Light and Temperature Independently Influence Methoxypyrazine Content of *Vitis vinifera* (cv. Cabernet Sauvignon) Berries

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Abstract. Methoxypyrazines (MPs) are fruit-derived extractable compounds that contribute to cultivar-specific aroma traits in wine, and greater concentrations can contribute to unpleasant vegetative aromas. Both light exposure and temperature have been reported to influence MP content in developing wine grapes, but individual effects of light and temperature are confounded. A novel method of manipulating light exposure with light-emitting diodes (LEDs) was used to impose light treatments with little or no effect on cluster temperature. Three treatments were imposed on developing fruit of *Vitis vinifera* (cv. Cabernet Sauvignon): 1) clusters exposed to direct sunlight, 2) clusters shaded by the grapevine canopy, and 3) clusters shaded by the canopy and exposed to supplemental LED light. Experiments were conducted over 3 years across pre- and postveraison periods of fruit development. A second experiment imposed the same light exposure treatments to ripening clusters on vines experiencing continual shoot growth during the postveraison period. Light exposure reduced 3-isobutyl-2-methoxypyrazine (IBMP) concentration of developing grape berries in the preveraison period independently of berry heating from solar radiation. Berry IBMP responded less to postveraison light levels, except on vines with active shoot growth, suggesting IBMP synthesis was continued during active vine growth but was suppressed by light. An inverse relationship of growing degree days (GDDs) with berry IBMP was observed, indicating high temperatures also reduce berry IBMP concentration. Response to temperature could result from either radiant heating of light-exposed clusters or from high ambient air temperature. Canopy management should consider the impact of both light and temperature on IBMP, and vine management practices should be adjusted appropriately to regional growing conditions and grape cultivars.

MPs are a class of aroma compounds responsible for vegetal aromas in fruit of some *Vitis vinifera* L. wine grape cultivars. MPs have been quantified in Grenache (López et al., 1999), Merlot (Sala et al., 2000), Cabernet Franc (Allen et al., 1994), and Cabernet Sauvignon (Bayonove et al., 1975). Sensory trials supplemented quantification in Carmenere (Belancic and Agosin, 2007) and Sauvignon blanc (Augustyn et al., 1982). Reciprocal grafting experiments demonstrated MPs or their precursors originate in berries, rather than being synthesized in

leaves and translocated to fruit (Koch et al., 2010). Maximum accumulation of MPs occurs 2 to 3 weeks before veraison, followed by a decline in MP concentration as berries ripen (Koch et al., 2012; Roujou de Boubée et al., 2000; Ryona et al., 2008).

MPs are highly extractable compounds that are stable during fermentation and perceived at low olfactory thresholds of about 1 to 2 ng/L in white wine and 10 to 16 ng/L in red wine (Roujou de Boubée et al., 2000; Sala et al., 2005). Although low MP concentrations contribute to cultivar-specific aroma traits, greater concentrations can contribute to unpleasant vegetative aromas that detract from wine quality and mask fruity characteristics in wine (Allen et al., 1996; Hein et al., 2009; Noble et al., 1995). A compound of particular concern is IBMP, which is responsible for green bell pepper aroma in wine (Roujou de Boubée et al., 2002).

Reported photodegradation of IBMP in clear-bottled white wine (Heymann et al., 1986) led to the assumption light exposure of

developing grape berries would reduce MP concentration via this mechanism. Consequently, Sauvignon blanc clusters were exposed to increased sunlight through leaf removal treatments, resulting in decreased herbaceous aromas of corresponding wines (Allen et al., 1988; Arnold and Bledsoe, 1990). Subsequent research examined various methods of canopy manipulation to increase sunlight exposure of fruit in attempts to reduce MP content, including basal leaf removal (Scheiner et al., 2010), shoot thinning (Ryona et al., 2008), pruning (Allen and Lacey, 1993; Chapman et al., 2004a, 2004b), different trellis and training systems (Kotseridis et al., 1999; Sala et al., 2004b), combinations of training systems and canopy management treatments (Balda and Martinez de Toda, 2013), shade-cloth (Koch et al., 2012; Sala et al., 2004b), and opaque boxes (Dunlevy et al., 2013a). Recent research suggests fruit light exposure may reduce MP accumulation through inhibition of biosynthesis (Koch et al., 2012; Ryona et al., 2010; Scheiner et al., 2010). Dunlevy et al. (2010, 2013a) demonstrated a methyl-transferase gene was involved in MP biosynthesis and light-reduced expression and concentration of an IBMP precursor (Dunlevy et al., 2013a). As a result of the apparent effect of sunlight exposure on biosynthesis of MP, preveraison is considered the critical time to decrease accumulation of MPs (Koch et al., 2012; Ryona et al., 2008) whereas postveraison light exposure has been reported to have little effect on reducing final MP content of grapes (Koch et al., 2012; Ryona et al., 2008; Scheiner et al., 2010).

It seems clear from these studies that sunlight exposure on developing fruit decreases MP content, but the potential confounding effect of radiant heat from sunlight on MP content has not been examined. Both light exposure and temperature have been reported to influence MP content in developing wine grapes (Allen and Lacey, 1993; Koch et al., 2012; Noble et al., 1995; Ryona et al., 2008), however the individual effect of each factor is difficult to distinguish as a result of the heat generated by light absorbance, as noted by Ryona et al. (2008). It is generally recognized that sun-exposed fruit typically have greater berry temperatures compared with shaded clusters. Smart and Sinclair (1976) reported berry temperature increases linearly with sunlight exposure. Temperatures of sunlight-exposed clusters on the south side of east–west rows in the central San Joaquin Valley of California were generally 3 to 4 °C greater when compared with clusters on the north side of the row (Bergqvist et al., 2001). In eastern Washington, berry temperature of sunlight-exposed clusters was 4 to 13 °C greater than ambient air temperature and shaded cluster temperatures regardless of row aspect (Spayd et al., 2002). Their study found west-exposed clusters sometimes exceeded temperatures of 40 °C. It is possible, therefore, that the reported association of reduced MP content with sunlight-exposed grape berries could be, at least in part, a result of temperature effects.

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Studies reporting effects of temperature on grape berry MP content have mostly involved broad comparisons of fruit or wines from regions or seasons with significant temperature differences (Allen and Lacey 1993; Falcão et al., 2007; Heymann and Noble, 1987; Lacey et al., 1988, 1991; Marais et al., 1999; Mendez-Costabel et al., 2013), all of which involved uncontrolled confounding factors of environment and vineyard management practices. Nevertheless, a trend is evident: MPs tend to be greater in fruit or wine from cooler growing regions and seasons (Allen and Lacey 1993; Falcão et al., 2007; Heymann and Noble, 1987; Lacey et al., 1991). Lower MP levels were observed in the warmest year of a 3-year field study on Cabernet Sauvignon fruit from a broad range of cluster light exposure treatments, and temperature effects on MP were examined in a companion study conducted in growth chambers (Koch et al., 2012). Two constant temperature regimes representing average daily temperatures for a warm and a cool growing region were imposed on potted Cabernet Sauvignon grapevines receiving either a low light (close to zero) treatment or a relatively high light ($500\text{--}600\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) treatment. Significant differences in MP levels were only detected between fruit from the high-light/high-temperature and low-light/low-temperature treatments (Koch et al., 2012). Separation of light and temperature effects were achieved in a field study by Spayd et al. (2002), indicating regardless of cluster temperature, light had the greatest influence on anthocyanin levels in Merlot berries, but MP levels were not measured.

Greater fruit MP content has also been associated with abundant soil moisture availability (Mendez-Costabel et al., 2014; Qian et al., 2009; Sala et al., 2005) and conditions conducive to active vine growth, which leads to shading (Mendez-Costabel et al., 2013; Roujou de Boubée et al., 2002; Scheiner et al., 2012; Wilkinson et al., 2008). Carmener was reported to have greater MPs in a season with late rainfall, which resulted in a second vegetative growth period, leading the authors to postulate that new growth promoted MP biosynthesis and transportation to berries (Belancic and Agosin, 2007). This explanation seems unlikely given subsequent reciprocal grafting experiments that demonstrated MPs are not translocated from vegetative tissues and are likely synthesized within grape berries (Koch et al., 2010).

To investigate light exposure effects on MP concentration of developing grape berries in the absence of radiant heat, small panels of LEDs were used to illuminate clusters that would otherwise be shaded from sunlight within the canopy. Shoot positioning was used to maintain cluster exposure to sunlight or shade in an experiment conducted across the pre- and postveraison periods of fruit development. A second experiment imposed similar treatments to ripening clusters on vines experiencing continual shoot growth during the postveraison period in response to supplemental irrigation. To elucidate light vs. temperature effects, at the

end of each developmental period, berry IBMP levels for shaded clusters were compared with shaded clusters receiving supplemental light from LEDs.

Materials and Methods

Experiments were conducted at the Texas A&M AgriLife Research and Extension Center, Lubbock, TX (lat. $33^{\circ}41'33''\text{N}$, long. $101^{\circ}49'17''\text{W}$) during the growing seasons of 2011, 2012, and 2013. Data vines were established on *V. vinifera* cv. Cabernet Sauvignon FPS 07 on 110R rootstock planted in 2008 at a $1.8 \times 3\text{-m}$ vine-by-row spacing with north-south row orientation and experimental clusters on the west side. Vines were bilateral cordon-trained and spur-pruned to two buds. Canopies were managed in a sprawl configuration with cordons established at $\approx 90\text{ cm}$, with single foliage catch wires at $\approx 15\text{ cm}$ and $\approx 35\text{ cm}$ above the cordon. Weather data, including GDD accumulation and annual precipitation were collected from a weather station located at Lubbock Preston Smith International Airport, located $\approx 3\text{ km}$ from the vineyard block (Weather Underground, 2016).

A novel method of manipulating light levels in the field with LEDs was used to impose light exposure treatments with little or no effect on cluster temperature (Plank et al., 2016). Three treatments were imposed: 1) clusters exposed to direct sunlight, 2) clusters shaded by the grapevine canopy, and 3) clusters shaded by the canopy and exposed to supplemental LED light. Treat-

ments were applied to one basalmost cluster per vine, with clusters selected for homogeneity among vines. Upper foliage wires were used to maintain the canopy around shade- and LED-treatment clusters, and to move shoots away from sun-exposed clusters throughout the growing season. Vines received standard vineyard management for the region, and light treatments were imposed at two stages: one cluster at preveraison (fruit set to veraison), which was harvested 50 d postanthesis; and one cluster postveraison (veraison to harvest), harvested at $^{\circ}\text{Brix}$ levels typical for commercial harvest parameters, with average $^{\circ}\text{Brix}$ ranging from 23 to 24.6, pH between 3.4 and 3.9, and between 5.3 and $7.2\text{ g}\cdot\text{L}^{-1}$ tartaric acid (TA). A second experiment was conducted to investigate the hypothesis of Belancic and Agosin (2007) that new growth promotes MP biosynthesis and transportation to berries. Cluster light exposure treatments were imposed during the postveraison period on vines receiving additional irrigation to encourage continuous shoot growth.

Preveraison and postveraison experiments. Treatments were assigned randomly to 45 vines among two adjacent rows of Cabernet Sauvignon, and each of three treatments was assigned randomly to 15 single-vine replicates. A single basal cluster per vine received the assigned light exposure treatment during the preveraison period imposed at fruit set. A second cluster on a different shoot of the same vine received the same light treatment during the postveraison period imposed at veraison. All fruit were harvested the same day.

Table 1. Growing degree-day (GDD) accumulation, annual precipitation, and phenology dates for Cabernet Sauvignon at Lubbock, TX, for 2011, 2012, and 2013.

Yr	GDD ($^{\circ}\text{C}$)			Annual precipitation (cm)	Avg maximum season wind speed ($\text{km}\cdot\text{h}^{-1}$) ^x	Phenology dates (veraison)
	Preveraison ^z	Postveraison ^y	Season ^x			
2011	1,511.4	1,098.0	3,042.7	14.88	39.9	15 July
2012	1,369.7	656.2	2,859.2	29.03	38.0	10 July
2013	1,445.8	692.5	2,699.1	32.03	39.1	25 July

^zCumulative 1 Apr. to veraison.

^yCumulative veraison to harvest.

^xCumulative 1 Apr. to 31 Oct.

Table 2. Photosynthetically active radiation (PAR) received by Cabernet Sauvignon grape clusters exposed to either direct sunlight, shaded by canopy, or shaded by canopy with supplemental light-emitting diode (LED) light. Exposure occurred in preveraison (fruit set to 50 d postanthesis), postveraison (veraison to harvest), and postveraison under a continuous shoot growth during 3 study years in Lubbock, TX.

Expt. ^x	Treatment ^y	Mean PAR ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) ^z		
		2011	2012	2013
Preveraison	Shade	47 c	29 b	20 c
	Shade + LED	1,479 b	1,874 a	1,988 b
	Sun	1,901 a	1,888 a	2,107 a
Postveraison	Shade	31 b	28 c	109 c
	Shade + LED	1,806 a	1,759 b	1,682 b
	Sun	1,851 a	1,920 a	1,844 a
Continuous growth	Shade	36 b	21 b	88 c
	Shade + LED	1,877 a	1,829 a	1,741 b
	Sun	1,814 a	1,813 a	1,929 a

^zMeans within columns followed by same letter are not significantly different at $P < 0.05$; $n = 15$ (least significant difference test).

^yShade = cluster shaded by canopy; Shade + LED = cluster shaded by canopy with supplemental LED light; Sun = clusters exposed to full sunlight.

^xData for all experiments analyzed independently.

LED panels were constructed as described in Plank et al. (2016). Cluster and canopy temperature were monitored continuously in 30-min intervals with Watchdog Temperature and Relative Humidity sensors and dataloggers (1000 Series Micro Stations; Spectrum Technologies, Aurora, IL). Interior cluster temperature was considered a good representation of all berries in a cluster; therefore, a fine-wire thermistor (Watchdog Temperature Micro Sensor, Spectrum Technologies) was threaded into the center of the cluster, secured with tying tape, and connected to a datalogger. The datalogger also recorded temperature of the air within the canopy, which served as ambient canopy temperature. Dataloggers were placed in radiation shields and secured to the T post between vines. Data were downloaded from the units using Spec 9 Basic software (version 9.02, Spectrum Technologies). Photosynthetically active radiation (*PAR*) exposure was measured weekly for each cluster with a Fieldsout 1400 Light Sensor Reader (Spectrum Technologies) on cloudless days during the solar noon period (1000–1400 HR). The *PAR* sensor was oriented to each cluster such that the sensor measured incident solar radiation or LED light.

Continuous growth experiment. Vines of Cabernet Sauvignon FPS 07 on 110R rootstock planted in 2006 in another location of the same vineyard were used for the continuous shoot growth study. Spacing and trellis/training configurations were the same as described previously. Continuous growth experiment vines were arranged in a randomized complete block design with four blocks. Each block consisted of a five-vine block, of which three data vines were selected randomly. Each of the three previously described cluster exposure treatments were assigned randomly to one vine per block, with a single basal cluster per vine receiving the assigned light treatment. A second drip irrigation line was installed in each block to provide supplemental water applications of 2.5 cm/week throughout the growing season. Light exposure treatments were imposed beginning at veraison and continued until berries were harvested at °Brix levels

(22.1–23.2) typical for commercial harvest. Cluster temperature and *PAR* were measured for each treatment, as described for the pre- and postveraison experiment. Shoot length was measured weekly from veraison to harvest on five shoots on the east side of experimental vines, and averaged among all shoots per block.

Total soluble solids, pH, TA, and berry weight. Clusters for MP analysis were too small to include in total soluble solids (TSS) content; therefore, other fruit was collected from the experimental vines to examine TA, pH, and TSS content on a single harvest date each year. TSS content was determined using a temperature-compensating digital refractometer (Atago PR-32; Bellevue, WA). Juice pH and TA were determined using an autotitrator (Metrohm 862 Compact Titrator; Herisau, Switzerland). TA was determined with 0.1 N NaOH to an endpoint of pH 8.2.

Fruit analysis. Fruit samples were prepared using methods adapted from Du et al. (2010). Fruit were frozen and stored at –18 °C until sample preparation, when they were thawed in a refrigerator overnight. Twenty-five grams of berries were removed from stems for each sample cluster. To inhibit enzymatic action, an equal weight of distilled water (25 g) and 1% (w/w) calcium chloride (final concentration) was added to thawed berries. Samples were pureed with a Tissuemaker Tissuemax #SIT-1810 (Tekmar Co., Cincinnati, OH) then placed in 50-mL centrifuge tubes and centrifuged in an Eppendorf 5804R centrifuge (Eppendorf, Hauppauge, NY) for 20 min. Supernatant was filtered through Whatman's No. 1 (particle retention, >11 µm) followed by VWR 413 filter paper (particle retention, >5 µm) (VWR, Radnor, PA). Supernatants from each sample were divided into triplicates of 10-mL aliquots and sealed in 20-mL amber headspace vials. To perform stir-bar sorptive extraction, a 10-mm polydimethylsiloxane stir bar (Gerstel, Inc., Germany), conditioned according to the manufacturer's directions, was added to each headspace vial. Because 2-ethoxy-3-ethylpyrazine (EEP) is similar to the target compounds but not found in grapes (Flamini and Traldi, 2010; Hartmann et al., 2002;

Sala et al., 2004a), EEP served as the internal standard. For each vial, 10 µL of 10 mg/L EEP (Pyrazine Specialties, Ellenwood, GA) diluted in ethanol was added. Vials were stirred for 35 min to absorb volatiles, then stir bars were removed, rinsed lightly with ultrapure water, and dried with a lint-free tissue.

Gas chromatography/mass spectrometry was used for quantification. IBMP and 3-isopropyl-2-methoxypyrazine (IPMP) were quantified concomitantly with one stir bar. Each stir bar was placed in an Agilent 5181-3316 single taper, deactivated splitless liner. The liner was then inserted into a splitless inlet and desorbed at a temperature of 200 °C in an Agilent Tech 7890A GC system (Agilent Technologies, Inc., Santa Clara, CA). Compound separation was achieved with an HP 5 MS column (30 m × 0.25 mm i.d., 0.25-µm thickness). The temperature program was set as follows: initial hold at 40 °C for 5 min, followed by 4 °C/min ramp to 124 °C with no hold, then 20 °C/min ramp to 220 °C with a 5-min hold. Helium served as the carrier gas, with a flow rate of 0.5 mL/min. Total runtime of the method was 35.8 min. Because retention times of compounds of interest eluted toward the end of the run, a 17-min solvent delay was used. Ion source temperature was 230 °C.

Quantification was accomplished with an Agilent Tech 5975C VL MSD with Triple-Axis Detector. Enhanced ChemStation E02.01.1177 software from Agilent Technologies, Inc., was used to visualize the output from the mass spectrometer. Quantification of MPs was based on peak area, and identification of compounds was achieved through select ion monitoring. Values were doubled as a result of 2-fold dilution by the addition of water to grape must (homogenized berry tissue). Selected mass channels were *m/z* = 124 (quantifier), 151, and 166 for IBMP; *m/z* = 124, 137 (quantifier), and 152 for IPMP; and *m/z* = 123 (quantifier), 124, and 152 for EEP (Flamini and Traldi, 2010). A six-point standard curve with concentrations ranging from 4 to 1000 ng/L (*n* = 6) was prepared for IBMP and IPMP in ultrapure water, resulting in *R*² = 0.9990 for IPMP and

Table 3. Temperature of grape clusters exposed to direct sunlight, shaded by the canopy, or shaded clusters exposed to supplemental LED light in preveraison or postveraison. Temperatures are shown for morning (0800 HR), midday (1300 HR), and late afternoon (1700 HR) for 2011, 2012, and 2013 in Lubbock, TX.

Expt. ^a	Treatment ^y	Mean cluster temperature (°C) ^z								
		2011			2012			2013		
		Time (HR)			Time (HR)			Time (HR)		
		0800	1300	1700	0800	1300	1700	0800	1300	1700
Preveraison	Shade	23.3	34.1	36.9	21.4	31.2	33.3	20.9	28.5	30.5
	Shade + LED	23.7	34.5	37.0	21.6	31.3	33.3	21.5	29.7	31.2
	Sun	23.3	34.3	37.9	22.4	31.2	35.0	21.6	29.7	31.0
Postveraison	Shade	20.4	31.8	33.7 b	22.2 b	33.4 b	35.9	19.4 b	30.8	32.4
	Shade + LED	20.5	31.7	33.8 b	22.9 a	34.1 a	36.8	20.2 a	30.6	32.9
	Sun	20.1	31.7	35.7 a	22.5 ab	34.0 a	36.2	20.0 ab	30.8	32.4
Continuous growth	Shade	18.1 b	29.4 b	31.7 c	21.5 b	32.7	34.9	19.7	31.2	31.2
	Shade + LED	20.2 a	33.5 a	34.5 a	23.0 a	33.0	35.2	19.9	30.6	31.7
	Sun	18.0 b	29.7 b	33.4 b	22.0 a	33.2	35.2	19.9	30.7	31.7

^aMeans within columns followed by the same letter are not significantly different at *P* < 0.05; *n* = 15 (least significant difference test).

^yShade = cluster shaded by canopy; Shade + LED = cluster shaded by canopy with supplemental LED light; Sun = clusters exposed to full sunlight.

^zExperiments analyzed independently.

$R^2 = 0.9999$ for IBMP. Standards of known concentration were used to determine accuracy and reproducibility of MP determinations. Limit of detection was determined to be less than 1.9 ng/L for IBMP, within range of previously reported values of 0.2 to 2 ng/L (Alberts et al., 2009). Peak area was used to determine concentration of MPs in the juice solution.

Statistics. Analysis of variance ($\alpha = 0.05$) was performed using SAS version 9.3 (SAS Institute, Cary, NC). Proc GLM was used to analyze *PAR*, cluster temperature, and MP concentration in fruit. The *PAR* model used the mean of three point measurements taken each sampling day for each treatment. Cluster temperatures were examined at three times during the day: 0800, 1300, and 1700 HR. Means were separated using least significant difference.

Results

Weather conditions at the experimental vineyard site varied over the 3 years of this study, with 2011 experiencing exceptionally high GDD accumulation, particularly postveraison, and record-low precipitation (Table 1). Postveraison GDD accumulation in 2011 was 67.3% and 58.6% greater than 2012 and 2013, respectively. This is attributable in part to the greater number of days between veraison and harvest in 2011, but total GDD accumulation for the season (1 Apr.–31 Oct.) was also greater in 2011 than subsequent years, and 6.4% to 12.7% greater than 2012 and 2013, respectively.

Pre- and postveraison experiment. Light interception by developing grape clusters was substantially greater for canopy-shaded clusters illuminated with LED (Shade + LED) compared with canopy-shaded clusters without supplemental lighting (Shade) during all experimental periods (Table 2). Incident *PAR* on clusters of Shade + LED treatments was consistently similar to sunlight exposed clusters (Sun), and exceeded that of the Shade treatment by an average factor of 55, and ranged from 15 to 99 times greater across all experimental periods.

Mean cluster temperature at three times of day for all experiments is reported in Table 3. No difference in cluster temperatures were found among treatments over 3 years in the preveraison experiment, although Shade clusters had slightly lower temperatures than one or both light treatments in the postveraison experiment in a few instances.

Fruit IBMP concentrations responded to cluster light exposure treatments during both preveraison and postveraison periods, although the response was greater and more consistent in preveraison treatments (Fig. 1). Preveraison Sun and Shade + LED treatments resulted in lower IBMP concentrations in fruit at the mid-development stage compared with Shade treatments in both 2012 and 2013. Shade treatment in 2012 had $\approx 43\%$ and 15% greater IBMP than Shade + LED and Sun treatments, respectively. In 2013, preveraison Shade had more than 50% greater IBMP

Fig. 1. Mean 3-isobutyl-2-methoxypyrazine (IBMP) concentration of Cabernet Sauvignon berry solutions with 1:1 dilution for clusters receiving light treatments during pre- and postveraison periods in (A) 2011, (B) 2012, and (C) 2013. Different letters within a year indicate significant treatment effects (Proc GLM; $\alpha = 0.05$, $n = 15$, $P \leq 0.05$). Error bars based on SEM. LED = light-emitting diode.

than both the Shade + LED and Sun treatments. All 2012 preveraison treatments and the 2013 preveraison Shade treatment produced fruit at mid development with IBMP exceeding the reported (Sala et al., 2005) sensory threshold range for red wine of 10 to 16 ng/L. Regardless of light treatment and

year, fruit IBMP concentration decreased between the mid-development stage (preveraison treatment period) and mature fruit (postveraison treatment period), with low final concentrations in the range of 4 to 8 ng/L. Light treatments imposed postveraison resulted in slightly lower IBMP in the Sun

and Shade + LED treatments compared with the Shade treatment in 2011, with a similar trend in 2012 and 2013. In all cases, IBMP levels of mature fruit subjected to light treatments postveraison were less than the sensory threshold for red wine. IPMP was not detected in fruit from either treatment period.

Continuous growth experiment. Active shoot growth from veraison to harvest was observed in all 3 years for blocks receiving weekly supplemental irrigation (Fig. 2), although total shoot length and growth rate were considerably less for all vines during the exceptionally warm and dry year of 2011. As with the preveraison and postveraison experiments, differential light treatments were imposed (Table 2), which resulted in few differences in cluster temperature among treatments (Table 3). Higher cluster temperature was observed for the Shade + LED treatments compared with Sun and Shade treatments in 2011 only.

Vines with continuous shoot growth produced mature fruit with low final concentrations of IBMP very similar to levels found in postveraison fruit from the other experiment—in the range of 4 to 6 ng/L. Light treatments imposed on actively growing vines during the postveraison period induced a response in IBMP concentration of mature fruit. The Sun and Shade + LED treatments had reduced mature berry IBMP concentration in 2 of 3 years compared with the Shade treatment (Fig. 3). Nevertheless, all light exposure treatments under continuous shoot growth produced mature fruit with IBMP levels less than the sensory threshold, and IPMP was not detected—results that are consistent with levels found in clusters postveraison in the other experiment.

Discussion

Light exposure reduced IBMP concentration of developing grape berries preveraison in concurrence with previous reports (Dunlevy et al., 2013b; Koch et al., 2012; Ryona et al., 2008; Scheiner et al., 2010), and our results demonstrate the light effect is independent of berry heating from solar radiation. The light response was quantified previously by Koch et al. (2012), who reported an inverse relationship of IBMP to light intensity from 0% to about 50% full sunlight with no further response as light increased, but their shaded clusters appeared to experience lower temperatures than exposed clusters. Both Ryona et al. (2008) and Dunlevy et al. (2013b) noted their methodology could not rule out lack of solar radiation in their shade treatments could have resulted in less radiant heating compared with sunlight-exposed berries. Our experiment was designed to eliminate radiant heat as a source of increased berry temperature, demonstrating light independently reduces IBMP concentration of fruit during the preveraison period.

Nevertheless, there appears to be an effect of ambient air temperature on IBMP, as our results also corroborate previous observations of an inverse correlation of GDD

Fig. 2. Mean shoot length recorded for five shoots within each five-vine block from postveraison to harvest. Blocks received 2.5 cm/week supplemental irrigation across each growing season over 3 years: (A) 2011, (B) 2012, and (C) 2013.

accumulation with IBMP of mature fruit (Alberts et al., 2009; Allen and Lacey 1993; Allen et al., 1994; Falcão et al., 2007; Heymann and Noble, 1987; Koch et al., 2012; Lacey et al., 1991). The greatest IBMP levels were found in fruit at the mid-development stage and at harvest under the lowest GDD in 2012, whereas the lowest

IBMP levels occurred in the warmest year (2011). Overall growing season temperatures were also likely responsible for low berry IBMP concentrations at harvest for all light treatments in all years. The Texas High Plains is a warm growing region (Hellman et al., 2011) and GDD accumulations ranged from 2699 to 3043 °C during the period of our

Fig. 3. Mean 3-isobutyl-2-methoxypyrazine (IBMP) concentration in Cabernet Sauvignon berry solutions with 1:1 dilution for clusters receiving light treatments in the postveraison period under continuous shoot growth conditions induced by supplemental irrigation. Different letters within each year indicate significant treatment effects (Proc GLM; $\alpha = 0.05$, $n = 15$, $P \leq 0.05$). Error bars based on SEM. LED = light-emitting diode.

study. Similarly, Koch et al. (2012) reported IBMP levels were lowest and less than detection levels in the warmest year of their study. Cluster temperature may have been influenced by wind speed. Average wind speed for the season (1 Apr.–31 Oct.) ranged from 38.0 to 39.9 km·h⁻¹ (Table 1). As noted in Plank et al. (2016), wind conditions in the field may have dissipated heat across the LED panels, and could have affected cluster temperatures of sun- and shade-exposed clusters, dissipating cluster temperature and decreasing the temperature difference of clusters across treatments.

Thus, both light and temperature affect IBMP before veraison, but it is unclear whether the mechanisms are similar. Dunlevy et al. (2013b) demonstrated preveraison sunlight exposure reduces berry IBMP accumulation through decreased expression of the methyltransferase gene *VvOMT3* responsible for the final step in MP biosynthesis. Because their study could not distinguish temperature effects, it is possible greater ambient temperatures also suppress *VvOMT3* expression. It has also been suggested IBMP biosynthesis and degradation occur simultaneously during the preveraison period (Scheiner et al., 2010), and it seems plausible degradation is enhanced by increased air temperature.

Berry IBMP concentration decreased from mid development (preveraison) to harvest, following the pattern reported by numerous authors (Allen et al., 1996; Dunlevy et al., 2013b; Koch et al., 2012; Roujou de Boubée et al., 2000; Ryona et al., 2008; Scheiner et al., 2010). By harvest, berry IBMP was low for all treatments, less than the sensory threshold levels for red wine,

with only a slight and inconsistent (1 of 3 years) postveraison response to light levels. These results confirm reduced (Dunlevy et al., 2013b; Sala et al., 2004b) or no response (Koch et al., 2012; Ryona et al., 2008) to postveraison light exposure on berry IBMP at harvest. Because expression of the *VvOMT3* gene involved in IBMP synthesis was reported to be much lower postveraison (Dunlevy et al., 2013b), the suppression response to light treatments would be expected to be less effective, and Dunlevy et al. (2013b) suggested degradation or metabolism of IBMP is responsible for the large declines in IBMP during the grape ripening period. It seems likely high ambient air temperatures, as described earlier, experienced during the postveraison period, could enhance degradation or metabolism of IBMP. Whether degradation is enzymatic or nonenzymatic has yet to be determined (Lei et al., 2018).

A more consistent response to postveraison cluster light exposure occurred in the continuous growth experiment, where shaded treatments had greater berry IBMP content in 2 of 3 years. Similarity of these results to our clusters harvested preveraison suggests berries on vines in a state of continuous growth continue to synthesize IBMP, and thus respond to the gene suppression effect of light treatments, but *VvOMT3* expression would need to be verified under such conditions. This mechanism would help explain reported association of berry IBMP in actively growing or highly vigorous vines (Belancic and Agosin, 2007; Mendez-Costabel et al., 2013; Roujou de Boubée et al., 2002), in addition to cluster shading resulting from a dense canopy. Despite the

response to cluster light exposure of continuously growing vines, harvested fruit had low IBMP levels, less than the threshold level at harvest, suggesting degradation or metabolism mechanisms possibly influenced by high ambient air temperatures.

Light exposure of clusters reduces IBMP concentration in developing grape berries independently of radiant heat. Response to light is greatest during the preveraison period, or when vines are actively growing postveraison, and appears to be related to suppression of *VvOMT3* expression during IBMP biosynthesis. High temperatures also reduce berry IBMP concentration, which could result from either radiant heating of light-exposed clusters or from high ambient air temperature. Vineyard managers should consider the impact of both light and temperature on IBMP and adjust canopy management practices appropriately to match regional growing conditions and grape cultivars. In cool climates prone to greater IBMP, hedging or leaf removal on both the morning and afternoon sides of the canopy could enhance sunlight exposure and radiant heat on developing clusters. It is likely that berry MP levels can also be minimized by vineyard management practices, resulting in limited shoot growth postveraison.

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