

Arrested Sugar Accumulation and Altered Organic Acid Metabolism in Grape Berries Affected by Berry Shriveling Syndrome

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Abstract: Berry samples were collected over four growing seasons from field-grown Cabernet Sauvignon grapevines in the Columbia Valley of southeastern Washington. Shoots were tagged prior to veraison and berries were sampled weekly from the same clusters. Symptoms of berry shrivel (BS) and bunch-stem necrosis (BSN) were monitored through harvest and berry samples were categorized as “healthy,” BS, or BSN for analysis of sugars, organic acids, potassium, and calcium. The vines of four adjacent rows were geolocated using GPS over three years to determine if BS was associated with specific vines or specific locations within the vineyard. Like BSN, the BS syndrome was seemingly restricted to individual clusters rather than individual vines and was spatially unpredictable from year to year. Vines propagated from BS-afflicted shoots only rarely displayed BS symptoms. Ripening-associated changes in berry solutes began simultaneously in healthy and BS clusters at veraison, but accumulation of sugars, K⁺, and oxalate ceased before shriveling symptoms became apparent on BS clusters. While BS did not affect tartrate, it was associated with slightly faster malate catabolism and slower decrease in soluble Ca²⁺ than in healthy berries. BS was not only associated with a cessation of phloem inflow into the berries, but also with altered organic acid metabolism and metal cation use.

Key words: fruit ripening, organic acids, potassium, ripening disorder, sugar, *Vitis vinifera*

Grape berries are somewhat unusual fleshy fruits because, unlike in most other species, their ripening period is associated with a second phase of expansive growth. Berry growth from veraison onward is coupled to sugar accumulation, which drives phloem influx (Keller et al. 2015). Phloem-derived water is used to sustain cell expansion and any surplus is transpired across the berry cuticle (Rogiers et al. 2004, Zhang and Keller 2015) or recycled via xylem backflow (Keller et al. 2006, 2015, Choat et al. 2009). Although xylem backflow has been considered a “pathological” condition that occurs due to berry cell membrane leakage (Tyerman et al. 2004), it now seems clear that such backflow accompanies normal ripening. Nevertheless, grape berries can shrink if water efflux exceeds influx. Various types of berry shrinkage have been described (Rogiers et al. 2004, Bondada and Keller 2012b). Arguably the first reference in the scientific

literature to an unusual and detrimental type of shrinkage, termed berry shrivel (BS), came in an account of bunch-stem necrosis (BSN) symptoms (Stellwaag-Kittler 1983). An infrequent form of “wilting” of the tip of grape clusters that was associated with postveraison berry shrinkage in the absence of any visible rachis necrosis was briefly described. Affected berries remained unripe and red cultivars remained poorly colored, but the causes were unknown. Despite considerable research into BS in the United States and Europe, the underlying causes of this ripening disorder remain elusive. No known viruses are associated with BS, suggesting that BS may be a strictly physiological disorder rather than a pathological condition (Krasnow et al. 2009). The idea that the syndrome is restricted to the ripening phase is supported by the finding that seeds of affected berries develop normally and retain their ability to germinate (Hall et al. 2011, Bondada and Keller 2012a).

Shrinkage of affected berries is associated with cell death in the mesocarp (Krasnow et al. 2008, Bondada and Keller 2012a) and in the rachis phloem (Hall et al. 2011, Zufferey et al. 2015, Bondada 2016), but it is not clear whether cell death in either the berries or the rachis is a cause or a consequence of the BS syndrome. Nevertheless, sugar accumulation in BS-affected grapes may cease shortly after veraison, up to three weeks before any shriveling symptoms become apparent (Knoll et al. 2010, Griesser et al. 2012). Consequently, BS berries have low sugar and anthocyanin contents (Krasnow et al. 2009, Knoll et al. 2010, Hall et al. 2011, Griesser et al. 2012). Some reports also suggest that the pH and amounts of potassium (K⁺) and, perhaps surprisingly, malate, may be low in affected berries, while the effects on calcium (Ca²⁺) and

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amino acids are more variable (Bondada and Keller 2012a, 2012b, Griesser et al. 2012, Bondada 2014). It may thus be hypothesized that berry metabolism is severely impacted in symptomatic berries. Based on the assumption that BS is a syndrome that often affects whole clusters rather than individual berries, the main goals of this study were to: (i) determine whether compositional changes in the berries could be detected at or before veraison, before the typical onset of BS; (ii) quantify changes in sugars, organic acids, and major soluble mineral ions before and during the progression of symptom expression; and (iii) track the occurrence of BS on the same vines over multiple growing seasons.

Materials and Methods

Vineyard site and GPS mapping. Berry samples were collected from 2005 through 2008 from own-rooted *Vitis vinifera* L. cv. Cabernet Sauvignon clone FPS 08 vines in a commercial vineyard with a history of high BS incidence. The Wallula vineyard (lat. 46.0°N; long. 119.1°W; 335 m asl) is located in the Horse Heaven Hills American Viticultural Area of southeastern Washington on a Shano silt loam (coarse-silty, mixed, superactive, mesic Xeric Haplocambid) of >2.4 m depth. The vineyard was planted in 1999 with a plant spacing of 1.8 m within rows and 2.4 m between rows that were oriented northeast-southwest on a southwest-facing slope. Vines were spur-pruned, trained to a relatively loose vertical shoot-positioning system, and drip-irrigated according to local industry standards for premium red wine production. All vineyard management practices were applied as uniformly across the vineyard as possible. Leaf, rachis, and berry samples were collected in early September and early October 2007 and immediately transferred to the Clean Plant Center Northwest (CPCNW) laboratory (<http://healthyplants.wsu.edu>) to test for phytoplasmas and bacterial pathogens.

Because soil depth and texture varied down the slope of the vineyard, partly due to leveling prior to vineyard establishment, BS incidence was visually assessed prior to harvest in 2005, 2006, and 2007. A GPS map was generated to determine whether or not the same vines consistently developed BS symptoms in consecutive years. A Trimble GeoXT (2005 Series, Trimble) was used to map all vines in four adjacent rows. In the first year, vines at the beginning and end of each row were marked. Visual assessment of each cluster on each vine was made each year, and vines with BS symptoms were marked with the GPS and given a ranking of 1, 2, 3, or 4 for the amount of shrivel visible on the southeastern side of the canopy, with 1 being low (shriveled berries noticeable on one cluster per vine) and 4 being high (every cluster had shriveled berries). Data were transferred to an Excel (Microsoft) file and unmarked vines were given a ranking of 0 for BS. The data were then exported to a .csv file format and imported into ArcGIS desktop (ESRI) for mapping. In addition, the mapped area of the vineyard was overlaid on a soil data layer (SURGO, USDA-NRCS Geospatial Data Gateway, <https://gdg.sc.gov.usda.gov>).

Berry sampling and analysis. Four adjacent rows were sampled extensively during the four growing seasons. Prior

to veraison, we randomly chose 50 shoots in 2005 and 2006 and tagged two clusters per shoot. The shoot number was increased to 200 in 2007 and further to 240 in 2008 to increase the likelihood of including potentially symptomatic clusters. All clusters were sampled weekly or biweekly through harvest. The appearance and progression of BS and BSN symptoms was assessed visually. Scissors were used to cut two to five berries with pedicel attached from each cluster. The berries were frozen at -80°C and later weighed and analyzed for total soluble solids (TSS), pH, sugars, organic acids, and soluble K^+ and Ca^{2+} as described (Keller and Shrestha 2014). Analysis of K^+ and Ca^{2+} was only conducted when sufficient material was available, and then only in 2006 and 2007. Berry volume was estimated as described (Keller et al. 2015). Solute data are expressed as both content (amount per berry) and concentration (amount per unit volume or weight). In addition, sap that exuded from the brush end when BS berries were pulled off and lightly squeezed between two fingers was collected one month before harvest in 2005 and analyzed separately from the bulk berry juice. At harvest in 2005, all clusters of the 50 sentinel vines were counted and weighed after they had been grouped into categories named “healthy” (i.e., free of visual symptoms) or “BS” (i.e., clusters with shriveled and sour-tasting berries). When visual symptoms were equivocal, berries were tasted to ensure proper categorization (Bondada and Keller 2012b). At harvest in 2006, 50 BS berries were immersed in distilled water to determine whether they could absorb and retain water. A superficial incision was made on 10 of these berries to expose the mesocarp. Rehydration of the berries was monitored visually over three days.

Periderm formation and cutting propagation. Because BS symptoms were more common on short shoots with poor periderm development, total and brown internodes were counted on 93 shoots before harvest in 2006. Of these, 27 had at least one BS cluster, 34 had no BS cluster but were on vines with BS clusters, and 32 were on vines without BS clusters. To test whether BS might be spread by propagation, we tagged the base of shoots that bore at least one cluster with clear BS symptoms prior to the 2006 harvest. During the following winter, 120 cuttings were collected from the tagged canes and rooted. Rooted cuttings were planted in pots the following spring alongside plants propagated directly from the virus-tested CPCNW foundation vineyard and monitored for BS each year through 2015. In the vineyard, all new shoots arising from the spurs produced from the shoots tagged in 2006 were monitored for BS development in 2007.

Weather data. Daily weather data were obtained from the nearest (<12 km) Washington State University AgWeatherNet (0) station at Eby (lat. 46.1°N; long. 119.1°W; 463 m asl). Growing degree days (GDD) for 1 April through 31 Oct were estimated from daily maximum and minimum temperatures, applying a base temperature of 10°C.

Data analysis. Berry composition data were analyzed using Statistica 64 (version 12; StatSoft). The pH values were converted to H^+ concentrations for data analysis and the means were converted back to pH for presentation. The data were initially analyzed by three-way analysis of variance (ANOVA),

applying a repeated-measures design to test for interactions between year, sampling date, and berry category. Because no BS was observed in 2005 and results were consistent across the remaining years, sampling date \times berry category interactions were analyzed using 2-way ANOVA across 2006, 2007, and 2008. Plot lines in figures were fitted using the distance-weighted least-squares method. Relationships between key response variables were tested using Pearson product moment correlation analysis. The GPS data were mapped for year to year variability.

Results

Berry sampling was conducted in growing seasons varying from warm (2006), to average (2005 and 2007), and to cool (2008). Both hot days ($T_{\max} > 30^{\circ}\text{C}$) and very hot days ($T_{\max} > 35^{\circ}\text{C}$) were generally much less frequent during grape ripening than before veraison (Table 1). There were more cool days ($T_{\max} < 15^{\circ}\text{C}$) in spring than in fall and they were especially common in 2008. However, the cool days were mostly confined to April and the late ripening phase in October and none occurred between bloom and the postveraison onset of BS symptoms. Cold days ($T_{\max} < 10^{\circ}\text{C}$) occurred very infrequently from April through October; all were limited to the periods before budbreak and after harvest. Annual precipitation was low and very little rain fell during the growing season; 2007 and 2008 were especially dry (Table 1).

Growers in southeastern Washington reported up to 50% BS incidence in some vineyards in each study year. Although Cabernet Sauvignon, Durif, and Sémillon were the cultivars most affected by BS, the locations of vineyards with high BS incidence were not consistent from year to year. Often, BS and BSN were noticed together in the same vineyard blocks. All leaf, rachis, and berry samples collected in this study tested negative for phytoplasmas and bacterial pathogens. Moreover, BS symptoms were observed on Cabernet Sauvignon and Durif vines in the virus-tested CPCNW foundation vineyard planted in 2003 and 2004, while a nearby Cabernet Sauvignon block planted in 1983 that was heavily infected with leafroll virus remained free of BS symptoms until it was removed in 2009. Contrasting with the grower observations and with the reported history of the Wallula vineyard, the BS incidence in this vineyard was $\leq 1\%$ in each

of the study years. While the rachis of BS clusters initially remained green and turgid, towards the end of the growing season it often became at least partly necrotic and flaccid, resembling BSN symptoms. As with BS symptoms, rachis necrosis associated with BSN sometimes initially appeared either near the cluster tip or on the shoulder and then spread toward the peduncle. Nevertheless, unlike the majority of BS symptoms, BSN symptoms rarely affected entire clusters. Moreover, additional BSN also appeared suddenly during the late ripening phase after apparently normal ripening; that is, without the early changes in berry size and fruit composition that characterize BS. Although all four types of “shrivel” described by Bondada and Keller (2012b) were usually observed, BSN incidence was too low to permit consistent berry sampling and analysis. Consequently, while a summary of harvest fruit composition is shown (Table 2), the remaining compositional data presented here are limited to healthy and BS berries.

No pattern of BS appearance could be identified on individual vines. Only occasionally did both clusters on a two-cluster shoot become symptomatic, and the basal cluster was as likely to be a BS cluster as was the apical cluster ($p = 0.63$; Figure 1). Both shaded and sun-exposed clusters sometimes developed BS symptoms. Categorizing all clusters as “healthy” or “BS” at harvest in 2005 showed that the yield loss associated with the removal of all BS clusters was $< 1\%$. Yield loss (in kg/vine) was a linear function of BS severity (in %; $y = 0.06x$, $r = 0.97$, $p < 0.001$). Of the 50 sentinel vines, 13 had between one and six BS clusters or $\leq 10\%$ of the total cluster number per vine, but despite a threefold range in the number of clusters per vine, there was no relationship between cluster number and BS severity ($r = -0.03$, $p = 0.83$). The vineyard is on a silt loam soil with steeper slopes on the western edge (Figure 2A). In 2005, shrivel was more prevalent in the eastern portion of the vineyard, with more severe shrivel clustered in two zones (Figure 2B). However, the pattern did not persist and while there was a range of shrivel symptoms in 2006, it was dispersed throughout the four mapped rows. In 2007, shrivel was less severe, never reaching a rating of 4, and much less widespread. Although in 2005 the vines with shriveling symptoms were clustered in areas with shallow soil and thus small canopies, the

Table 1 Weather conditions for the Wallula vineyard in southeastern Washington. Data were collected by an AgWeatherNet station located ~11 km from the vineyard.

Year	GDD ($^{\circ}\text{C}$) ^b	Veraison (Date)	Seasonal temperatures (d) ^a				Precipitation (mm)	
			>30°C PV/RP	>35°C PV/RP	<15°C S/F	<10°C S/F	Annual	Seasonal ^c
2005	1480	22 Aug	39/5	10/0	15/9	0/0	231	98
2006	1685	18 Aug	43/14	17/2	14/13	0/2	265	119
2007	1552	20 Aug	38/8	10/1	18/15	1/1	98	43
2008	1344	25 Aug	32/1	5/0	25/17	7/0	127	54
2000 to 2015	1501		44	0	13/10	0/0	250	119

^aNumber of days from 1 April to 31 Oct with maximum temperatures above or below four threshold temperatures. PV: preveraison; RP: ripening; S: spring; F: fall.

^bCumulative growing degree days ($>10^{\circ}\text{C}$) from 1 April to 31 Oct.

^cCumulative rainfall from 1 April to 31 Oct.

Table 2 Berry size and composition at harvest of field-grown Cabernet Sauvignon grapevines over four years. Berries were collected from apparently healthy clusters (n = 99) and clusters with symptoms of berry shivel (BS: n = 24) or bunch-stem necrosis (BSN: n = 16).

	Berry weight (g)	Berry volume (mL)	TSS (Brix)	Sugar/berry (mg)	Tartrate (g/L)	Malate (g/L)	pH
Healthy	0.96 a ^a	0.87 a	25.1 a	239 a	4.4 b	3.8 b	3.50 a
BS	0.66 b	0.62 b	16.8 c	115 c	5.8 a	3.3 c	3.25 b
BSN	0.70 b	0.63 b	27.1 b	188 b	4.6 b	4.8 a	3.26 b
<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	0.06	<0.001
2005	0.82 b	0.75 b	26.5 a	221 a	5.8 a	3.9 b	na
2006	0.83 b	0.75 b	21.3 c	184 b	4.4 b	2.2 c	3.54 a
2007	0.94 a	0.85 a	24.2 b	231 a	4.6 b	3.9 b	3.46 a
2008	0.89 ab	0.81 ab	24.2 b	215 a	4.5 b	4.6 a	3.34 b
<i>p</i>	0.11	0.12	0.44	0.17	0.001	<0.001	<0.001
SE ^b	0.06	0.05	1.4	16	0.4	0.4	0.06
C × Y ^c	0.90	0.95	<0.001	0.04	0.08	0.25	0.26

^aMeans followed by different letters differ significantly at $p < 0.05$ by Fisher's LSD test.

^bLargest standard error (SE) of any berry category or year mean.

^cSignificance (p value) of berry category (C) × year (Y) interaction. Significant interactions were entirely due to BSN: Intermediate TSS in 2006, highest in 2008; no BSN in 2005 and 2007.



Figure 1 Cabernet Sauvignon shoot with one healthy cluster and one cluster with berry shivel (BS). The inset shows sap exuding from a BS berry upon light squeezing.

majority of shivel was due to late-season shrinkage caused by dehydration. Those clusters usually continued to accumulate sugar, unlike typical BS clusters.

In 2006, many shoots with BS clusters were relatively short and had poor periderm formation. Preharvest counts showed that shoots with at least one BS cluster had on average 8 ± 0.9 brown internodes (out of 14 ± 1 internodes), compared with 16 ± 0.5 brown internodes (out of 20 ± 1 internodes) on shoots with no BS clusters ($p < 0.001$), regardless of whether the latter grew on vines with or without BS. A similar trend was observed in 2007, but was not quantified. None of the shoots arising from 120 spurs produced from BS

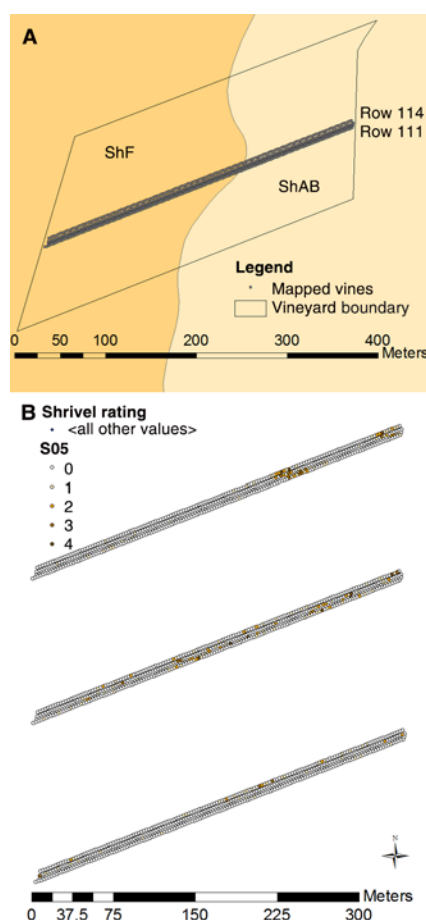


Figure 2 Map of the vineyard boundary and the rows GPS-mapped in 2005, 2006, and 2007 for severity of shivel (A). The vineyard boundary is laid over the SURGO soils map, with ShAB and ShF abbreviations for slight (0 to 5%) and very severe (35 to 60%) slope classifications for Shano silt loam soil. Four vineyard rows mapped with GPS in 2005 (top), 2006 (middle) and 2007 (bottom) for shivel (B). Shivel ratings of 0 (none) to 4 (most) indicate very little (1 = shivel on 1 cluster, ≤ 5 berries) to severe (4 = shivel on every visible cluster).

shoots tagged in 2006 produced clusters with BS symptoms in 2007. However, BS symptoms appeared suddenly following postveraison mechanical leaf removal in the fruit zone in mid-September in an adjacent Cabernet Sauvignon block, but incidence was not quantified since there was no control treatment against which to compare the data. Of the 120 cuttings propagated from “BS shoots” and grown in pots since spring

of 2007, the maximum number of vines that ever displayed BS symptoms in any year through 2015 was three, similar to the two symptomatic vines propagated from the CPCNW foundation vineyard. Moreover, symptoms were not observed on the same pot-grown vines from year to year.

Berries with severe BS symptoms that were cut superficially and immersed in distilled water swelled up at least partly over three days, but rehydration was much less evident for intact BS berries. Relatively clear sap exuded through the pedicel end when detached BS berries were lightly squeezed, but no sap exuded from healthy berries. The composition of the exuded sap was rather similar to the bulk juice of BS berries; however, the exuded sap contained somewhat more tartrate ($p = 0.026$), less citrate ($p = 0.004$), and had a lower glucose:fructose ratio ($p < 0.001$).

Across all years and sampling dates, and irrespective of the presence or absence of BS, the amount of sugar per berry as estimated from berry weight and TSS (s_{Brix}) was directly proportional to that derived from HPLC analysis (s_{HPLC}) over a measured range of $0 < s_{\text{HPLC}} < 320$ mg/berry ($r = 0.99$, $p < 0.001$, $n = 686$). Nevertheless, the former method overestimated the sugar content in preveraison berries with TSS < 9 Brix and $s_{\text{HPLC}} < 50$ mg ($s_{\text{Brix}} = 19.33 + 0.99 \times s_{\text{HPLC}}$). When organic acids were added to s_{HPLC} , this discrepancy almost disappeared ($s_{\text{Brix}} = 3.85 + 1.03 \times s_{\text{HPLC}}$). The sugars in both healthy and BS berries were almost exclusively (and in equal amounts) glucose and fructose, with only traces of sucrose (< 10 g/L). Sugar accumulation usually started in mid- or late-August, preceding by a few days the change in color associated with veraison (Table 1), and continued through mid- to late-October in healthy clusters (Figures 3 and 4). Although sugar accumulation began at the same time and rate in BS clusters, accumulation generally slowed suddenly and then stopped completely by early to mid-September (Figure 4). The slight increases in TSS and the concentrations of sugars and K^+ observed in BS berries toward the end of the growing season were due entirely to berry shrinkage. Despite the cessation of sugar accumulation in BS clusters, the berries on healthy clusters, even if they were on the same shoot as BS clusters, continued to ripen normally. By harvest, healthy berries had accumulated ~ 240 mg sugar, while BS berries reached a plateau at < 120 mg (Table 2). The average amount of sugar in BSN berries at harvest was intermediate between those numbers, reflecting the fact that BSN often did not appear until relatively late in the growing season. In addition, the berry shrinkage that accompanied BSN led to a concentration effect that increased TSS in BSN berries (Table 2).

The postveraison increase in berry volume was tightly coupled to sugar accumulation. K^+ is unlikely to have significantly impacted berry expansion, since its molar concentration was 15 to 40 times lower than that of sugars (data not shown). As sugar accumulation slowed in BS clusters (Figure 4), so did berry growth, and within one to three weeks after the cessation of sugar accumulation, the berries started shrinking (Figure 3). Nevertheless, even healthy berries did not expand after mid- to late-September, although they continued to accumulate sugar (Figure 4) and K^+ (Figure 5A) for

another month. Each year, the K^+ content per berry correlated positively with total sugar content ($r = 0.79$, $p < 0.001$), but the berries accumulated less K^+ per unit sugar (both in mg/berry) in 2006 ($\text{K}^+ = 0.29 + 0.0042 \times \text{sugar}$) than in 2007 ($\text{K}^+ = 0.29 + 0.0079 \times \text{sugar}$). Both the content and the concentration of soluble K^+ increased throughout ripening in healthy berries, while the amount of K^+ remained essentially constant in BS berries (Figure 5A). Unlike K^+ , the soluble Ca^{2+} content per berry decreased during early ripening, but the decrease was less pronounced in BS berries (Figure 5B). The difference in the change of Ca^{2+} concentration was even greater than that in the change of Ca^{2+} content (data not shown).

Organic acid contents and concentrations were lower in 2006 than in other years, even at the same sugar content. Organic acids were dominated by malate and tartrate: these two acids accounted for 98% of the total before veraison, decreasing slightly to 93% by harvest. Malate declined during ripening of both healthy and BS berries, but the decrease was

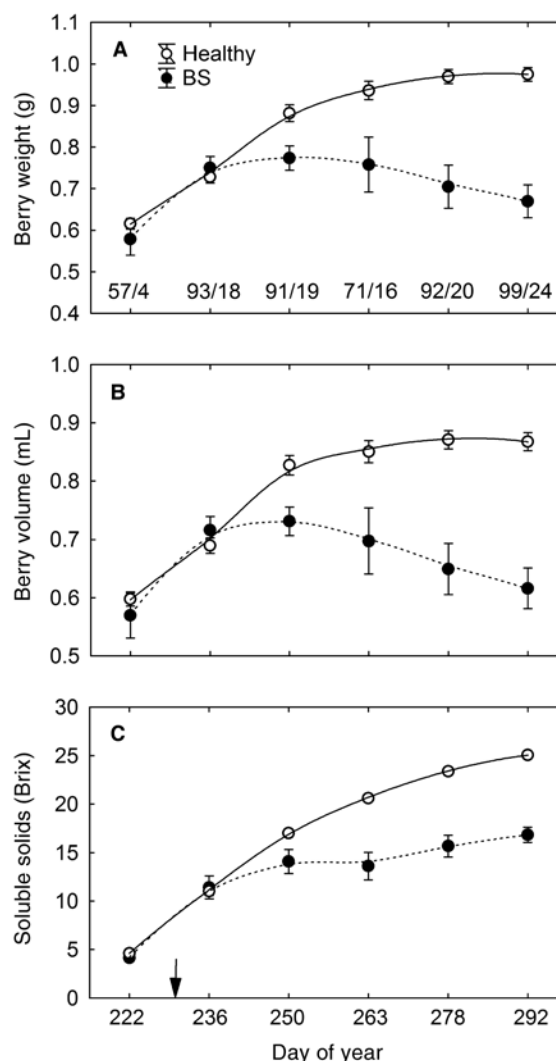


Figure 3 Changes in berry weight (A), berry volume (B), and soluble solids (C) during ripening of field-grown Cabernet Sauvignon grapes with and without symptoms of berry shrivel (BS). Values are means \pm SE from data pooled across four years, with n (healthy/BS) shown above lower x-axis in A. The arrow indicates the average date of veraison.

slightly faster in BS berries (Figure 6A). The tartrate content also decreased somewhat during early ripening, but tartrate remained unaltered by BS (Figure 6B). Oxalate accumulated in healthy berries during ripening, while accumulation ceased concurrently with sugar accumulation in BS berries (Figure 6C). No clear trends were found for citrate content, although citrate remained lower in BS berries than in healthy berries throughout the sampling period (Figure 6D). Across all samples, and irrespective of the year, the concentration of free H^+ ions (range 0.06 to 3.2 mM) was proportional to that of total organic acids (range 28 to 320 mM, $r = 0.95$, $p < 0.001$). However, at similarly low malate concentrations late in the growing season, BS berries had more H^+ than healthy berries. Although H^+ concentrations (and hence pH) were similar in healthy and BS berries before veraison, they increasingly diverged: later on during ripening, the H^+ concentration in BS berries was approximately twice that of healthy berries. By contrast with organic acids, the (negative) correlation between K^+ and H^+ depended on the growing season ($r = -0.57$ in 2006 and $r = -0.63$ in 2007, $p < 0.001$); the K^+ (and Ca^{2+}) and H^+ contents were lower in 2006 than in 2007.

This study also tested the assumption that BS berries taste sour because they are high in organic acids. Contrary to this assumption, the total organic acid concentration was similar in healthy and BS berries (Figure 7A). However, due to the cessation of sugar accumulation, the sugar:acid ratio stopped increasing in BS berries soon after veraison and then remained almost constant (Figure 7B). The same trend was observed for

the pH (Figure 7C); it remained lower in BS berries, mostly because berry shrinkage led to a concentration effect on H^+ .

Discussion

Several lines of evidence in this study lend support to the idea that BS is a physiological disorder rather than of pathogenic origin (see also Krasnow et al. 2009). First, all tests conducted on symptomatic vines for phytoplasmas and bacterial pathogens returned negative results. Second, BS symptoms on plants propagated from symptomatic Cabernet Sauvignon shoots were rare and no more frequent than those on vines propagated from the CPCNW foundation vineyard. Moreover, BS symptoms were also observed on vines in the CPCNW vineyard itself. Finally, BS was not observed on the same vines from year to year, neither in the vineyard nor in pots. Nevertheless, we cannot exclude the possibility that BS may be caused by an as-yet unknown pathogen or by an interaction between biotic and abiotic stress factors that is not currently understood. Unlike previously presented data (Krasnow et al. 2009), the present study supports the conclusion that BS is a syndrome that affects individual clusters rather than entire vines (Knoll et al. 2010, Griesser et al. 2012). This study also demonstrated that Cabernet Sauvignon berries that would develop BS symptoms shortly after veraison begin the ripening process like berries on clusters that remain free of this ripening disorder. Initial accumulation of hexose sugars and phloem-mobile K^+ in BS clusters did not lag behind that of healthy clusters and proceeded at the same rate both within

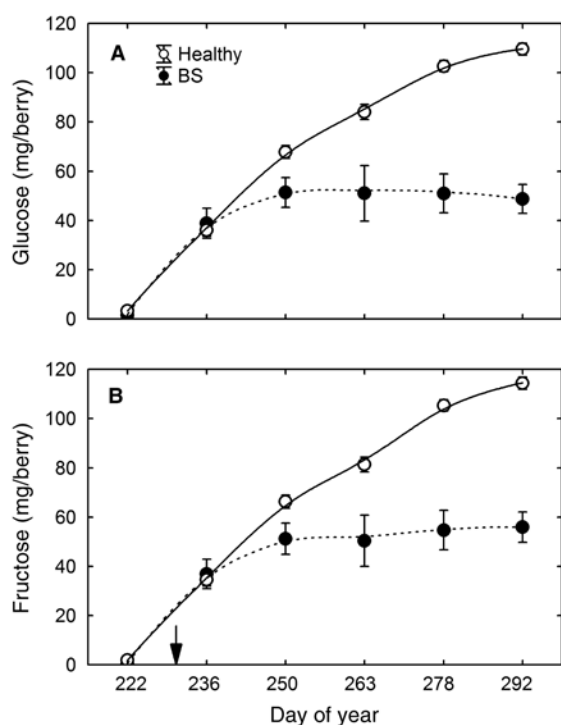


Figure 4 Changes in the contents of glucose (A) and fructose (B) during ripening of field-grown Cabernet Sauvignon grapes with and without symptoms of berry shrivel (BS). Values are means \pm SE from data pooled across four years. The arrow indicates the average date of veraison.

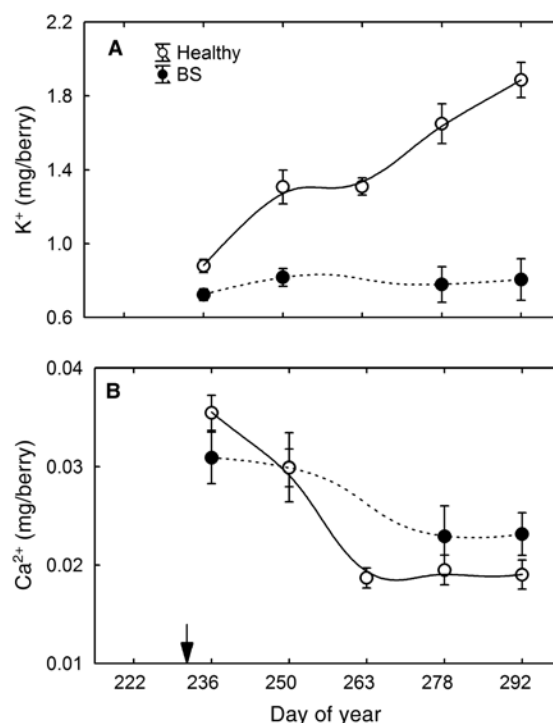


Figure 5 Changes in the contents of potassium (A) and soluble calcium (B) during ripening of field-grown Cabernet Sauvignon grapes with and without symptoms of berry shrivel (BS). Values are means \pm SE from data pooled across two years. The arrow indicates the average date of veraison.

and between vines. However, solute accumulation apparently may cease at any point during ripening and give rise to the BS syndrome. The increase in sink strength of grape berries at veraison is normally so strong as to override the influence of even severe water deficit (Keller et al. 2015) or defoliation (Candolfi-Vasconcelos et al. 1994). This change in sink strength triggers a marked increase in phloem influx, whether from the leaves or from storage reserves, and is associated with both sugar and K^+ accumulation and berry expansion. The beginning of sugar accumulation precedes, and

is a prerequisite for, anthocyanin accumulation in the skin of red grape cultivars (Castellarin et al. 2011, Keller 2015). Consequently, the poor coloration often observed in BS berries is likely a result of their low sugar concentration. Earlier work in our laboratory found that *Vitis* sp. berries typically do not become fully blue-colored until their TSS exceeds ~15 Brix (Keller and Shrestha 2014, Zhang and Keller 2015). Physiological disorders, it seems, are rare or absent in grapes between fruit set and veraison: that is, between seed initiation and seed maturity (Keller 2015). Indeed, seed weight and viability were unaffected by BS (Hall et al. 2011, Bondada and Keller 2012a).

The simple s_{Brix} proved a robust estimate of the actual sugar content of ripening berries. The fact that s_{Brix} overestimated s_{HPLC} before veraison may be because of the contribution of organic acids to the TSS measurement (this study, Keller and Shrestha 2014, Keller et al. 2015). Although s_{Brix} might be more appropriately termed “solutes per berry”

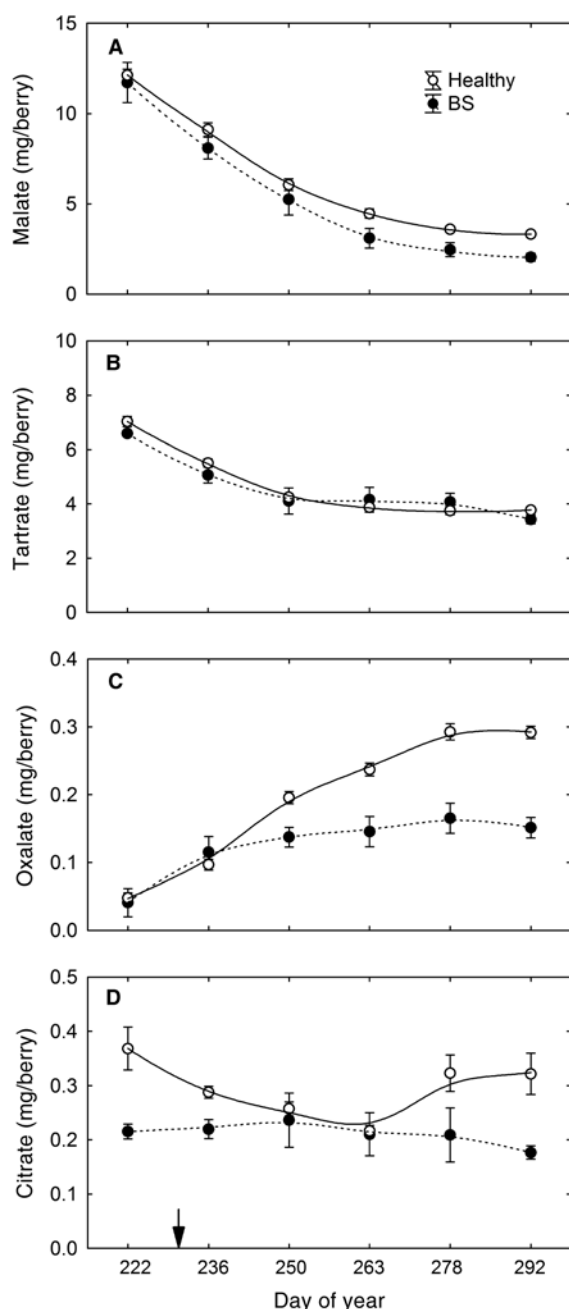


Figure 6 Changes in the contents of malate (A), tartrate (B), oxalate (C), and citrate (D) during ripening of field-grown Cabernet Sauvignon grapes with and without symptoms of berry shrivel (BS). Values are means \pm SE from data pooled across four years. Arrow indicates average date of veraison.

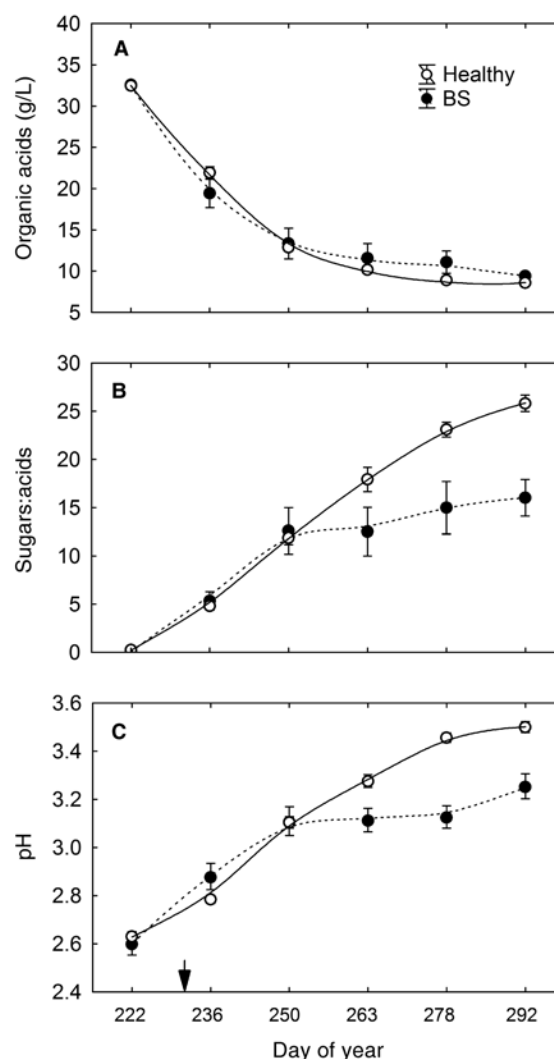


Figure 7 Changes in total organic acid concentration (A), molar sugar:organic-acid ratio (B), and pH (C) during ripening of field-grown Cabernet Sauvignon grapes with and without symptoms of berry shrivel (BS). Values are means \pm SE from data pooled across two years. The arrow indicates the average date of veraison.

rather than “sugar per berry,” this distinction becomes unimportant after veraison, when sugars dominate the berry solutes. As in other *V. vinifera* cultivars (Knoll et al. 2010, Griesser et al. 2012, Zufferey et al. 2015), cessation of sugar accumulation in berries approximately two weeks prior to the appearance of shriveling symptoms appears to be a hallmark of BS. As shown here and elsewhere (Keller et al. 2015), sugar accumulation and postveraison berry growth are tightly linked. The cessation of sugar and K^+ accumulation in BS clusters implies that phloem influx into the berries is interrupted. This interruption seems unlikely to originate in the shoots or perennial plant organs, considering the frequent observation that only one of two clusters on the same shoot develops BS. It remains unknown whether the interruption of phloem influx originates within the berries themselves or within the subtending rachis. Cell death has been demonstrated independently in both the berries (Krasnow et al. 2008, 2009, Bondada and Keller 2012a) and rachis (Hall et al. 2011, Zufferey et al. 2015, Bondada 2016). If the BS syndrome originates within the berries, however, it is unclear why the berries of entire clusters, or at least the tip portions of the clusters, typically develop symptoms simultaneously. It is possible that events occurring in one or a few berries lead to a rapid “chain reaction” that triggers cessation of phloem flow to other berries in the same cluster. However, removal of individual berries, as was done in this and many other studies, does not stop ripening in the rest of the cluster. Moreover, neither pedicel girdling (Rogiers et al. 2006b) nor rachis girdling on a shoulder of a cluster (M. Biondi and M. Keller, unpublished data) altered the progression of ripening in the remaining berries.

The characteristic shrinkage of BS and BSN berries is likely a result of uncontrolled water loss combined with interrupted phloem influx. The phloem is the major route for water supply to ripening berries and water loss may occur by cuticular transpiration or xylem backflow toward the leaves (Rogiers et al. 2004, Keller et al. 2006, 2015). Because berry transpiration is strongly driven by atmospheric vapor pressure deficit (VPD), dehydration is rapid under warm and dry conditions (high VPD) but is less severe under cool and humid conditions (low VPD; Zhang and Keller 2015). If some berry water is indeed lost via xylem backflow, and considering that membrane death occurs in BS berries (Krasnow et al. 2008, 2009, Bondada and Keller 2012a), the question arises why the amounts of sugar and soluble K^+ remain constant in BS berries. Sugars and other solutes accumulate in the berry apoplast during normal ripening (Wada et al. 2008, Keller and Shrestha 2014), so one might expect solutes to be lost along with water during BS symptom development. This is evidently not the case. One potential explanation is that residual solute important via any remaining functional phloem might simply balance solute loss via the xylem. Alternatively, or additionally, it is at least hypothetically possible that the observed phloem deterioration in the rachis (Hall et al. 2011, Zufferey et al. 2015) might be a response to loss of membrane integrity in the berries. This would prevent solute leakage but also stop phloem flow to the remaining berries. Nonetheless,

despite the decrease in mesocarp cell vitality in BS berries, cell death is far from complete (Krasnow et al. 2008, 2009). The partial rehydration of late-season BS berries immersed in water demonstrates that mesocarp membranes retained at least some semipermeability. The rehydration also suggests that the detrimental consequences of BS for harvest fruit composition may be exacerbated by dilution resulting from late-season rainfall. However, even with leaky membranes in many mesocarp cells, sucrose and malate catabolism evidently continued.

The near-absence of sucrose in all berry samples demonstrates that invertase activity continues to be high in BS berries. Non-functional invertase could lead to accumulation of sucrose supplied by the phloem, which could have caused phloem influx to stop. The absence of sucrose and the low hexose contents in BS berries are consistent with inhibition of phloem transport to the berries (Hall et al. 2011). Unlike the present study, a previous study found higher amounts of malate in postveraison berries that subsequently showed symptoms of BS, and no difference in tartrate contents per berry (Krasnow et al. 2009). Nevertheless, both studies showed that malate contents and concentrations declined after veraison in both healthy berries and BS berries. In addition, the impact of BS on tartrate concentration was rather minor. The distinctive sour taste of BS berries is thus not due to high organic acid concentration but instead is a result of a low sugar:acid ratio and low pH. The low pH in turn results from a combination of high H^+ and low K^+ concentrations (Boulton 1980). While accumulation of hexoses and K^+ during ripening increasingly masks the sour taste in healthy berries, this does not occur in BS berries.

Unlike the K^+ content, the amount of soluble Ca^{2+} in berry juice found here was an order of magnitude lower than the total Ca^{2+} present in grape berries (Rogiers et al. 2006a, 2006b, Bondada and Keller 2012a, Bondada 2016). This is not surprising, since the bulk of a berry's Ca^{2+} accumulates in the seeds and much of the remainder is bound to cell wall pectins or in Ca-oxalate crystals in the skin and mesocarp. In contrast, most K^+ occurs in solution in the mesocarp vacuoles (Rogiers et al. 2006a, Bondada and Keller 2012a, Keller 2015). Cell expansion during the early ripening phase is associated with an increase in pectin content (Silacci and Morrison 1990). The observed decline in soluble Ca^{2+} content during early ripening in this study mirrored the simultaneous increase in berry size. It is conceivable that this was due to Ca^{2+} incorporation into cell walls at a time when its influx via the xylem had ceased (Rogiers et al. 2006b, De Freitas et al. 2012). Less Ca^{2+} would presumably be required for this process in BS berries that stop expanding soon after veraison and then begin to disintegrate. Despite the potential association of Ca^{2+} and oxalate, however, the reason for the cessation of oxalate accumulation in BS berries is currently unknown. One putative possibility is that the presumed lower Ca^{2+} requirement for integration in cell walls of BS berries resulted in some of the “surplus” soluble Ca^{2+} being sequestered in insoluble Ca-oxalate crystals (c.f. Bondada and Keller 2012a).

Conclusions

This study monitored changes in BS and BSN symptom development and fruit composition of Cabernet Sauvignon in a commercial vineyard in arid southeastern Washington over four growing seasons. The results support the idea that BS is a physiological rather than pathological disorder and is restricted to the ripening period. No consistent patterns were observed for the appearance of BS within vines, between vines, or between years. Detailed observations on symptom development are in partial agreement with the idea that BS and BSN might be related physiological disorders: it is conceivable that BS might be a partly asymptomatic or early form of BSN. Nevertheless, unlike the majority of BS symptoms, BSN symptoms rarely affected entire clusters. Moreover, BSN symptoms also appeared suddenly during the late ripening phase after apparently normal ripening; that is, without the early changes in berry size and fruit composition that characterized BS. Visible BS symptoms were preceded by cessation of sugar, K^+ , and oxalate accumulation in the berries, but other organic acids differed little between healthy and BS berries. The present data suggest that the characteristic sour taste of BS berries is not due to high organic acid concentrations, but instead arises from a combination of low sugar:acid ratio due to low sugar content, low pH due to low K^+ , and a concentration effect on H^+ as a result of berry shrinkage.

Literature Cited

- Bondada B. 2014. Structural and compositional characterization of suppression of uniform ripening in grapevine: A paradoxical ripening disorder of grape berries with no known causative clues. *J Am Soc Hort Sci* 139:567-581.
- Bondada B. 2016. Nutritional aspects of grape (*Vitis vinifera* L.) clusters afflicted with SOUR shrivel is related to functionality of its vascular tissues. *Am J Plant Sci* 7:194-200.
- Bondada BR and Keller M. 2012a. Morphoanatomical symptomatology and osmotic behavior of grape berry shrivel. *J Am Soc Hort Sci* 137:20-30.
- Bondada BR and Keller M. 2012b. Not all shrivels are created equal—morpho-anatomical and compositional characteristics differ among different shrivel types that develop during ripening of grape (*Vitis vinifera* L.) berries. *Am J Plant Sci* 3:879-898.
- Boulton R. 1980. The general relationship between potassium, sodium and pH in grape juice and wine. *Am J Enol Vitic* 31:182-186.
- Candolfi-Vasconcelos MC, Candolfi MP and Koblet W. 1994. Retranslocation of carbon reserves from the woody storage tissues into the fruit as a response to defoliation stress during the ripening period in *Vitis vinifera* L. *Planta* 192:567-573.
- Castellarin SD, Gambetta GA, Wada H, Shackel KA and Matthews MA. 2011. Fruit ripening in *Vitis vinifera*: Spatiotemporal relationships among turgor, sugar accumulation, and anthocyanin biosynthesis. *J Exp Bot* 62:4345-4354.
- Choat B, Gambetta GA, Shackel KA and Matthews MA. 2009. Vascular function in grape berries across development and its relevance to apparent hydraulic isolation. *Plant Physiol* 151:1677-1687.
- De Freitas ST, Handa AK, Wu Q, Park S and Mitcham EJ. 2012. Role of pectin methylesterases in cellular calcium distribution and blossom-end rot development in tomato fruit. *Plant J* 71:824-835.
- Griesser M, Eder R, Besser S and Forneck A. 2012. Berry shrivel of grapes in Austria – Aspects of the physiological disorder with cultivar Zweigelt (*Vitis vinifera* L.). *Sci Hort* 145:87-93.
- Hall GE, Bondada BR and Keller M. 2011. Loss of rachis cell viability is associated with ripening disorders in grapes. *J Exp Bot* 62:1145-1153.
- Keller M. 2015. *The Science of Grapevines. Anatomy and Physiology*. 2nd ed. Elsevier Academic Press, London.
- Keller M and Shrestha PM. 2014. Solute accumulation differs in the vacuoles and apoplast of ripening grape berries. *Planta* 239:633-642.
- Keller M, Smith JP and Bondada BR. 2006. Ripening grape berries remain hydraulically connected to the shoot. *J Exp Bot* 57:2577-2587.
- Keller M, Zhang Y, Shrestha PM, Biondi M and Bondada BR. 2015. Sugar demand of ripening grape berries leads to recycling of surplus phloem water via the xylem. *Plant Cell Environ* 38:1048-1059.
- Knoll M, Achleitner D and Redl H. 2010. Sugar accumulation in ‘Zweigelt’ grapes as affected by “Traubenwelke.” *Vitis* 49:101-106.
- Krasnow M, Matthews M and Shackel K. 2008. Evidence for substantial maintenance of membrane integrity and cell viability in normally developing grape (*Vitis vinifera* L.) berries throughout development. *J Exp Bot* 59:849-859.
- Krasnow M, Weis N, Smith RJ, Benz MJ, Matthews M and Shackel K. 2009. Inception, progression, and compositional consequences of a berry shrivel disorder. *Am J Enol Vitic* 60:24-34.
- Rogiers SY, Hatfield JM, Jaudzems VG, White RG and Keller M. 2004. Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and preharvest weight loss. *Am J Enol Vitic* 55:121-127.
- Rogiers SY, Greer DH, Hatfield JM, Orchard BA and Keller M. 2006a. Mineral sinks within ripening grape berries (*Vitis vinifera* L.). *Vitis* 45:115-123.
- Rogiers SY, Greer DH, Hatfield JM, Orchard BA and Keller M. 2006b. Solute transport into Shiraz berries during development and late-ripening shrinkage. *Am J Enol Vitic* 57:73-80.
- Silacci MW and Morrison JC. 1990. Changes in pectin content of Cabernet Sauvignon grape berries during maturation. *Am J Enol Vitic* 41:111-115.
- Stellwaag-Kittler F. 1983. Äussere Symptomatik der Stiellähme an Trauben. *Mitt Klosterneuburg* 33:94-99.
- Tyerman SD, Tilbrook J, Pardo C, Kotula L, Sullivan W and Steudle E. 2004. Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. *Aust J Grape Wine Res* 10:170-181.
- Wada H, Shackel KA and Matthews MA. 2008. Fruit ripening in *Vitis vinifera*: Apoplastic solute accumulation accounts for pre-veraison turgor loss in berries. *Planta* 227:1351-1361.
- Zhang Y and Keller M. 2015. Grape berry transpiration is determined by vapor pressure deficit, cuticular conductance, and berry size. *Am J Enol Vitic* 66:454-462.
- Zufferey V, Spring JL, Voinesco F, Viret O and Gindro K. 2015. Physiological and histological approaches to study berry shrivel in grapes. *J Int Sci Vigne Vin* 49:113-125.