Inception, Progression, and Compositional Consequences of a Berry Shrivel Disorder

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Abstract: Berry shrivel (BS) is a ripening disorder of grapes; symptoms include shriveling of berries and low soluble solids before and at harvest. BS is distinct from bunchstem necrosis (BSN) in that BS rachis tissue remains green and does not undergo necrosis. BS berries were somewhat firmer than normally developing berries until just after veraison, but then softened faster than normally developing fruit. Soluble solids increased in all berries after veraison, but sugar accumulation on a per berry basis essentially stopped in BS berries several weeks before visible symptoms. Compared to normally developing berries, BS berries lost weight through water loss and mesocarp cell viability began to decline at about the same time as shriveling became apparent. BS berries had reduced anthocyanins in the skin, but had more skin tannin at harvest. Juice pH of BS berries was lower than that of normally developing berries, although amounts of tartrate and malate per berry were similar at harvest. Nonshriveled berries on vines that had BS symptomatic clusters had composition intermediate between BS and normally developing berries for sugar per berry, soluble solids, pH, anthocyanins, and skin tannins, indicating that BS disorder affects the entire vine rather than individual clusters or berries. BS vines at one site had consistently less negative water potentials than normally developing vines, but that was not the case at a second site. BS and normally developing vines were tested for common viruses as well as *Xylella fastidiosa* bacteria, but no differences were found.

Key words: berry shrivel, bunchstem necrosis, sugar accumulation, ripening, sugar accumulation disorder

Shriveling of grape berries during ripening is not uncommon in vineyards throughout the world. Little is known about the developmental pattern or physiological basis of the disorders. All shriveling is presumably the result of water loss exceeding uptake, but the relative contributions of xylem flow, phloem flow, and transpiration are generally unknown. Furthermore, the consequences of shriveling for fruit ripening remain largely unresolved.

One type of shrivel, late season dehydration of berries, has been studied in Shiraz (McCarthy 1999, Rogiers et al. 2001), but it also occurs in other varieties, such as Carignane (Freeman and Kliewer 1983). The onset of weight loss in Shiraz was shown to be closely associated with a constant number of days after anthesis and not closely associated with other parameters such as soluble solids or accumulated heat units (McCarthy 1999). The hydraulic conductance of the berry and pedicel remained higher in shriveling Shiraz than in nonshriveling Chardonnay. Water efflux through the xylem may be involved in this type of shriveling (Tyerman et al. 2004).

A second type of berry shriveling, bunchstem necrosis (BSN), is a widespread grape disorder that occurs in perhaps all grapegrowing regions. In California's Central Valley the term *waterberry* has been used to describe BSN in table grapes (Christensen and Boggero 1985), and in other countries the same disorder is termed *Stiellähme* (Germany), *palo negro* (Chile), *shanking* (New Zealand), and *desséchement de la rafle* (France). BSN is characterized by the rachis becoming necrotic and by berries on the necrotic portions of the rachis subsequently shriveling to a hard raisin state. The first visible symptoms of BSN are necrotic lesions on the rachis tissue that spread and eventually encircle the rachis. Several studies have examined possible causes of BSN, primarily focused on mineral nutrition, and have shown associations of BSN with an imbalance of potassium, calcium, nitrogen, and magnesium (Ureta et al. 1981, Christensen and Boggero 1985, Cocucci et al. 1988, Morrison and Iodi 1990, Capps and Wolf 2000). However, it is not clear if these compositional differences are consequences or causes of the disorder. The susceptibility of different cultivars to BSN has also been associated with a reduction in the amount of xylem distal to branch points in the peduncle (During and Lang 1993). The visible symptoms of BSN can occur

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at any point during grape berry development, from flowering (Jackson and Coombe 1995) until harvest (Ureta et al. 1981, Christensen and Boggero 1985, Cocucci et al. 1988, Morrison and Iodi 1990, Capps and Wolf 2000).

Other sources of shrivel are sunburn and a disorder termed *berry shrivel* (BS) (see Jensen 1970). Although sunburn has long been a concern (e.g., Winkler 1954) and has been frequently mentioned in the literature, the specific environmental and cultural conditions that give rise to sunburn of grape berries have not been evaluated. The first description of BS was by Jenson in 1970, although he refers to grower anecdotes from the beginning of the 20th century. In Jenson's description, BS in table grapes is characterized by clusters with flaccid berries with low sugar accumulation and poor color development in red varieties. The disorder only visibly affects some clusters, and occasionally only parts of clusters, on a symptomatic vine, while other clusters appear outwardly normal. More recent work has shown that potassium and magnesium foliar fertilization had no effect on the incidence of berry shrivel (termed *Traubenwelke* in the paper) on Zweigelt grapevines (Knoll et al. 2006). Winegrapes affected with berry shrivel have low sugar accumulation, often with excessive acid, and the fruit of red varieties has poor color. These compositional differences of berry shrivel fruit make it undesirable for high-quality wine production, and the fruit is usually removed from the vineyard before harvest at significant cost to growers.

Shiraz clusters with late-season dehydration are harvested for wine, but fruit afflicted with BSN, sunburn, or BS is often removed with several passes through the vineyard before harvest or at a sorting table before crushing. Although these operations are conducted at considerable cost and loss of yield, sunburn and BS have been little studied in part because the conditions necessary for the disorders to occur are not known, and therefore, the disorders cannot be readily imposed as experimental treatments. The culling takes place on a cluster basis, although Jensen (1970) did note reports of increased incidence on heavier cropped vines. However, specific vines have exhibited symptoms of BS every year since 1998 at the UC Davis Oakville Experimental Vineyard in Napa Valley, CA. We used the Oakville Vineyard to study the development of BS berries throughout ripening during two growing seasons (2004 and 2005). There is a perception among some growers that BS incidence is induced by vine stresses, such as limited water. To test this hypothesis, in 2005 we also undertook an irrigation experiment at a vineyard in the Alexander Valley (Sonoma County, CA) to analyze any effects of supplemental irrigation on the incidence of BS and/or BSN. If stressed vines have a propensity to show BS, then vines receiving supplemental irrigation should show a lower incidence. This work was conducted to assess the underlying physical and compositional changes associated with the berry shrivel disorder. Since the disorder seems to affect single clusters, while the vine appears outwardly healthy, we hypothesized that berry shrivel affects specific clusters on the vines and not clusters without visible symptoms. This research tests this hypothesis by documenting the progression of the berry shrivel disorder, its effects on the physical and chemical properties of affected and unaffected fruit on the same vine, and the influence of irrigation on the incidence of BS.

Materials and Methods

Selection of experimental vines, Oakville. The vineyard site for this study was a Cabernet Sauvignon clone 8 rootstock/vine spacing/training block, in which vines were grown on seven different rootstocks (Table 1), with various vine spacing (1 to 2 m between vines and 2 to 4 m between rows), and trained as either unilateral cordons or bilateral cordons on vertical shoot-positioned trellises with a north/south row orientation in a completely randomized design. There were 2199 vines on this 1-ha plot. Of these, 22 vines with a history of BS in the preceding five years were chosen as "likely to shrivel" (LTS) vines. Twenty-two normally developing vines were chosen based on lack of a history of BS. Normally developing vines were chosen so that they were on the same rootstock and had the same training system and the same vine spacing as LTS vines. Normally developing vines were either directly adjacent to LTS vines or directly across the row from LTS vines in order to reduce any effects of soil heterogeneity. All experimental vines had leaves pulled from the cluster zone on the east side to allow morning sun exposure and better air movement around the clusters, a common practice in the Napa Valley.

Selection of vines and irrigation regime, Sonoma. The trial was set up as a randomized complete block with four blocks, and two data vines (subsamples) per plot, randomly assigned to a 2 x 2 factorial design, with one factor being shrivel affliction and the other being irrigation level. Shrivel vines displayed some shrivel

Table 1 Total number of vines on each rootstock at the Oakville site, number of likely to shrivel (LTS) vines, number of LTS vines that exhibited berry shrivel (BS) in each year for each rootstock, total number of monitored BS clusters in 2005, and the range in the number of experimental BS clusters on data vines.

Rootstock	Vines in block	LTS vines	LTS vines with BS symptoms			Total BS clusters 2005 (per vine range)
			2003	2004	2005	
420A	303	8	7	7	8	47 (2–10)
5C	303	6	1	2	3	5 (1–2)
3309	309	4	2	2	3	10 (1–5)
O39-16	291	3	0	1	2	7 (1–6)
110R	371	1	0	0	0	0
1103P	316	0	0	0	0	0
1616C	306	0	0	0	0	0
Total	2199	22	10	12	16	69

clusters in the previous year (2004) whereas normally developing vines did not. All vines were irrigated once per week for 6 to 8 hr, with standard irrigation of 14.2 L/irrigation, and supplemental irrigation of 110.2 L/irrigation. The vines were Cabernet Sauvignon, clone 15, grafted onto 101-14 rootstock and planted in 1998 at 1.63 x 2.95 m vine spacing with an east/west row orientation. ET_c (evapotranspiration) was calculated using a seasonally adjusted crop coefficient for a VSP trellis with 2.95 m row spacing (L.E. Williams, personal communication, 2007) and the reference ET_o(reference evapotranspiration) from the CIMIS station located in Windsor, CA, 12 km southeast of the study block.

Berry sampling for composition, Oakville. In 2004, 44 data vines were sampled throughout the season. Of these, 22 vines had a history of BS (LTS vines), while the other 22 had no history of BS (normally developing vines). Two experimental clusters were tagged on each vine for sampling. On bilateral-trained vines, one cluster was chosen from each cordon. For unilateral-trained vines, two clusters were chosen on the single cordon. Prior to symptom onset, 104 days after anthesis (DAA), 20 berry samples were collected from each vine once per week. Once visual symptoms appeared, 7 to 10 berry samples were collected from individual symptomatic clusters and analyzed separately. For normally developing vines, 20 berry samples were collected throughout the season.

In 2005, the same 44 data vines were used, but with a different sampling protocol in order to more closely follow development of BS fruit prior to symptom expression. Ten experimental clusters were chosen on LTS vines, and five experimental clusters were chosen on normally developing vines. Two berries were subsampled from each of the 15 experimental clusters of each of the 22 vine pairs (330 clusters total) each week beginning just after veraison (74 DAA). Collected berries were put into a ziptop bag, placed directly on ice for transport back to the lab, and frozen at -20°C for later analysis. After BS symptoms developed, berry subsamples were identified as coming from BS affected clusters, from nonshriveled clusters on vines which had BS clusters, and normally developing berries. To ensure enough material for compositional analyses, skins and flesh from 8 to 12 berries (i.e., from 4 to 6 subsamples) were pooled to make a sample. Whenever possible berries from the same BS vine were pooled, otherwise berries from BS affected clusters on different vines were pooled. Each normally developing sample came from the same normally developing vine, and each sample from nonshriveled clusters on vines that had BS clusters came from the same LTS vine. Overall, there were 15 samples of 8 to 10 BS berries from 16 vines, six samples of 8 to 10 nonshriveled berries from LTS vines from six vines, and six samples of 10 normally developing berries from six vines. Unless otherwise stated, all 2005 compositional parameters were obtained from these samples.

Differentiation of BS from BSN, Oakville and Sonoma. Clusters were identified as having BS based on the presence of an outwardly healthy appearing rachis, low soluble solids at harvest, and shriveled berries on all sides of the cluster (Figure 1). Our experience is that sunburn develops only on the sides of clusters that are exposed to direct sunlight. Clusters with shriveled berries on all sides and a necrotic rachis were classified as BSN clusters.

Determination of BS. For Oakville, clusters on LTS vines that showed visible symptoms of BS at any point in the season were classified as BS clusters. Clusters on these vines that did not show visible symptoms were classified as LTS clusters. BS symptoms were not present in any of the normally developing clusters in either year. For Sonoma, at the end of the season (30 Sept, 122 DAA) all clusters were harvested from data vines and the number of normally developing, BS, and BSN clusters determined by counting.

Firmness measurement, Oakville. Firmness of the fruit was measured nondestructively with a custom-fabricated caliper device (Weis 2006) that simultaneously measured berry diameter and firmness. Briefly, the device consisted of a force transducer (FSG-15N1A; Honeywell, Morristown, NJ) attached to a linear actuator/stepper motor (Airpax, Cambridge, MD) operated by a CR10 datalogger (Campbell Scientific, Logan, UT). A switch closure initiated advancement of the force transducer toward the berry in ~50 µm steps; the number of steps made until berry contact were counted, allowing determination of berry diameter. After contact, the force at each step was recorded as the berry was deformed, until the stepper motor had either moved ~1 mm or a force of 100 g was reached. The firmness of the berry was calculated by the slope of a linear regression between force and deformation and was expressed as grams of force per percent deformation.

In 2004, a representative berry on each of the two experimental clusters on each data vine was tagged for firmness measurement, for a total of 88 berries. Firm-

Figure 1 Clusters displaying symptoms of bunchstem necrosis (BSN) (left) and berry shrivel (BS) (right).

ness/diameter readings were taken at least twice per week between 7:00 and 11:00 hr local time.

In 2005, a representative berry for each experimental cluster was selected and tagged for firmness measurements throughout development, for a total of 220 LTS clusters and 110 normally developing clusters. Firmness/diameter readings were taken once per week between 7:00 and 11:00 hr local time. Firmness values from individual clusters were kept separate until the BS status of that cluster was known, after which the values were averaged based on the status of the cluster (i.e., either BS, LTS, or normally developing). Data were obtained from 69 BS clusters, 137 LTS clusters, and 105 normally developing clusters. Eleven LTS clusters and five normally developing clusters developed BSN during the season. Three LTS clusters were damaged during the season and were removed from the study.

Weight determination, Oakville. Fresh berries were weighed. Berry weights were determined for, and corresponded to, the same samples used for compositional parameters. Skin and seed weights were determined directly and flesh weight was inferred by subtraction of skin and seed weight from the total berry weight. Flesh was homogenized using a homogenizer (Omni International GLH, Marietta, GA) for approximately 2 min in 50-mL polypropylene tubes.

Organic acid determination, Oakville. A 1-mL aliquot of flesh homogenate was incubated at 80°C for 20 min to redissolve acids that might have precipitated during the freezing process. Solid cell material was removed by centrifugation and the supernatant used for all assays. Organic acids (tartrate and malate) were analyzed by HPLC (Hewlett Packard 1100, Santa Clara, CA) according to a published method (Vlassides et al. 2001). Concentrations were determined using curves constructed with pure standards (Sigma Chemical, St. Louis, MO).

Soluble solids determination, Oakville. In 2004, samples were collected in ziptop bags and crushed by hand before being assayed for soluble solids concentration (Brix) with a hand-held refractometer (Valley Vintners, Livermore, CA). In 2005, a 100-µL aliquot of flesh homogenate was centrifuged to remove solid cell material, and a 25-µL aliquot of the supernatant was used to measure soluble solids using a temperature-corrected digital refractometer (RFM 110; Bellingham Stanley, London).

Water and sugar per berry, Oakville. Skins or an aliquot of flesh homogenate (100 µL) were weighed fresh, lyophilized overnight, and weighed again to determine dry weight. Water weight was determined by subtraction of the dry weight from the fresh weight. Sugar per berry wes calculated by multiplying the fresh weight of the berry minus the weight of the seeds by the Brix value divided by 100.

Mineral analysis, Oakville. Samples from eight BS clusters and four normally developing clusters were analyzed by the University of California ANR analytical lab (http://danranlab.ucdavis.edu/). BS and normally developing rachises and berries were collected from samples harvested on 169 DAA in 2005. These samples were lyophilized overnight and ground to a powder. Total nitrogen was analyzed by a nitrogen gas analyzer using an induction furnace and thermal conductivity. Potassium was extracted from the sample with 2% acetic acid and was quantified by atomic emission spectroscopy. Phosphorous, sulfur, boron, calcium, and magnesium were analyzed using a nitric acid/hydrogen peroxide microwave digestion and quantified by inductively coupled plasma atomic emission spectrometry.

Skin tannin and total phenolic measurements, Oakville. Lyophilized skins were ground to a powder in liquid nitrogen with a mortar and pestle. Ground skins were extracted in 50 µL of 70% acetone ([v/v] in water) per mg of dried skin tissue, agitated overnight on a rotary shaker (150 rpm) in microfuge tubes. Solid cell material was removed by centrifugation. The acetone in the supernatant was removed in a speedvac concentrator (Savant Laboratory Equipment Company, Hayward, CA), and distilled water was added to bring extracts up to the original volume. Extracts were diluted 1:10 and used for the tannin/total phenolic/anthocyanin assay (Harbertson et al. 2003).

Specific phenolic analyses, Oakville. Lyophilized skins were ground to a powder in liquid nitrogen with a mortar and pestle. Ground skins were extracted overnight in 50 µL of 50% methanol ([v/v] in water) per mg of dried skin tissue, agitated overnight on a rotary shaker (150 rpm) in microfuge tubes. Specific phenolic compounds were analyzed by reverse-phase HPLC (Hewlett Packard) (Bogs et al. 2005) with a 50 µL injection volume. Standard curves of malvidin-3-glucoside were constructed using authentic standards (Carl Roth, Karlsruhe, Germany) and the extinction coefficients from these curves were used to quantify all anthocyanins.

Water potential measurements. In 2004, vine water status was monitored as midday leaf water potential with the pressure chamber technique (Soil Moisture, Santa Barbara, CA) at least every 10 days pre- and postveraison and every 5 days for a 15-day window around veraison. Measurements were performed on mature, well-exposed leaves. Leaves were covered with a plastic bag, immediately severed at the petiole, and sealed inside the pressure chamber for determination of the balancing pressure. Measurements were taken on three leaves per vine of 3 to 8 LTS and normally developing vines, and were taken between 11:00 and 15:00 hr on days during which the sun was not obscured. Measurements were made on paired LTS and normally developing vines alternately to reduce the effects of changing water potentials during the measurement period.

In 2005, leaf water potential at Oakville was measured by the same method used in 2004. Duplicate leaves per vine were measured. Sixteen vines (eight LTS vines and their paired normally developing vines) were measured at Oakville and 32 vines (16 shrivel and 16 normally

developing) were measured at Sonoma. All measurements were taken between 12:00 and 15:00 hr on days during which the sun was not obscured.

Pathogen testing, Oakville. Dormant cane tissue was collected in 2004 from four vines with a history of BS and their respective paired normally developing vines for PCR testing of the following pathogens: grapevine leafroll-associated viruses 1 through 5, grapevine vitiviruses A, B, and D, grapevine fanleaf virus, tomato ringspot virus, arabis mosaic virus, *Xylella fastidiosa*, grapevine rootstock stem lesion-associated virus, grapevine rupestris stem-pitting-associated virus, and grapevine fleck virus. Testing was performed by Foundation Plant Services (http://fpms.ucdavis.edu/).

Fluorescein diacetate staining, Oakville. A previously published protocol was followed for berry staining (Krasnow et al. 2008). Briefly, berries were sectioned longitudinally between the seeds and stained with a 9.6 µM fluorescein diacetate staining (FDA) solution balanced to the osmolarity of the berry. Berries were stained for 20 min before visualization on a Leica MZ12 stereomicroscope with illumination from a Leica HBO 100 mercury lamp fitted with a Leica GFP Plus Fluor filter (450–490 nm). Photographs were taken with a Leica DC 300F camera attached to the microscope (Leica, Allendale, NJ). Fluorescent areas (presumed to be viable cells) were quantified from the photos using Image J software (http://rsbweb.nih.gov/ij/).

Statistical analysis. Values for berry firmness and chemical components were analyzed by ANOVA (SAS Institute, Cary, NC) as appropriate for the experimental designs. Means comparisons were by Dunnett's test at $p = 0.01$.

Results

Rootstocks of LTS vines, Oakville. Not all rootstocks had vines that regularly displayed BS. Of the 22 LTS vines, eight were grafted onto 420A, six onto 5C, four onto 3309, three onto O39-16, and one onto 110R (Table 1). No LTS vines were on either 1103P or 1616C. Regardless of the year, a majority of the vines actually displaying BS were on 420A and 5C. In 2003 there were 10 symptomatic vines among the 22 LTS vines. In 2004 there were 12 symptomatic vines and in 2005 there were 16.

Incidence and timing of BS and BSN. In the 2004 season, a total of 12 vines exhibited BS symptoms (Table 1), but only on six of the 44 monitored LTS clusters. These symptoms became apparent ~24 Aug (104 DAA), with no new symptoms appearing after this date (data not shown). BSN clusters were not counted in 2004. In the 2005 season, there was a much greater incidence of BS at Oakville. Of the 220 monitored LTS clusters, 69 exhibited BS symptoms. The first visibly symptomatic BS clusters appeared on 2 Sept (112 DAA), and newly symptomatic clusters continued to appear until 16 Sept (126 DAA), after which no new LTS clusters shriveled (data not shown). A total of 16 clusters became symptomatic with BSN during the 2005 season, the first of which appeared 21 Aug (99 DAA) and the last of which appeared 5 Oct (145 DAA). There was no sunburn in the monitored clusters in 2005.

Berry firmness. In 2004 the firmness of both BS and normally developing berries reached a peak just prior to veraison, at which point berry firmness began to decrease (Figure 2A). Overall, BS and normally developing berries had similar firmness early in the season and very late in the season, but BS fruit had lower firmness for the period between 83 and 100 DAA in 2004 and between 112 and 136 DAA in 2005. At essentially every point in the 2005 season, LTS berries were intermediate in firmness between BS and normally developing berries (Figure 2B).

Soluble solids. In both the 2004 and 2005 growing season, the postveraison concentration of soluble solids (Brix) in BS berries was substantially less than in normally developing berries (Figure 3). In both years, the soluble solids of nonshriveled berries from LTS vines was intermediate between BS and normally developing fruit, although this trend was much clearer in the 2005 season. In general, soluble solids for all berries continued to rise throughout the experimental period.

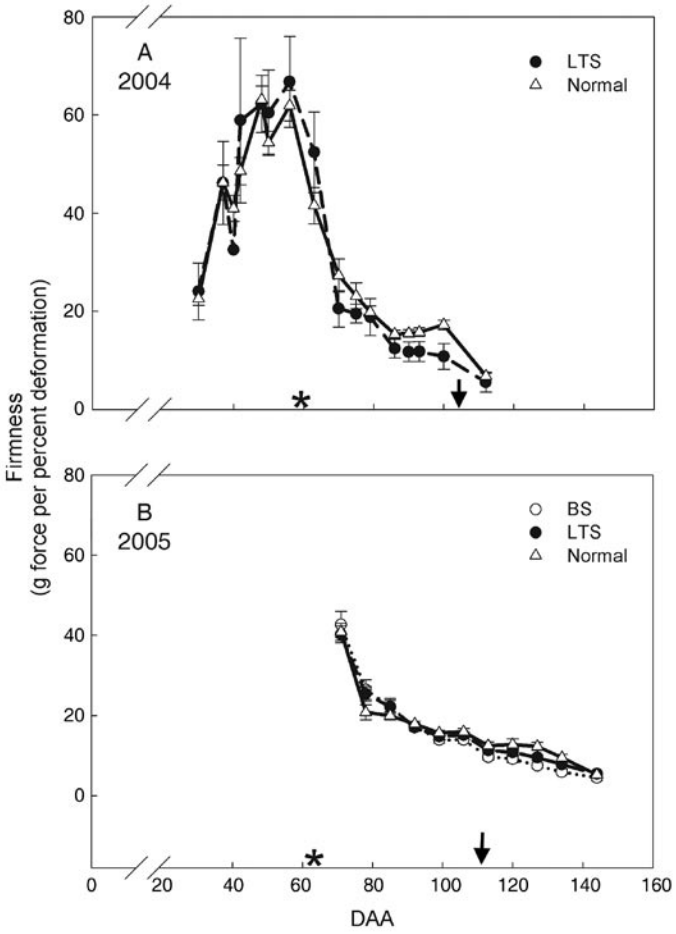

Figure 2 Berry firmness at various days after anthesis (DAA) in 2004 (**A**) and 2005 (**B**). Arrows indicate the date of first visible shrivel symptoms and asterisks indicate the approximate date of veraison. Some error bars are hidden behind the symbols. Data are means ± 95% confidence limits.

Berry weight and water per berry. In 2005 all berries reached a maximum weight at 99 DAA. From 112 DAA on, all berries began to lose weight (Figure 4A), with BS berries losing more weight than LTS or normally developing berries at the point at which the first visible wrinkling and puckering became apparent on BS clusters. BS berries lost weight largely because of water loss (Figure 4B). Water was lost primarily from the flesh, as skin weights were not different between samples (data not shown).

Sugar per berry. In 2005, all berries accumulated sugar during the first part of the season, although BS and nonshriveled berries from LTS vines did so at a slower rate than normally developing berries. The accumulation of sugar ceased in BS berries at ~99 DAA, while normally developing berries continued to accumulate sugar until at least 146 DAA (Figure 4C). In nonshriveled berries from LTS vines, sugar accumulation dramatically slowed at ~99 DAA, even though visible symptoms were not apparent at any time. Cessation of sugar accumulation occurred about two weeks before visible symptoms in BS berries.

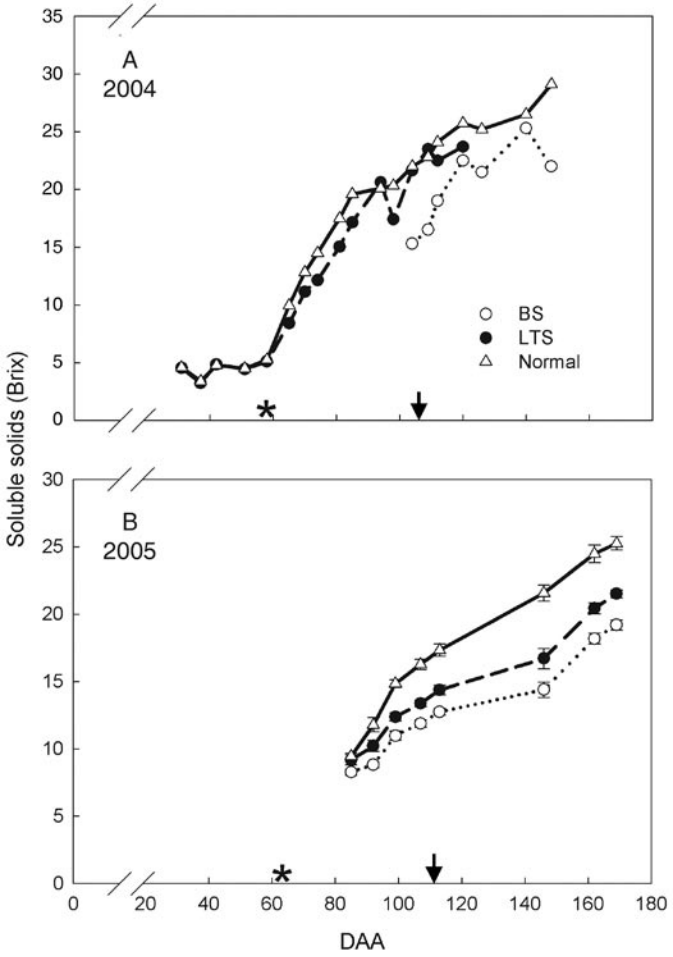

Figure 3 Soluble solids at various DAA for 2004 (**A**) and 2005 (**B**) seasons. Arrows indicate the date of first visible shrivel symptoms and asterisks indicate the approximate date of veraison. Data are means ± 95% confidence limits.

pH, organic acids, and minerals. All berries in 2005 showed the same trend in pH, with an initial steep rise, a lag from 112 to 146 DAA, and another steep rise after that period until harvest (Figure 5A). The pH of juice from BS berries was lower than that of normally developing berries at every point measured. Nonshriveled berries from LTS vines had an intermediate pH between BS and normally developing berries.

Tartrate concentrations per berry were similar for normally developing, LTS, and BS berries throughout the

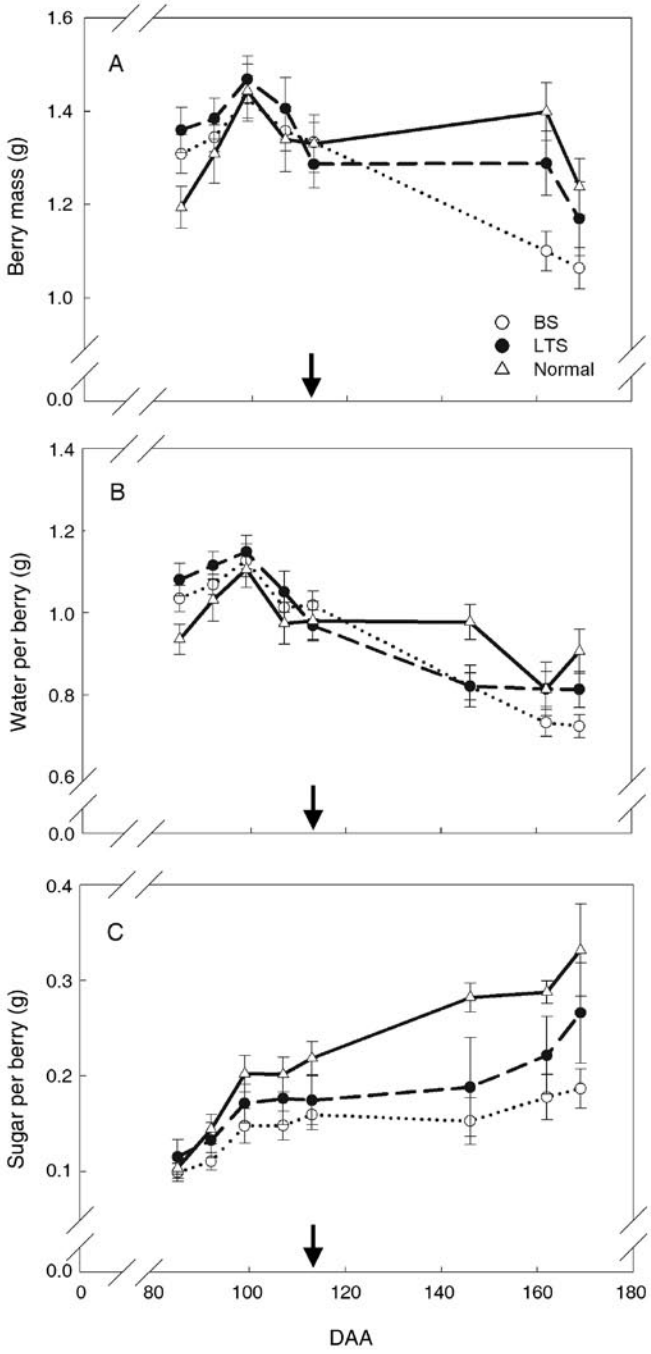

Figure 4 Berry mass (**A**), water per berry (**B**), and sugar per berry (**C**) at various DAA for the 2005 season. Arrows indicate the date of first visible shrivel symptoms. Data are means ± 95% confidence limits.

season and remained somewhat constant at 6 to 10 mg per berry (Figure 5B). At the onset of the experiment, normally developing berries had less malate per berry than BS and nonshriveled berries from LTS vines (Figure 5C). Malate loss occurred at about the same rate in fruit from LTS vines and normally developing berries early in ripening (from 85 to 112 DAA). After this point, malate loss was more rapid in all berries from LTS vines than in normally developing berries, leading to similar malate concentrations at the end of the season. In general, non-

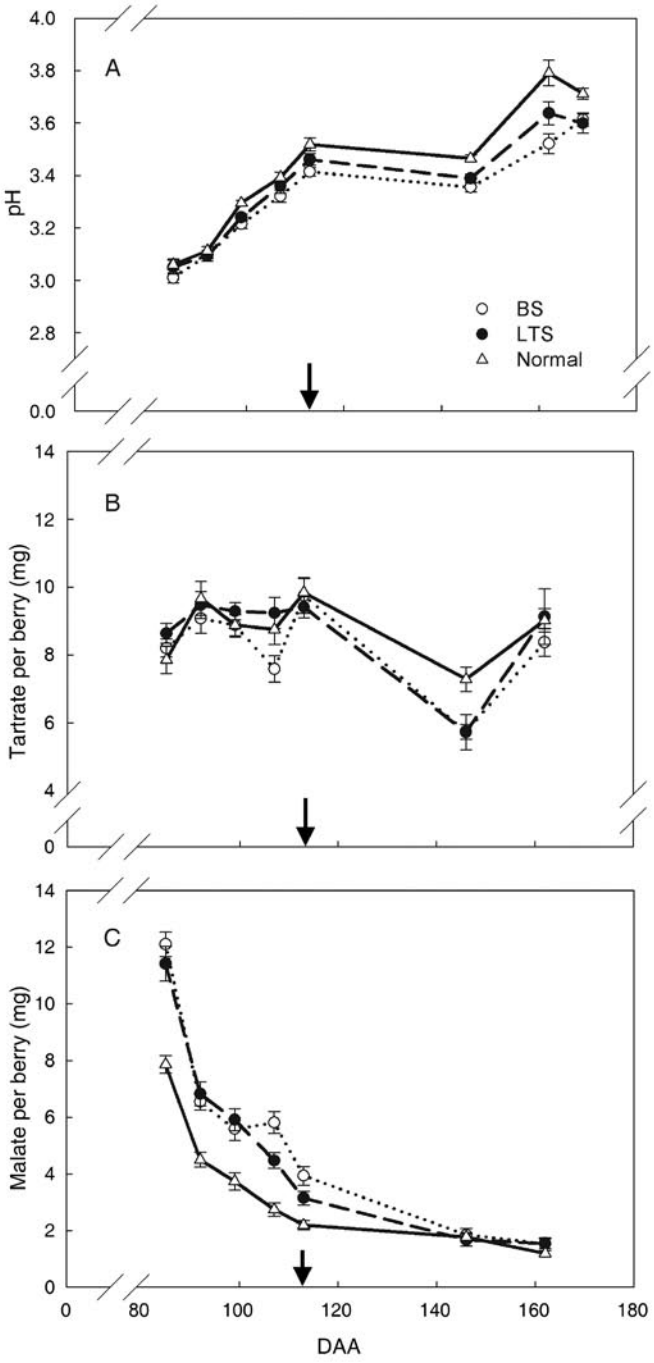

Figure 5 Juice pH (**A**), tartrate per berry (**B**), and malate per berry (**C**) at various DAA in the 2005 season. Arrows indicate the date of first visible shrivel symptoms. Data are means ± 95% confidence limits.

shriveled berries from LTS vines had intermediate levels of malate compared with BS and normally developing berries.

In 2005, there was more calcium in the rachis tissue of normally developing clusters than in that of BS clusters (Table 2). There were no differences in total nitrogen, phosphorous, potassium, boron, sulfur, or magnesium in rachis tissue between BS and normally developing samples. On a per berry basis, there were no differences in total nitrogen, phosphorous, potassium, boron, sulfur, magnesium, or calcium (Table 3).

Skin tannin and total anthocyanin. Skin tannin per berry was similar at the beginning of the experiment (~8 mg/berry) for all berries (Figure 6A). There was little change over the season, but overall, all three categories lost skin tannin on a mg skin tannin per berry basis throughout the season. BS and nonshriveled berries from LTS vines had more skin tannin than normally developing berries late in the season.

Total anthocyanins were higher in normally developing berries than BS berries throughout most of the season (Figure 6B). Nonshriveled berries from LTS vines, beginning at 92 DAA, also had less anthocyanin than normally developing berries until 162 DAA when values of the two categories began to converge again and were not different. At the end of the season (169 DAA), there were no differences in the amount of anthocyanin per berry among any of the categories.

Individual anthocyanins by HPLC. Combined amounts of all nonacylated anthocyanins were higher in normally developing berries than berries from LTS vines from 85 to 146 DAA (Figure 7A). After 146 DAA the values tended to converge, although values for cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside remained higher in normally developing berries than in BS berries (data not shown). Maximal concentrations of nonacylated anthocyanins, with the exception of malvidin-3-glucoside, occurred at 100 to 110 DAA for all berries (data not shown). The concentration of malvidin-3-glucoside continued to increase until day 146 in normally developing and nonshriveled berries on LTS vines and day 162 in BS berries. By 162 DAA the concentration of malvidin-3-glucoside was not different among BS, LTS, and normally developing berries (data not shown).

Combined amounts of acylated anthocyanins showed less difference among treatments (Figure 7B). Concentrations of malvidin-3-acetylglucoside were higher in normally developing berries than in berries from LTS vines from 85 to 113 DAA. After that point there were no differences among the treatments. There were no differences between berries from LTS vines and normally developing berries in the amount of malvidin-3-coumarylglucoside at any point in the season (data not shown).

Water potential. At the Oakville site, BS vines typically had higher (less negative) water potentials than normally developing vines, particularly in 2005 (Figure 8). At the Sonoma site in 2005, there was a very narrow

Table 2 Mineral concentration in rachis tissue from BS and normally developing vines sampled in 2005 (169 DAA). Data are means ± SE (n = 8 for BS, n = 4 for normal); only Ca showed a statistical significance ($p = 0.009$).

	K (%)	P (%)	Total N (%)	Ca (%)	Mg (%)	B (ppm)	S (ppm)
BS rachis	2.85 ± 0.38	0.30 ± 0.023	0.54 ± 0.043	0.49 ± 0.023	0.11 ± 0.013	24.2 ± 2.63	379.4 ± 14.1
Normal rachis	3.16 ± 0.046	0.25 ± 0.0087	0.45 ± 0.026	0.30 ± 0.024	0.07 ± 0.0063	22.0 ± 2.48	365.0 ± 6.45

Table 3 Mineral concentration in berries from BS and normally developing vines sampled in 2005 (169 DAA). Data are means ± SE (n = 8 for BS, n = 4 for normal); no difference was statistically significant.

	K (mg/berry)	P (mg/berry)	Total N (mg/berry)	Ca (mg/berry)	Mg (mg/berry)	B (mg/berry)	S (mg/berry)
BS berry	3.98 ± 0.26	0.42 ± 0.022	1.88 ± 0.18	0.35 ± 0.035	0.17 ± 0.011	0.0074 ± 0.00065	0.18 ± 0.01
Normal berry	4.58 ± 0.15	0.47 ± 0.022	2.15 ± 0.15	0.37 ± 0.031	0.19 ± 0.0037	0.0099 ± 0.0014	0.20 ± 0.014

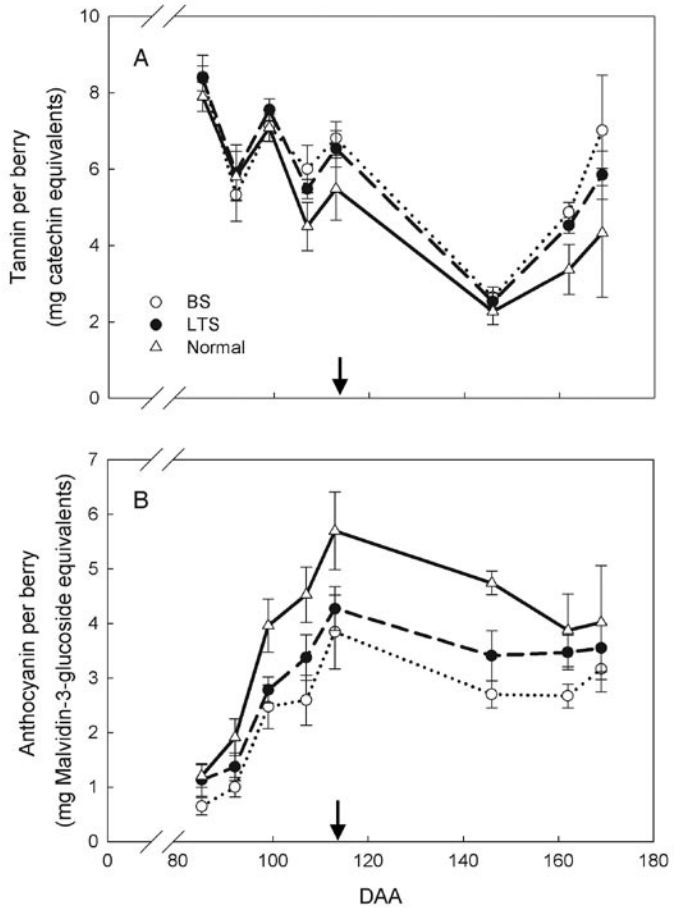

Figure 6 Skin tannin (**A**) and anthocyanin (**B**) per berry in the 2005 season. Arrows indicate the date of first visible shrivel symptoms. Data are means ± 95% confidence limits.

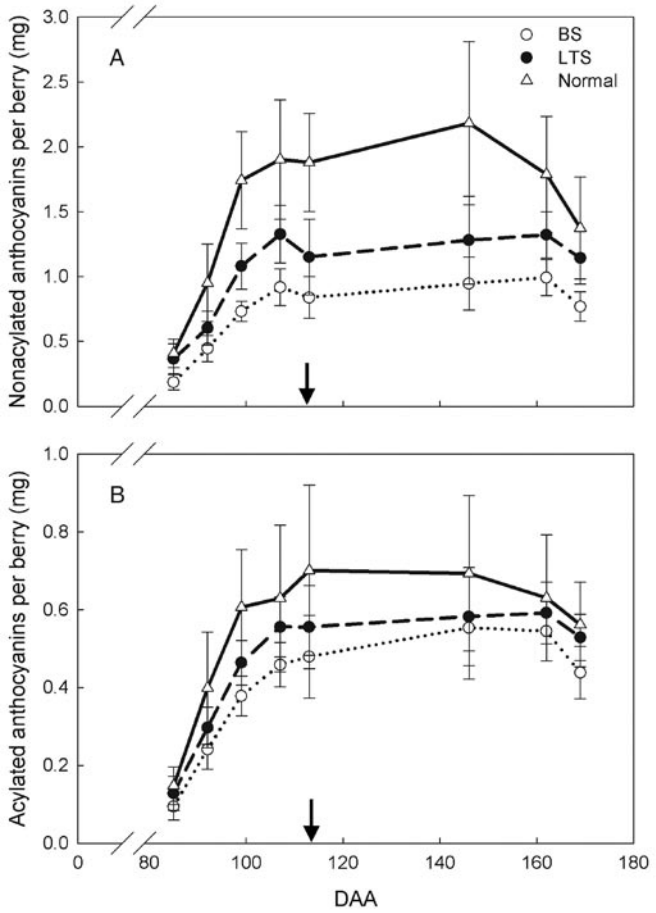

Figure 7 Total nonacylated anthocyanins (**A**) and total acylated anthocyanins (**B**) per berry in the 2005 season. Arrows indicate the date of first visible shrivel symptoms. Data are means ± 95% confidence limits.

range of average water potentials across all treatments, with no statistically significant effect of either BS status or irrigation level (Table 4). Irrigation at Sonoma was calculated as 21.4% of ET_c for the standard irrigation and as 166.7% for supplementally irrigated vines when calculated from the date of the first irrigation (24 June), and 15.9% and 123.6% of ET_c, respectively, when calculated from the date of budburst (1 Apr). The significant interaction of irrigation X BS status (Table 4) indicated that BS vines exhibited a significantly lower LWP compared with nonsymptomatic vines, but only under supplemental irrigation (analysis not shown), in contrast to the generally higher LWP by BS vines at Oakville (Figure 8).

FDA staining of berries. As reported previously (Krasnow et al. 2008), cell viability of BS fruit was high and similar to normally developing berries before shrivel symptoms appeared but was lower than normally developing berries thereafter (Figure 9).

Discussion

These results show that the BS disorder is a whole-vine phenomenon that arrests sugar accumulation during ripening and eventually leads to cell death in the mesocarp. Nonshriveled berries from LTS vines did not exhibit visible symptoms of BS, but many berry parameters evaluated in this fruit, such as firmness, skin phenolics, soluble solids, sugar per berry, pH, and berry size, were intermediate between BS and normally developing fruit. LTS clusters were on vines with BS fruit and on vines that have consistently exhibited BS over several seasons. Thus, the BS phenomenon is a disorder of whole vines and not a disorder of individual clusters as was previously thought.

The considerable evidence that the BS disorder affects an entire vine suggests the possibility of a pathogen causing the disorder. However, there were no obvious outward symptoms of known diseases present on the LTS vines, with the exception of one vine displaying symptoms of Botryosphaeria canker. All LTS vines tested were negative for grapevine leafroll-associated viruses 1, 2, 3, 4, and 5, grapevine vitivirus A, B, and D, grapevine fanleaf virus, tomato ringspot virus, arabis mosaic virus, *Xylella fastidiosa*, and grapevine rootstock stem lesion-associated virus. Both normally developing and LTS vines gave positive results for grapevine *rupestris* stem-pitting-associated virus and grapevine fleck virus. Thus, BS is apparently not due to any of the known pathogens for which there are standard diagnostic tests, although these tests do not rule out other unknown pathogens.

The fact that LTS vines were found on some rootstocks at Oakville and not others may indicate an effect of rootstock on the incidence of BS. Although the total number of vines grafted on a given rootstock are similar in this vineyard, a majority of the LTS vines (14 of the 22) were

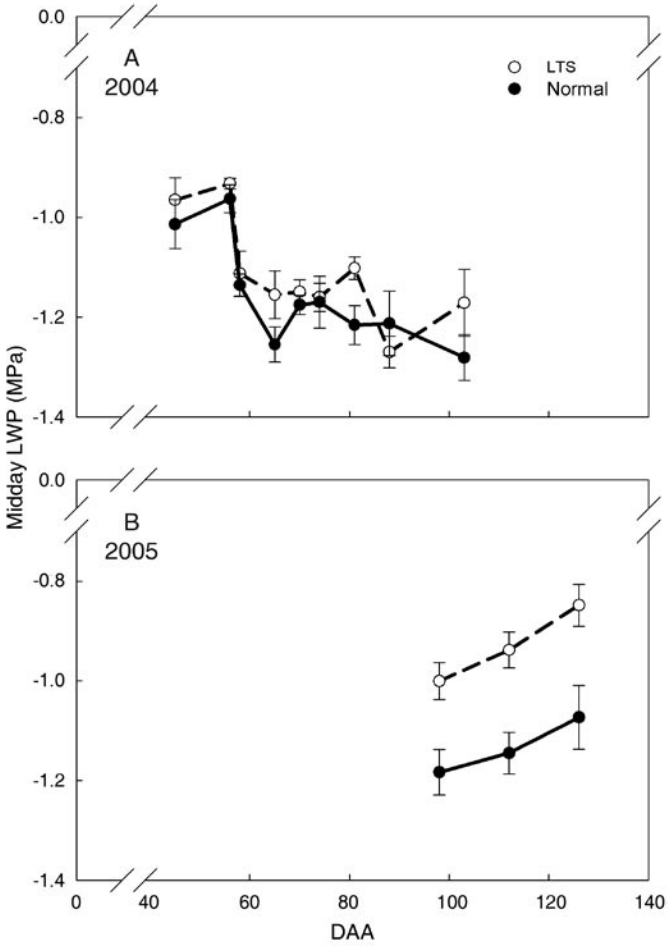

Figure 8 Midday leaf water potential of vines at Oakville at various DAA for the 2004 (**A**) and 2005 (**B**) seasons. Data are means ± SE.

Table 4 Midday leaf water potential and percent of clusters affected with BS or BSN of vines in Sonoma County, California (std = standard; supp = supplementally irrigated vines). Normally developing (N) vines had no incidence of shrivel in the 2004 season; BS vines had at least one shrivel cluster in 2004. Percent BS and BSN were obtained at harvest (30 Sept 2005) from cluster counts. Data are means ± SD (n = 8); *p* values are from a 2 x 2 factorial randomized complete block analysis.

Treatment	Average midday LWP (MPa)	% BS clusters	% BSN clusters
N std	-1.04 ± 0.04	7.6 ± 5.4	0.8 ± 1.3
BS std	-1.07 ± 0.10	8.8 ± 8.0	1.4 ± 2.0
N supp	-0.95 ± 0.09	5.8 ± 8.7	2.6 ± 3.1
BS supp	-1.05 ± 0.10	8.0 ± 10.1	1.5 ± 1.6
Factor		***p* value**	
Irrigation	0.10	0.56	0.15
BS status	0.06	0.47	0.72
Irrigation x BS status	0.03	0.80	0.13

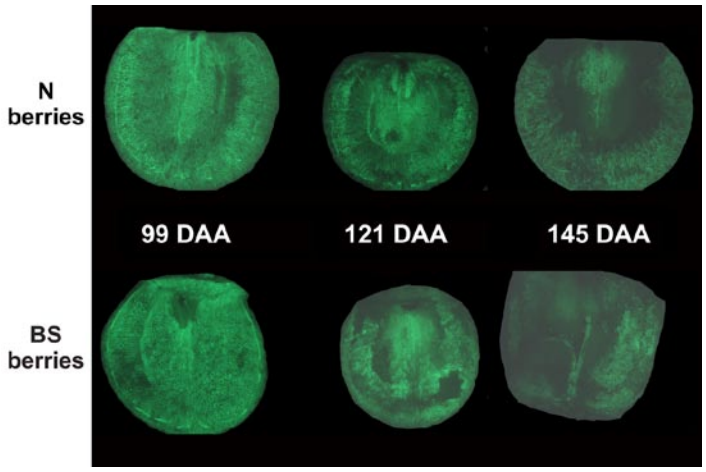

Figure 9 Images of FDA stained normally developing (N) and BS berries from before symptom expression (99 DAA; 97% and 96% viable, respectively), shortly after symptom expression (121 DAA; 90% and 74% viable, respectively), and long after symptom expression (145 DAA; 68% and 49% viable, respectively).

on either 420A or 5C, while none of the vines were on 1103P or 1616C (Table 1). Even among the rootstocks that displayed BS, there were differences in the number of experimental clusters that became symptomatic (Table 1), indicating that rootstock might also affect the severity of BS. Jensen (1970) presented no data on rootstocks but reported that vines on rootstocks, especially the variety Emperor on 1613C, were markedly more prone to BS than own-rooted vines.

Studies using vital stains (Krasnow et al. 2008) have shown a higher degree of cell death in visibly shriveled berries than in nonshriveled berries on the same cluster as well as compared to normally developing berries (Figure 9). Whether cell death leads directly to the visible shriveling of fruit has not been determined, although the death of cells around the vasculature would impair sugar import into the berry, leading to the observed differences in soluble solids.

In addition to differences in mesocarp development, biochemistry, and viability, there was a difference in skin metabolism such that there was less total anthocyanin and more tannin in skin of BS berries than in skin of normally developing ones. There also appeared to be metabolic channeling within the anthocyanin pathway itself, as BS berries had similar amounts of acylated anthocyanins as normally developing berries even though they had less total anthocyanin. Fruit from nonshriveled clusters on LTS vines had anthocyanin and skin tannin that was intermediate between BS and normally developing fruit.

The BS disorder is distinct from BSN in that the rachis remains green and healthy appearing throughout the ripening period (Figure 1), which was until at least 169 DAA at Oakville. No differences in mineral composition between BS and normally developing fruit were found, in contrast to a number of studies reporting differences between BSN and normally developing fruit (Ureta et al. 1981, Christensen and Boggero 1985, Cocucci et al. 1988, Morrison and Iodi 1990, Capps and Wolf 2000). The timing of the appearance of the disorders in 2005 was also different, although it is unknown how typical that difference may be. There was a relatively narrow time window in ripening during which monitored clusters first displayed BS symptoms (112 to 126 DAA), while there was a much wider window for BSN symptom expression in the monitored clusters (99 to 145 DAA).

It is still not clear whether or not the BS symptoms were caused by a pathogen. Previous work has shown that external symptoms appear about the same time as a loss in cell viability is observed (Krasnow et al. 2008), but the physical symptom of shriveling is due to the loss of water from the berries. Visible shriveling occurred at the same time as net water loss began (112 DAA) and two weeks after sugar accumulation ceased in BS berries. Other studies showed that after veraison a majority of the water entering the berry was through the phloem (Greenspan et al. 1994, 1996). Based on the cessation of sugar import into BS berries, it is not unreasonable to assume a disruption of phloem water influx to BS berries. However, the extent of the disruption may be variable and occur over many days, because there are several indications of an incomplete disruption. The net loss in water from BS berries in 2005 was ~4 mg/day per berry from 92 to 162 DAA, whereas transpirational water loss from postveraison Cabernet Sauvignon has been reported as ~110 mg/day per berry (Greenspan et al. 1996). Potassium, a phloem mobile element, was not different between BS and normally developing berries, although sugar accumulation was significantly less in BS fruit, suggesting that there is not a wholesale disruption of phloem flow to BS berries, but that sugar accumulation specifically is affected more than potassium and water uptake. It may be that all compositional and physical symptoms of BS are the consequence of slowing or cessation of phloem sugar and/or water import into berries, although this possibility has not been investigated. Regardless of the underlying cause of the abnormal development of berries from LTS vines, their composition (low sugar, low pH, and low color) makes them undesirable for winemaking, and they are usually removed from the vineyard before harvest or from the sorting table in the winery before crushing.

In addition to the altered composition of berries from LTS vines, another difference at Oakville was the trend for LTS vines to have higher water status than normally developing vines. This trend was consistent for both 2004 and 2005 (Figure 8). It is unknown why the site in the Alexander Valley (Sonoma County) displaying high BS incidence did not show a similar trend (Table 4). It must be noted that at the Sonoma site, unlike Oakville, vines that showed BS symptoms in one year did not necessarily show symptoms the previous year or the next year. It is possible that all the vines at the Sonoma site were "LTS" vines, with the potential to show BS in any given year, and there were no "normally developing" vines for comparison. There is also the possibility that there are multiple causes of the symptoms associated with BS.

Supplemental irrigation had only small effects on the water potential at the Sonoma site, especially considering the standard irrigation regime in the vineyard was ~20% ET_c. It is surprising that vines receiving this little water had relatively high values for water potential throughout the season. As the vineyard is not far from a river, these vines may have had access to a water table, which would explain the small effect of supplemental irrigation. In any case, the irrigation treatments did not affect the percent of clusters with BS or BSN at the end of the season (Table 4).

Conclusion

The BS disorder leads to many changes in fruit composition in addition to visible shriveling. Fruit from BS clusters had increased calcium concentrations in the rachis tissue, lower berry weights, and water per berry late in the season, less anthocyanin in the skins, and lower juice pH than normally developing fruit. Moreover, symptoms

of BS were present in a less severe form in all clusters of vines that had BS clusters (LTS clusters), indicating that it is a whole vine rather than a berry or a cluster phenomenon. The most consistent and clear consequence of BS is sugar accumulation that is slowed and ceases early in the season. Given that there are several possible causes of shriveling fruit, that the disorder is present in unshriveled berries on affected vines, and that the most consistent and clear effect of the disorder is reduced sugar accumulation, we suggest that the name "sugar accumulation disorder" (SAD) be assigned to this disorder which is identified by these symptoms. Future work should focus on finding a causal agent and attempting to determine the biochemical sequence of events leading to the altered composition and outward appearance of BS/SAD fruit in order to eliminate or mitigate its effects.

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