hoover 1991, Ferguson et al. 2011, 2014, Schnabel and Wample 1987).

Concentrations of NSC are closely associated with cold hardiness in grapevine buds and canes (Hamman et al. 1996,

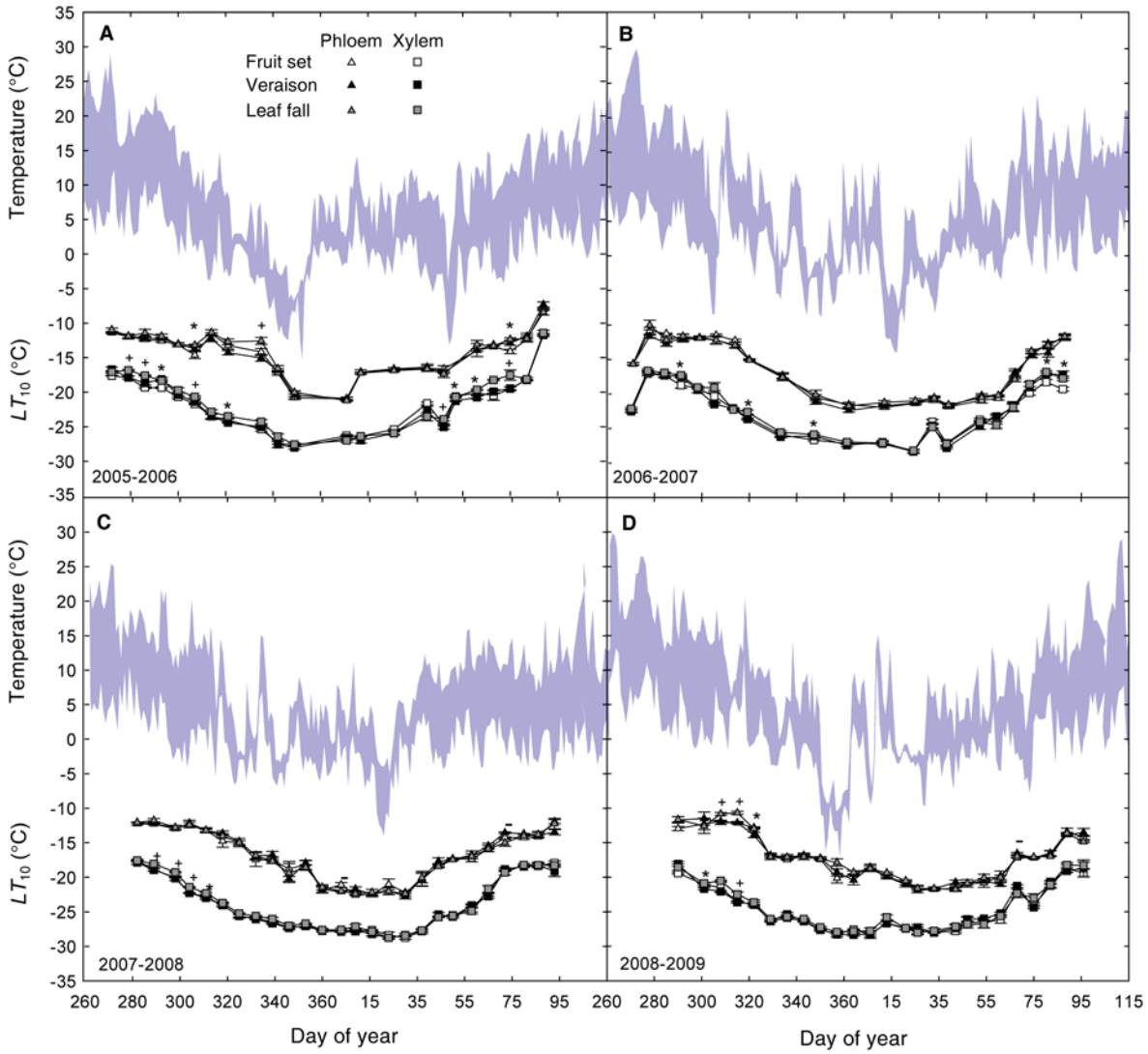

Figure 4 Dormant-season changes in minimum/maximum temperatures and cold hardiness of cane phloem and xylem (LT_{10}) of field-grown Cabernet Sauvignon harvested at fruit set, veraison, or leaf fall in 2005 (**A**), 2006 (**B**), 2007 (**C**), and 2008 (**D**). Values are means ± SE (n = 4). Symbols above *LT* values indicate significant treatment effect at $p < 0.05$: * = any treatment difference; + = late harvest least hardy; – = late harvest most hardy.

Jones et al. 1999, Wample and Bary 1992). The lack of treatment effect on NSC and the minor effect on cold hardiness in this study is consistent with earlier research that found no influence of commercial harvest time (Hamman et al. 1996, Wample and Bary 1992). Our results extend those earlier findings by showing that even complete fruit removal before the massive sugar accumulation phase in the berries did little to enhance cold acclimation and improve midwinter hardiness of grapevine buds and vascular tissues. In practical terms, the infrequent and inconsistent gain of 1 to 2ºC in bud LT_{50} and xylem LT_{10} when the fruit was harvested after fruit set or at veraison is very minor. For comparison, the range from 10 to 90% bud damage was typically ~3 to 5°C, which is in the same range as the short-term changes in bud LT_{50} and phloem or xylem LT_{10} due to temperature-driven deacclimation/reacclimation (this study and Ferguson et al. 2011, 2014). Once the vines had filled up their pool of storage reserves, they simply began to shut down and initiated a premature senescence program in the leaves. Williams and Smith (1985) found that leaf nitrogen declined in concert with photosynthesis during postharvest leaf senescence in grapevines. We suggest that early leaf senescence brought about by low overall sink strength may be an adaptive strategy: vines may benefit from the ability to remobilize leaf nitrogen, as well as other nutrients, sooner for storage in the perennial structure. This reduces the risk of loss of valuable nutrients when the leaves are killed by early fall frosts. In this study, photosynthesis was not simply switched off once leaf senescence started, but declined gradually, and thus continued to serve as a source of energy, carbon, and nutrients well into the senescence program. Regardless of the applied cropping treatment, the leaves evidently provided sufficient carbon to replenish the NSC storage pool in the canes and to permit adequate cold acclimation of the buds and cane tissues. The delayed leaf senescence on the nonharvested vines suggests that, under the conditions of this study, inadequate cold acclimation may be limited to years with unusually early fall frosts.

Conclusions

This four-year study found that cropping itself or extended hang time, at least within a commercially acceptable range in the arid western regions of the United States, are not detrimental to grapevine cold acclimation and to the maximal extent of midwinter hardiness. Only sporadic gains of 1 to 2ºC in bud hardiness and about 1°C in xylem hardiness were observed following the drastic intervention of removing all clusters after fruit set or at veraison. On most measurement dates (89% for the buds, 93% for the phloem, 78% for the xylem), such early crop removal failed to enhance cold hardiness. This means that if measurements had been taken only infrequently, or if only partial crop removal (e.g., cluster thinning) had been applied, the probability of finding any effect at all would have been very low. It seems that grapevines are able to adjust their source activity to overall sink demand. Cool temperatures in autumn favor cold acclimation, regardless of whether or not the vines are simultaneously ripening a crop. At present it is unclear, however, whether these findings also apply to much more heavily cropped vines or to regions with much cooler or shorter growing seasons, low light intensity, or high soil water status.

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