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Influence of Plant Water Status on the Production of C_{13}-Norisoprenoid Precursors in *Vitis vinifera* L. Cv. Cabernet Sauvignon Grape Berries

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The influence of irrigation strategy on grape berry carotenoids and C_{13}-norisoprenoid precursors was investigated for *Vitis vinifera* L. cv. Cabernet Sauvignon. Two irrigation treatments were compared, one in which vines received reduced irrigation applied alternately to either side of the vine (partial rootzone drying, PRD) and a second control treatment in which water was applied to both sides of the vine. Over the two years of the experiments, PRD vines received on average 66% of the water applied to the controls. Initially, the PRD treatment did not alter midday leaf (ψ_L) and stem (ψ_S) water potential relative to the control, but decreased stomatal conductance (gs). Continued exposure to the PRD treatment resulted in treated grapevines experiencing hydraulic water deficit relative to the control treatment and induced lowered midday ψ_L and ψ_S, which was also reflected in decreased berry weight at harvest. In both irrigation treatments, the most abundant grape berry carotenoids, β-carotene and lutein, followed the developmental pattern typical of other grape varieties, decreasing post-veraison. At certain points in time, as the fruit approached maturity, the concentration of these carotenoids was increased in fruit of PRD-treated vines relative to the controls. This effect was greater for lutein than for β-carotene. PRD consistently caused increases in the concentration of hydrolytically released C_{13}-norisoprenoids β-damascenone, β-ionone, and 1,1,6-trimethyl-1,2-dihydronaphthalene in fruit at harvest (24 °Brix) over two seasons. The effect of the PRD treatment on the concentration of hydrolytically released C_{13}-norisoprenoids was greater in the second of the two seasons of the experiment and was also reflected in an increase in total C_{13}-norisoprenoid content per berry. This suggests that the increases in the concentration of the C_{13}-norisoprenoids in response to PRD were independent of water deficit induced changes in berry size and were not the result of an altered berry surface area to volume ratio.

KEYWORDS: Grape; berry; *Vitis vinifera*; PRD; partial rootzone drying; water deficit; carotenoid; β-carotene; lutein; C_{13}-norisoprenoid; β-damascenone; β-ionone; 1,1,6-trimethyl-1,2-dihydronaphthalene; TDN

INTRODUCTION

Fruit-derived C_{13}-norisoprenoids are important odorants in wines and are thought to originate from carotenoid precursors in grapes (*1*−*5*). The biosynthetic formation of glycosidically bound C_{13}-norisoprenoids in grape berries is proposed to follow a stepwise process of enzymatically mediated oxidation followed by glycosylation (*5*−*7*). Until recently, this was speculative, but increasing evidence suggests that the region-specific cleavage of carotenoids yields characteristic C_{13}-norisoprenoids, thereby reducing the likelihood of their formation by degradation through the action of oxidases and/or chemical and photochemical means (*5*, *7*, *8*). The identification and characterization of a specific carotenoid cleavage dioxygenase gene in grape berries yielding a C_{13}-norisoprenoid compound has strengthened this school of thought (*7*). The induction of this gene occurs at veraison, which coincides with the characteristic sharp decrease in total carotenoid content and concomitant increase in the formation of C_{13}-norisoprenoid precursors at this stage of berry development (*7*, *9*−*13*).

Applied research on grape C_{13}-norisoprenoids has attempted to link environmental conditions with the metabolism of carotenoids and their products (*5*). Evidence has been put forward that increased incidence of sunlight on developing grape bunches mediates the accelerated decrease of carotenoids after veraison (*5*, *11*, *14*, *15*). In particular, increased UV exposure

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has been associated with a reduction in carotenoid content of berry skins at maturity, due to their accelerated decline post-veraison rather than increased synthesis in green berries pre-veraison (*16*). In the case of C_{13}-norisoprenoids, full sunlight can increase the concentration of a range of these compounds (16−36%) in berries, compared with shaded conditions (90% shade) (*5*, *17*). However, comparison of naturally shaded and sun-exposed bunches showed that the accumulation of certain C_{13}-norisoprenoids is more strongly affected by sunlight than others, namely, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) being increased up to 52%, whereas β-damascenone concentration has been shown to be unaffected by sun exposure (*18*).

In grapevines, water stress can indirectly affect the light environment of developing fruit, through a reduction in shoot growth rate and vine leaf area (*19*). This may therefore lead to an increase in sunlight penetration to the fruiting zone of the canopy, which in itself may have the potential to influence the metabolism of carotenoids and thus precursors to the C_{13}-norisoprenoids. However, due to the close relationship of the metabolic pathways for carotenoids (*20*) and stress-related plant hormones such as abscisic acid (ABA) (*21*, *22*), it is conceivable that there may also be a direct effect of water stress on the metabolism of carotenoids and C_{13}-norisoprenoids. A nonirrigated treatment was shown to increase the berry-derived carotenoids lutein, β-carotene, neoxanthin, violaxanthin, and luteoxanthin up to 60% in comparison with an irrigated treatment, but only when the soil had a low water-holding capacity (*23*). This phenomenon indicates the potential for a physiological response elicited within the root system that necessitates sufficient drying of the soil. The conditions required to mediate such a response are similar to those described for partial rootzone drying (PRD) (*24*−*27*). The PRD technique applies irrigation water alternately to each half of the rootzone, typically in cycles of 7−14 days (*22*, *25*−*29*). As such, part of the root system is maintained in drying soil, which causes sustained transmission of nonhydraulic signals such as ABA in the transpiration stream (*25*). This leads to a reduction in canopy growth and stomatal conductance, with potential effects on fruit development (*22*, *25*−*29*). The potential therefore exists that water deficit can result in increases in carotenoid content (*23*), but as yet there are no reported data on the production of C_{13}-norisoprenoids under these conditions.

Using the PRD technique, the current study aimed to explore the effect of water deficit on the most abundant carotenoids in *Vitis vinifera* L. cv. Cabernet Sauvignon fruit, namely, β-carotene and lutein, as well as its potential effect on hydrolytically derived C_{13}-norisoprenoids in grape berries. Interpretation of the results are made with reference to changes in grapevine water status and light penetration to the bunch zone in response to the irrigation treatment.

MATERIALS AND METHODS

Field Experiments. The field experiment was carried out over two seasons at a commercial site managed by Orlando-Wyndham, Langhorne Creek, South Australia. The "A" horizon of the soil at this site was a loamy sand up to approximately 60 cm depth with a "B" horizon with a higher clay content, either a clay loam or a medium clay. The experiment compared PRD and control irrigation treatments on 5-year-old Cabernet Sauvignon vines pruned to 60 nodes, with sprawling canopies. The experimental design was set up as three irrigation replicates of randomly positioned rows within the vineyard, within which were contained seven sets of two-vine subplots. The row and vine spacing were 2.4 and 1.8 m, respectively. PRD irrigation was applied to separate halves of the vine's root system using intermittent irrigations from two 2 L/h drippers/vine, positioned 30 cm from the vine trunk, for 7-day cycle periods. The control treatment received water to both sides of the vine's root system at any time throughout the season. In 2001/2002, the control and PRD treatments received 1.19 and 0.84 ML/ha respectively. In 2002/2003, the control and PRD treatments received 1.37 and 0.85 ML/ha, respectively. Fruit was collected at 23−24 °Brix, and 50-berry samples were weighed and then frozen at −20 °C for later analysis. A measure of photosynthetically active radiation (PAR) within the fruiting zone of PRD- and control-treated vines was made at both veraison and harvest using a ceptometer (model SF-80, Decagon Devices, Cambridge, U.K.) positioned at five different angles within the fruiting zone along the planting line. Stomatal conductance of leaves was determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, U.K.).

Measures of Plant Water Status. A restricted sample size was used to determine stomatal conductance (gs) ($n = 6$, 14) and midday leaf (Ψ_L) and stem (Ψ_S) water potentials ($n = 28$) to give a reference measure of plant water status in response to the treatments. This was due to time constraints on the period during the day during which readings could be taken. Diurnal measurements of stomatal conductance (gs) were taken at two stages of a single growing season (2001/2002), termed "early" and "advanced", corresponding to pre- and post-veraison, respectively. Both readings were taken on days corresponding to the end of the 7-day PRD cycle period, that is, when the soil on the "dry" side of the PRD vines was at its driest prior to switching of the "dry" and "wet" sides for the following irrigation cycle. For the duration of the study there was excessive cloud coverage in the mornings, allowing only midday readings (solar noon) of gs, which were taken at regular intervals throughout the season. For all measures of gs, six reference leaves were randomly selected per subplot. Selected leaves were sun-exposed leaves of similar maturity, approximately the fifth leaf from the shoot apex. The terminal part of the main lobe was placed into the cup on the head unit, which was positioned normal to the sun. The porometer was calibrated prior to each use and was recalibrated within the daily period subject to changes in environmental conditions, for example, relative humidity or temperature. Leaf (Ψ_L) and stem (Ψ_S) water potentials were measured with a manual pump-up pressure chamber (PMS Instrument Co). Measurements were made at solar noon on leaves of maturity similar to those selected for gas exchange measurements. For the measurement of stem water potential, clear plastic bags were placed over two selected leaves per treatment replicate at 9:00 a.m., followed by a second opaque bag. The opaque bags were constructed from plastic that was black on the interior and white on the exterior to prevent light penetration to the leaf and minimize leaf heating. These leaves were left to equilibrate until readings were taken at midday. Selected leaves were detached from the shoot by cutting through the base of the petiole, and for the Ψ_L measurements an additional two leaves per treatment replicate were immediately transferred to a plastic bag and measured within 1 min. Water potential pressure readings were recorded when sap was first observed to exude from the cut end of the petiole.

Extraction and HPLC Analysis of Carotenoids. Twenty-five berries taken from frozen berry samples of each treatment replicate were defrosted over 30 min and immediately homogenized using an Ultra-Turrax T 25 (IKA Labortechnik, Staufen, Germany), ensuring that both seeds and flesh were completely crushed. The extraction procedure for carotenoids was adapted from the methods of refs *30* and *31*. A 1 g sample of grape homogenate was extracted in 10 mL of extraction solvent (hexanee/acetone/ethanol, 50:25:25, v/v) in the dark for 1 h on an orbital shaker. The extracts were centrifuged for 5 min at 8000 rpm, and the hexane layer was removed using a syringe. Recovery of carotenoids in the hexane fraction was tested using samples spiked with pure β-carotene (Sigma-Aldrich) and was 96%. The hexane extracts were then spiked with a known amount of β-apo-8′-carotenal (Fluka Biochemica) as an internal standard and dried in a Savant Speed Vac Plus at ambient temperature. The dried extracts were resuspended in 1 mL of diethyl ether, to which was then added 1 mL of 5% methanolic KOH. Saponification was carried out over an 18 h period in the dark at 4 °C. Following saponification, an additional 1 mL of diethyl ether was added to the extract, which was then transferred to a 10 mL centrifuge tube. The methanol−KOH fraction was removed with two successive 3 mL water washes. The water fractions were removed

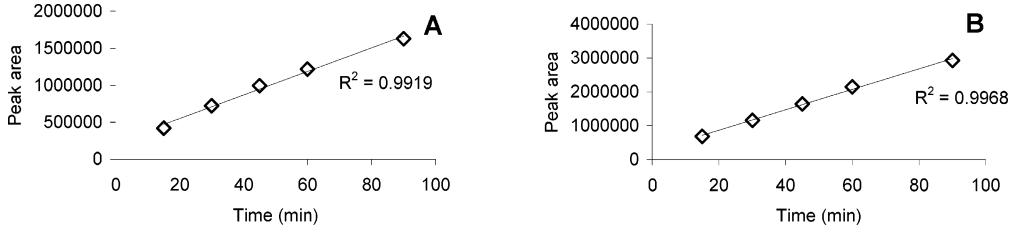

Figure 1. Equilibration of (**A**) β-damascenone and (**B**) β-ionone in the headspace during 90 min at 40 °C from a 25 ng/mL solution in water.

with a syringe, and the final diethyl ether fraction was dried in a fume hood under a nitrogen stream. The dried samples were redissolved in 250 μL of HPLC-grade acetone and centrifuged at 10000*g* for 5 min. The samples were transferred to HPLC vials, and 100 μL was injected onto a LiChrosopher 100 RP-18 (5 μm) 4 × 250 mm HPLC column with photodiode array detection at 450 nm. The HPLC system used was a Hewlett-Packard HP 1100. The method was a ramped gradient of acetone/water: 70% acetone to 100% acetone from 0 to 25 min. The flow was maintained at 100% acetone from 25 to 35 min and returned to 70% acetone at 36 min. The final run time was 38 min. The flow rate was maintained at 1 mL/min, and the column temperature was 40 °C. Increased resolution of carotenoid peaks was observed at this column temperature and was not accompanied by degradation or isomerization of carotenoids. The elution times of lutein and β-carotene standards (Sigma-Aldrich) were 13.34 and 24.52 min, respectively. Carotenoids were quantified according to a standard curve of β-apo-8′-carotenal, which had a linear response within the range of concentrations injected onto the column (0−10 μg), giving an R^2 value of 0.9994.

Direct SPME Sampling of Hydrolytically Released C_{13}-Norisoprenoids from Berry Homogenates. For analysis of grape and wine C_{13}-norisoprenoids, a solid-phase microextraction (SPME) technique was developed. A 2 g sample of berry homogenate was placed in a 10 mL glass vial. Berry homogenates were diluted with 500 μL of deionized water to ensure a suspension of the homogenate formed and manually adjusted to pH 1. Deuterated analogues of C_{13}-norisoprenoids were obtained from the Australian Wine Research Institute (AWRI), including [$^2H_4$]β-damascenone and [$^2H_3$]β-ionone as internal standards for β-damascenone and β-ionone, respectively (*32*), and d_8-naphthalene (Sigma-Aldrich Pty. Ltd.) was used as an internal standard for TDN. To grape samples was added 10 μL of a 5 μg/mL solution of deuterated standard mix, and the samples were sealed with a PTFE/silicone septum (Supelco). Sampling vials were then transferred to a heating block for 1 h at 100 °C and then transferred to 40 °C. SPME sampling was then performed manually using a 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Supelco). The fiber was exposed at a constant level above the sample surface for 1 h. SPME-GC-MS analysis was performed using a Hewlett-Packard HP6890 gas chromatograph fitted with a 30 m fused silica SGE BP20 column (0.25 mm i.d. and 0.25 μm film thickness). The SPME fiber containing adsorbed headspace volatiles was manually transferred to the GC injection port for 1 min. The splitless/split injection port was heated to 220 °C. Ultrahigh-purity helium was used as a carrier gas at a constant flow rate of 1 mL/min, with a column head pressure of 6.98 psi. The temperature program was initially 40 °C for 4 min and then increased to 240 °C at a rate of 12 °C/min. The temperature was held at 240 °C for 5 min, giving a total run time of 25.67 min. The GC instrument was coupled to a Hewlett-Packard HP5972 mass selective detector. For quantitation of C_{13}-norisoprenoids using selective ion monitoring, *m*/*z* 69 was used for β-damascenone, *m*/*z* 73 for [$^2H_4$]β-damascenone, *m*/*z* 177 for β-ionone, *m*/*z* 180 for [$^2H_3$]β-ionone, *m*/*z* 136 for TDN, and *m*/*z* 157 for d_8-naphthalene.

Statistical Analysis. The experimental design was three spatially distinct irrigation treatment replicates (PRD and control), each with seven subplots of two vines for which data were collected. One-way ANOVA was used to compare treatment differences for all analyses ($n = 42$), apart from the reference measures of gas exchange and plant water status, which used a restricted sample size and for which Student's *t* test was used. The Genstat 6 software package was used.

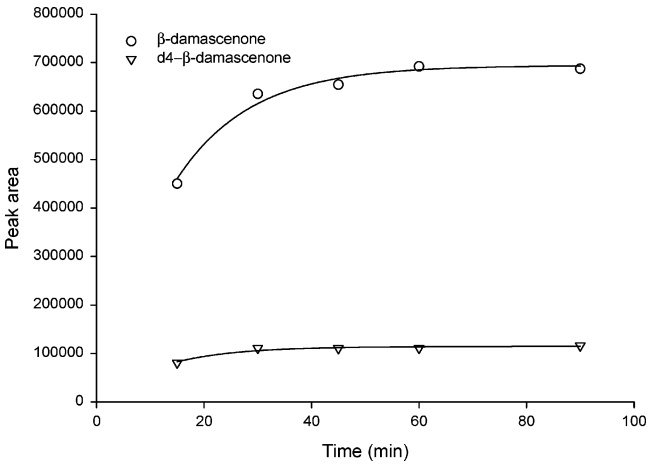

Figure 2. Influence of the time of acid hydrolysis on the release of free β-damascenone in the presence of [$^2H_4$]β-damascenone from a berry homogenate over 90 min at 100 °C and pH 1.

RESULTS AND DISCUSSION

Critique of the Direct SPME Sampling Technique. Three different SPME fibers were tested, with the stationary phases 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 100 μm nonbonded polydimethylsiloxane (PDMS), and 65 μm carbowax/divinylbenzene (CW/DVB), all from Supelco. In the case of the standards used in this study, the PDMS/DVB coating on the fiber had the maximum affinity when the peak areas of the bound compounds were compared (results not shown). The adsorption for β-damascenone, β-ionone, and TDN to the PDMS/DVB fiber was assessed at 40 °C over 90 min (**Figure 1**). The volatiles did not reach equilibrium during this period, and the binding response was essentially linear. Given this, the standard adsorption time would not be critical, so long as it fell within this linear range. This is possible because the internal standards were of known concentration. Thus, a standard adsorption time of 60 min was selected. For this 60 min adsorption time, standard curves of the standards in water were constructed for the approximate range of concentrations to be found in grapes (results not shown). In the case of each standard, a linear relationship was observed for the range of dilutions analyzed in water, where $R^2 > 0.99$. Reproducibility of the recovery of each compound was compared for four sample replicates, and the error was found to be <5% for all of the standards tested. Hydrolytic release of the three C_{13}-norisoprenoids was tested when homogenates were adjusted to different pH values and temperatures (results not shown). Maximal release of all compounds was at 100 °C, which is the same temperature previously described for hydrolytic release of glycosylated β-damascenone (*33*). The pH of hydrolysis used is lower than that previously described (*33*), 1 as compared with 2.2, but this pH was found to be optimal for the release of TDN in particular. The stability of the deuterated standards under these conditions indicated that no degradation of the volatiles would

Table 1. Effect of PRD on Measures of Plant Water Status (Ψ_S and Ψ_L) and Gas Exchange Measured as Stomatal Conductance (gs) in Cabernet Sauvignon Vines during Irrigation Cycles of the 2003 Growing Season (A, ANOVA, $n = 28$; B, Student's t test; $n = 14$)[a]

irrigation stage	cycle no.	date of analysis	plant water status meaurements[a] (A) (–Bar) Ψ_S control	Ψ_S PRD	Ψ_L control	Ψ_L PRD	P	gas exchange measurements[a] (B) (mmol·m^{-2} s^{-1}) gs control	gs PRD	P
end cycle	I	Jan 7, 2003	11.5 a	12.2 a	14.3 b	13.8 b	<0.001	229.6 a	181.5 b	<0.05
switch	I	Jan 9, 2003	7.7 a	8.6 b	10.5 c	10.9 c	<0.001	304.4 a	276.6 b	<0.05
end cycle	II	Jan 14, 2003	11.1 a	10.5 a	13.1 b	13.0 b	<0.001	308.6 a	334.2 a	ns[b]
switch	III	Jan 21, 2003	7.4 a	7.2 a	9.6 b	9.9 b	<0.001	294.3 a	288.7 a	ns
end cycle	IV	Jan 28, 2003	11.7 a	14.1 b	14.8 b	15.7 c	<0.001	135.7 a	77.9 b	<0.001
end cycle	V	Feb 3, 2003	12.0 a	14.0 b	14.9 b	16.3 c	<0.001	229.8 a	154.2 b	<0.01
switch	V	Feb 5, 2003	9.3 ab	8.9 a	10.9 c	10.0 b	<0.001	nd[c]	nd	nd

[a] Differences in the letters a–c indicate significant differences. [b] ns, not significant. [c] nd, not determined.

Table 2. Berry Weight, Carotenoid and C_{13}-Norisoprenoid Concentration in PRD-Treated and Standard-Irrigated Cabernet Sauvignon for the 2001/2002 and 2002/2003 Seasons[a]

component	unit	2001/2002 control	PRD	% PRD> control	P	2002/2003 control	PRD	% PRD> control	P
berry weight	g	0.94	0.84	–12.0	<0.001	0.87	0.79	–11	<0.05
lutein	μg/g	0.76	0.84	10.0	<0.01	0.70	0.76	8.1	ns[b]
	μg/berry	0.72	0.72	–0.4	ns	0.64	0.62	–2.4	ns
β-carotene	μg/g	1.54	1.78	13.5	<0.01	1.58	1.73	8.4	<0.05
	μg/berry	1.46	1.54	5.3	ns	1.44	1.41	–2.7	ns
β-damascenone	ng/g	30.44	34.94	12.9	<0.05	34.80	45.60	23.7	<0.01
	ng/berry	28.17	30.30	7.0	ns	31.20	37.40	16.6	<0.05
β-ionone	ng/g	17.11	19.53	12.4	ns	12.57	15.51	19.0	<0.05
	ng/berry	15.99	17.03	6.1	ns	11.35	12.70	10.6	ns
TDN	ng/g	5.90	7.46	20.9	<0.05	4.19	6.25	33.0	<0.01
	ng/berry	5.48	6.44	14.9	ns	3.69	5.11	27.8	<0.05

[a] ANOVA; $n = 42$. [b] ns, not significant.

have potentially occurred during hydrolysis (**Figure 2**). Release of C_{13}-norisoprenoids from grape samples occurred maximally after 60 min and remained constant to 90 min (**Figure 2**). A potential shortfall of the technique is the assumption that under the conditions of the experiment that there was no de novo synthesis of C_{13}-norisoprenoids from carotenoids through oxidative degradation, and if so, that it would be minor. This was tested through preparation of a sample with nitrogen passed through the homogenate and headspace to remove oxygen. Compared to the amount released from a standard sample preparation in air (78% nitrogen, 21% oxygen) there was not a significant increase in the relative levels of norisoprenoids formed. The release of small amounts of β-ionone have been reported from oxidative, thermal, and/or hydrolytic degradation of β-carotene (*34*, *35*). The hydrolysis products of 20 μL of a 100 μg/mL solution of pure β-carotene in hexane (Sigma-Aldrich) suspended in 2 mL of water were investigated under the conditions of this experiment; 0.09 ng of β-ionone was formed. For the purposes of the current study, it was concluded that this amount would be negligible relative to the levels of β-carotene and β-ionone observed (**Table 2**), and the assumption is therefore made that the reported levels of C_{13}-norisoprenoids are derived from existing aglycones or hydrolysis of glycosylated precursors alone.

Plant Water Status. The diurnal pattern of gs was as expected for grapevines (*36*, *37*), with the highest gs measures recorded in the morning, dropping to low levels at midday, and then increasing again toward the late afternoon (**Figure 3**). Because the readings were taken at the end of the 7-day PRD cycle, it would be expected that at this stage the differences in gs between PRD and control treatments would be greatest (*37*). For both the early and advanced stages, PRD significantly reduced gs at certain times of the day (**Figure 3**). At the advanced stage of the season, the midday gs levels were lower than those recorded during the early part of the season for both the PRD and control treatments. Ambient temperature was 6 °C higher on the second sampling date (data not shown).

In the second season of the study (2002/2003), midday gs measurements were compared to midday measures of Ψ_L and Ψ_S to assess the effects of the treatment on plant water status. The use of a midday Ψ_S rather than a predawn measure of Ψ_L was selected on the basis of the premise that this is a more sensitive indicator of water deficit in grapevines (*38*). The readings were taken for four consecutive PRD cycles within the irrigation period between November 5, 2002 and March 12, 2003, termed cycle I to cycle V for discussion purposes (**Table 1**). In cycle I, midday gs was reduced by the PRD treatment, with no significant effect on either Ψ_L or Ψ_S (**Table 1**). This potentially indicates that a nonhydraulic signal was operating to bring about the observed reduction in gs, independent of plant water status for cycle I, which is an expected response using the PRD irrigation technique (*19*, *26*, *29*). However, later measures of gs, Ψ_L and Ψ_S taken at the end of cycles IV and V, indicated that the PRD vines had begun to experience a water deficit relative to the control vines, suggesting that changes in gs were in response to a hydraulically mediated effect (**Table 1**) (*25*, *26*). This was evident from reduced midday gs readings, which corresponded to significantly more negative measures of both Ψ_L and Ψ_S for PRD. At both stages, Ψ_S was more sensitive than Ψ_L to changes in soil water status, showing greater differences between the PRD and control treatments. Measurements were also taken during the period of the PRD cycle, termed the "switch" (**Table 1**), when the irrigation lines, and thus the sides of the root system under irrigation, were alternated.

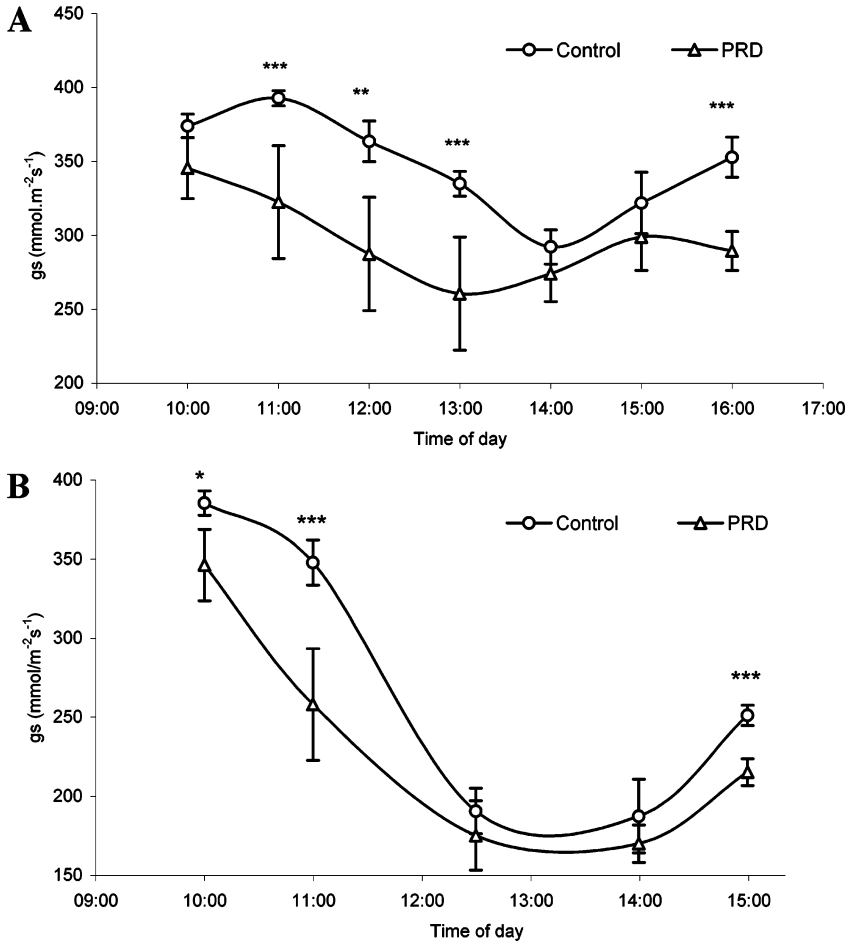

Figure 3. Effect of PRD on diurnal changes in gas exchange measured as stomatal conductance (gs) in Cabernet Sauvignon vines at two stages of the growing season: (**A**) early, Jan 31, 2002; (**B**) advanced, Feb 24, 2002. (Student's *t* test; **A**, $n = 36$; **B**, $n = 30$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.)

This theoretically represents a period when both sides of the vine would have been wet, and measures of vine water status in PRD-treated vines should not have differed significantly from those observed for the control treatment. During the switch period, stomatal conductance was higher than at other stages of the irrigation cycle, and measures of Ψ_L and Ψ_S were less negative. Generally, there was no observed effect of the PRD treatment on gs or plant water status during the switch period. Some small changes in these measures were observed in some instances, but these were minor and most likely reflect differences in equilibration of the soil water profile between treatments, following irrigation.

Berry Carotenoids and C_{13}-Norisoprenoid Precursors. In both seasons, berry weights compared at similar °Brix values were significantly decreased by PRD at harvest (**Table 2**). The reduction in berry weight in the current data suggests that water stress was induced by the PRD treatment, which is consistent with other studies in which a reduction in berry weight occurs due to restriction of pericarp expansion post-veraison, under conditions of water deficit (*39*−*46*). Early studies with PRD showed no change in berry size at harvest when predawn Ψ_L remained constant relative to fully irrigated controls (*24*, *37*, *47*). However, later studies using the PRD irrigation technique have shown a significant reduction in berry size from treated vines relative to fully irrigated vines, either when the treatment was associated with a reduction in predawn Ψ_L (*48*) or when there was no significant change in predawn Ψ_L caused by the irrigation treatment (*22*, *29*). The current data are therefore consistent with that from other studies using this technique, and it cannot be directly inferred that the reduction in berry weight is due to a hydraulically mediated response alone, although it remains a strong likelihood.

At harvest, the concentration of the grape carotenoid β-carotene, measured per gram of berry homogenate, was significantly higher in PRD-treated berries for both the 2001/2002 and 2002/2003 seasons (**Table 2**). For the carotenoid lutein, mature PRD-treated berries had a higher concentration in 2001/2002 only (**Table 2**). For both seasons, the changes in the concentration of lutein and β-carotene were not reflected in changes per berry (**Table 2**), due to the decrease in berry weight in response to PRD. The concentrations of the carotenoids lutein and β-carotene were determined at five different stages of development from approximately 15−16 °Brix until harvest at 23.5 °Brix in 2002/2003 (**Figure 4**). In that season, both carotenoids showed the expected decline in concentration as the fruit matured (*9*, *49*, *50*), and PRD-treated berries had a higher concentration throughout development, although this was not statistically significant on every occasion (**Figure 4**). However, significant differences did occur during the later stages of development for both carotenoid types, which is the period of C_{13}-norisoprenoid accumulation (*5*, *12*).

The response of C_{13}-norisoprenoids in fruit of PRD-treated vines reflected the changes seen in the carotenoids in both seasons (**Table 2**). Hydrolytically released β-damascenone and TDN were increased in PRD-treated fruit in both 2001/2002 and 2002/2003, although the magnitude of the increase relative to the control was greater for the latter season. β-Ionone was not significantly affected by the PRD treatment in 2001/2002,

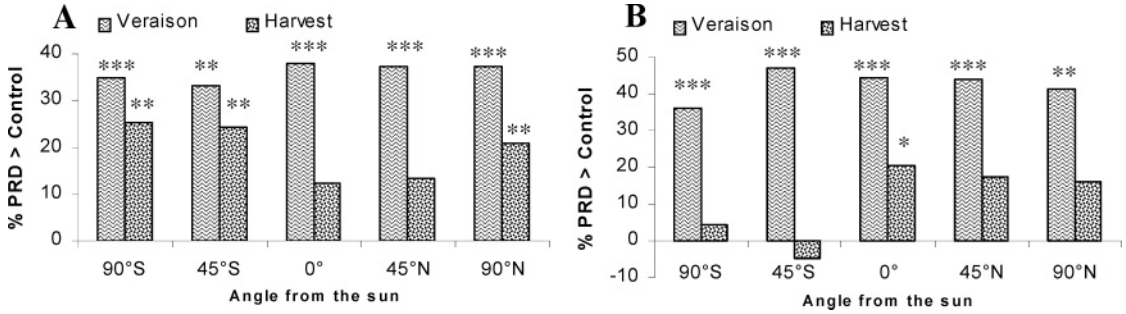

Figure 4. Effect of PRD on the percentage increase in within-canopy PAR (μmol·m^{-2}·s^{-1}) measured at different angles within the fruiting zone at veraison and harvest in (**A**) 2002 and (**B**) 2003. (ANOVA, $n = 42$; * indicates a significant difference: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.)

although levels were significantly higher in PRD-treated fruit in 2002/2003. TDN was the compound most significantly affected by the PRD treatment. It should be noted that fruit weight decreased 10−12% in response to the PRD treatment in both seasons. Levels of C_{13}-norisoprenoids per berry were not significantly different in 2001/2002. On the bssis of 2001/2002 data, it can be speculated that the relative increase in the concentration of β-damascenone and TDN in response to PRD was mainly due to a reduction in berry size. This is because a greater proportion of the carotenoid precursors to the C_{13}-norisoprenoids are concentrated in the berry skin (*9*) such that a change in skin to fruit weight ratio could increase the relative concentration of these compounds per gram in smaller fruit. However, despite equivalent decreases in berry weight in response to PRD in both seasons of the study, there was a more significant increase in the concentration of β-damascenone and TDN per gram of fruit in 2002/2003 than in 2001/2002 (**Table 2**). This was also reflected in a significant increase in the content of these components per berry, despite the fruit weight reduction with PRD. It is therefore more likely that biochemical changes induced by PRD caused the changes in C_{13}-norisoprenoid concentration, rather than a change in berry weight alone.

In the two seasons of the Cabernet Sauvignon experiment at Langhorne Creek, sunlight penetration (PAR) measured at different angles within the fruiting zone was increased approximately 40% within the canopies of PRD-treated vines relative to control-treated vines (**Figure 3**). These increases in incident sunlight to the fruiting zone observed in response to PRD treatment could have potentially influenced the composition of both carotenoids and C_{13}-norisoprenoids (*5*, *14*, *15*, *17*, *18*). However, increases in sunlight are usually associated with accelerated degradation of carotenoids, resulting in a decreased final concentration (*5*, *11*, *14*, *15*, *50*) rather than the converse, as is observed in the current study. Additionally, the increased accumulation of C_{13}-norisoprenoids in grapes grown under sunny conditions has been associated with this loss in carotenoids, leading to the speculation that this reflects, at least in part, the enzymatically mediated conversion of carotenoid-derived precursors to glycosylated C_{13}-norisoprenoids (*11*, *14*, *17*, *18*). The interpretation of the current data in the light of this hypothesis is tricky. The pattern of carotenoid decline in PRD-treated berry samples closely follows that of the control treatment (**Figure 5**). Rather, total levels of the primary carotenoids were increased from the onset of veraison, and it can be speculated that the pool of carotenoid precursors for the formation of C_{13}-norisoprenoid glycosides was potentially increased in response to PRD. This cannot be conclusively demonstrated unless their interconversion is demonstrated using ^{13}C or ^{14}C markers (*5*). However, it is evident from the current study that the effect of incident sunlight on developing bunches could not have operated in isolation to bring about the observed

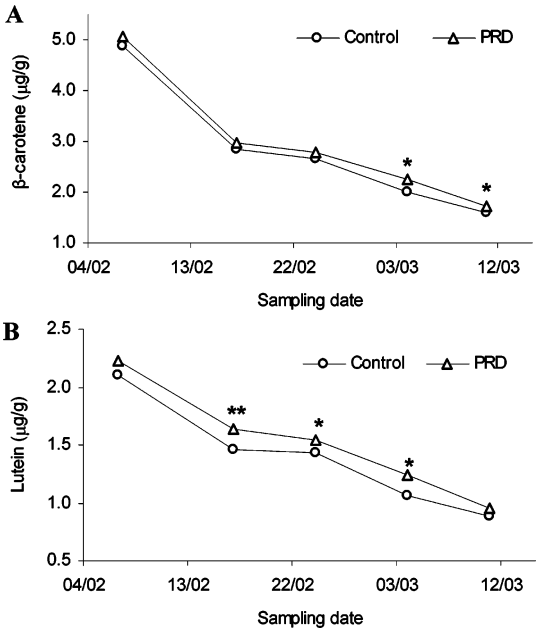

Figure 5. Effect of PRD on the concentration of carotenoids during berry development from veraison to harvest in Cabernet Sauvignon at 24 °Brix in 2002/2003: (**A**) β-carotene; (**B**) lutein. (ANOVA, $n = 42$; * indicates a significant difference: * = $P < 0.05$; ** = $P < 0.01$.)

changes in C_{13}-norisoprenoid concentration. Two possibilities exist. First, a combination of stress-related signaling in conjunction with high light intensities on developing fruit may have activated metabolism within the non-mevalonate pathway of isoprenoid synthesis (*25*, *51*). Second, this could potentially reflect increased operation of thermal dissipation mechanisms such as the xanthophyll cycle, under conditions of high light and water stress, which could be verified by a more thorough investigation of the carotenoid profile and pre-veraison non-photochemical quenching (NPQ) (*52*−*58*).

The findings of the current study suggest that irrigation strategy can induce changes in the glycosylated precursors to volatile C_{13}-norisoprenoids in grapes, which could potentially be recovered in wines during crushing and fermentation. It should be noted that the reported levels in this study represent hydrolytically released C_{13}-norisoprenoids, which gives an estimate of the maximum amount of precursor available for hydrolytic release during the aging process in wines. It is therefore not an exact representation of the aroma or flavor of the wine, but gives an indication of the potential of irrigation management to influence the volatile profile of fruit and resultant wines. Deficit irrigation has been shown to be associated with increases in the fruity characteristics in *Vitis vinifera* L. cv. Cabernet Sauvignon wines determined sensorially (*59*). A chemical candidate for this response could in part be β-dama-

scenone, which has a complex fragrance of flowers, tropical fruit, or stewed apple (*1*). It can be detected by the human senses at low concentration, with perception thresholds of 2 ng/L in water and 45 ng/L in dilute alcohol solution (*1*). Although β-damascenone and β-ionone denote positive sensory characteristics in wines (*60*), TDN may impart a negative, kerosene-like odor at high concentration (*61*). This compound has mainly been studied in *Vitis vinifera* L. cv. Riesling wines, where TDN imparts an important varietal character to the aroma. However, this is only below a threshold of 20 ppb, over which concentration it can become negative (*61*). There has been little attention given to the effect of TDN on the aroma and quality of red wines, and the implications of the results of the current study for this component in red wines are thus unknown.

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The original posting of May 1, 2007, contained an error in Table 1. This has been corrected with the posting of May 17, 2007.

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