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Fruit ripening in *Vitis vinifera*: light intensity before and not during ripening determines the concentration of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon berries

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The roles of light and temperature in the accumulation of the vegetal impact compound 2-methoxy-3-isobutylpyrazine (MIBP) in grape (*Vitis vinifera* L.) berries were determined. Individual clusters were exposed to various light intensities using neutral density shade cloth before ripening, during ripening or throughout the season in three growing seasons. A recently developed method using headspace solid-phase microextraction combined with GC-MS in the selected ion-monitoring mode was employed to measure MIBP in berries. Berry MIBP concentration increased subsequent to berry set, reached a maximum prior to onset of ripening, and then decreased thereafter until harvest. Complete shading of clusters increased the concentration of MIBP more than 100% compared to unshaded controls in 2 out of 3 years. Light increasingly inhibited MIBP concentrations up to 25–50% of ambient light intensities (1500 µmol photons m^{-2} s^{-1}). However, only changes in light intensity before ripening had any effect on MIBP accumulation or final MIBP concentration. Analyses of weather data showed that the 1 year in which shading was ineffective was unusually warm, warm early in the season, and had more hot days and higher early season degree days than the other 2 years. In controlled environment experiments, warm growth conditions reduced MIBP concentrations in fruit about as much as light exposure reduced MIBP concentrations in the field experiments. The results indicate that both light and temperature significantly affect MIBP in harvested fruit, but that the light environment during ripening does not significantly affect MIBP concentrations in the berries at harvest.

Introduction

Pyrazines are thought to be involved in aposematism (Kaye et al. 1989, Lindstrom et al. 2000), and methoxypyrazine compounds have long been associated with vegetal flavor and aroma in reproductive plant organs (Buttery et al. 1969, Murray et al. 1970). One in particular, 2-methoxy-3-isobutylpyrazine (MIBP), has been found consistently in Sauvignon blanc and Cabernet Sauvignon grapes and wines, typically at a concentration of 2–30 ng l^{-1} (Harris et al. 1987, Allen et al. 1991, 1994, Lacey et al. 1991, Chapman et al. 2004a). MIBP is an impact compound in fresh fruit and vegetables, producing a vegetal, bell pepper flavor and aroma with a very low sensory threshold. The vegetal attribute in wines is variously described as positive or negative depending upon the era, population sampled, and winegrape variety. The concentration of MIBP in wines is a function of its concentration in berries (Roujou de Boubée et al. 2000, Ryona et al. 2008). Therefore,

Abbreviations – DD, degree days; dMIBP, 2-($^2D_3$)-methoxy-3-isobutylpyrazine; DOY, day of year; HTHL, high temperature/high light; LTLL, low temperature/low light regime; MIBP, 2-methoxy-3-isobutylpyrazine; PFD, photon flux density.

it is important to understand those factors that increase or decrease the concentration of MIBP in harvested winegrapes.

Heymann et al. (1986) found that MIBP in wine is photolabile. They exposed clear glass bottles of wine to fluorescent light for 120 h and found that MIBP decreased 28% compared to the control. In comparable experiments, Maga (1990) reported similar results with red wines. Perhaps as a result of that work in wines and solutions, light was thought to control the concentration of MIBP in grape berries in the field via photodegradation (Noble et al. 1995). However, Blake et al. (2010) found no evidence for light-affecting MIBP in stored red or white wines. Although several field studies manipulated vine shoots and leaves to alter shading of fruit and reported corresponding changes in either MIBP concentrations in resultant wines (Allen and Lacey 1993) or in wine vegetal aroma (Arnold and Bledsoe 1990, Morrison and Noble 1990), MIBP in the fruit was not investigated. When excised grapes were exposed to low light intensities, MIBP decreased in ripe grapes compared to controls exposed to no light, but increased in unripe grapes (Hashizume and Samuta 1999). These results raise questions about the role of light in MIBP metabolism in berries and suggest that the stage of berry development may be important in how the berries respond to light. Indeed, precedents exist in which early (before ripening) water deficits (Matthews and Anderson 1988, Castellarin et al. 2007) and cluster-shading treatments (Dokoozlian 1990) had greater impact on final fruit composition than late (during ripening) treatments. Furthermore, although light inhibits stem elongation, much precedent exists for light in promoting accumulation of volatiles in plants (Chang et al. 2008) and accumulation of non-volatile solutes in berries (Kliewer 1977, Morrison and Noble 1990).

The situation was somewhat clarified by Ryona et al. (2008) who shoot-thinned Merlot grapevines to create 'shaded' and 'exposed' clusters, and showed clearly an inhibitory effect of exposure on the concentration of MIBP in fruit. Ryona et al. (2008) further showed that most MIBP is made before the onset of ripening, and deduced that the early light environment may be more important than late light, during ripening. However, no studies have tested the effects of light at different stages of fruit development. Furthermore, no studies have quantified the light response of the putative inhibition of MIBP accumulation. The light environment of clusters in commercial vineyards is highly variable, ranging from full, direct sunlight to as low as 1% of full sunlight (Dokoozlian and Kliewer 1996). Moreover, the grapevine canopy in production systems is subject to much manipulation, trellising and training are commonly used by viticulturists and winegrape production often involves more intensive practices such as shoot positioning and leaf pulling.

Knowledge of both the timing and light intensities that affect MIBP in fruit is important for understanding how the plant responds to light cues in fruit development and to design canopy management systems that appropriately take into account the plant responses to light. A better understanding of the effects of light environment on berry composition is clearly a necessary precursor to the design of an optimal canopy structure. In addition, light may affect fruit metabolism via the heat generated by its absorbance. Both light and temperature may alter allocation of recent photosynthate (Grechi et al. 2007) toward or away from the synthesis of volatiles. Although no studies of berry temperature responses are published, Allen and Lacey (1993) reported data that reflect a negative correlation of mean air temperature during the growing season of various vineyards and the concentration of MIBP in commercial wines produced from those vineyards. Therefore, this study was conducted to evaluate effects of light and temperature on MIBP in field-grown and chamber-grown Cabernet Sauvignon grapes.

Materials and methods

Field and growth chamber experiments

The study was conducted from 2005 to 2007 in a commercial Napa Valley vineyard [*Vitis vinifera* L. cv. Cabernet Sauvignon (clone 7) grapevines grafted onto 039-16 rootstock and planted in 1998 near Rutherford, CA (38°27′21″N, 122°25′29″W, elevation 30 m)] with vine and row spacings of 1.83 and 2.44 m, respectively, in an approximate east/west row direction. The vines were trained to a unilateral cordon with vertical shoot positioning and pruned to a single bud at each of 18 spurs per vine. Crop yield varied from 7.4 to 9.9 t ha^{-1}. Experimental clusters were selected to be fully exposed to morning sunlight (south side of vines). Treatments were imposed by covering selected clusters with air permeable, black polyethylene shade cloth. Spectral analysis of light passing through the shade cloth indicated no deviation in quality from natural sunlight. The nominal treatments were 0, 10, 25, 50 and 100% of unshaded controls' PFD (photon flux density). At the initiation of treatments, PFD for the control was 1500 μmol photons m^{-2} s^{-1} t (measured with a Model 185b quantum sensor; LI-COR Inc., Lincoln, NE). Daily time-course of PFD during the growing season was measured with a quantum sensor (model G2711-01 photodiode; Hamamatsu Corporation, Middlesex, NJ) placed beneath the shade cloth covering the clusters and positioned on the upper, outside surface of the cluster. Berry temperature was measured with

a thermocouple (Omega HH23 thermocouple; Omega Engineering Inc., Stamford, CT) inserted into a berry located on the exterior of the cluster directly beneath the shade cloth. Light and temperature probes were connected to a Campbell Scientific data-logger (CR10; Campbell Scientific Inc., Logan, UT). The shade treatments were imposed on individual clusters from: berry set-to-veraison (onset of ripening), S–V; berry set-to-harvest, S–H; and veraison to harvest, V–H. The experimental design was a completely randomized design. Treatments were imposed across 12 rows and individual treatments replicated down the row. Each treatment was replicated four times. Each plot consisted of six contiguous vines where 8–10 south facing clusters were shaded.

Degree days (DD) were calculated using a base temperature of 10°C and the single sine method and air temperature data obtained from a California Irrigation Management Information System weather station (#77) located approximately 8 km from the vineyard site. During the 2007 growing season, leaf and cluster water potentials were measured twice (once in July and once in August) as previously described (Greenspan et al. 1996, Williams and Araujo 2002). Briefly, leaf water potential was measured on mature leaves exposed to direct sunlight (on the south side of the canopy) or completely in the shade (on the north side of the canopy). Leaf blades were enclosed in plastic bags, the petioles immediately cut, and leaf and plastic bag placed into a pressure chamber. The procedures to measure cluster water potential were similar to those used to measure leaf water potential. For the July water potential measurements, clusters were enclosed in shade cloth to exclude all light 3 h prior to measurement. In August, sampled clusters were from the S–H 0% treatment. The same vines were sampled for leaves and clusters on both dates.

At commercial harvest, the soluble solids concentration in the fruit varied only slightly among treatments within a given year and among years, and 150–200 g berry samples were randomly selected from the exterior portion of all experimental clusters by cutting the pedicel with a pair of scissors. The berries were placed into 50 ml Falcon® tubes (Falcon Plastics, Los Angeles, Calif., USA) or plastic bags and then into a chest-containing dry ice for transport to the laboratory where the samples were stored at −65°C until analyses were performed. Soluble solids were determined on berry samples with a temperature-compensating refractometer (Reichert model AR200 digital refractometer; Reichert Analytical Instruments, Depew, NY). The concentration of MIBP in berries was determined as described in Koch et al. (2010) using headspace solid-phase microextraction (SPME) combined with GC-MS in the selected ion-monitoring mode (Ebeler 2001), as described below.

For the controlled environment study, temperature and light treatments were imposed on 3-year-old Cabernet Sauvignon potted vines growing in two identical growth chambers. The temperature regime was set to the 30-year average daily temperatures found at Davis, CA, during June, July and August in one chamber, and to the 30-year average for Santa Maria, CA, in the other chamber (Table S1, supporting information). Half of the clusters were covered with air permeable, black polyethylene shade cloth. The PFD measured at the height of the clusters was close to zero for the shade cloth treatment and 500–600 μmol photons m^{-2} s^{-1} for the non-shaded clusters. Each growth chamber contained eight fruiting vines, four with each light treatment. The diurnal photoperiod in each chamber was from 06:00 to 20:00 h with light slowly increasing during the morning hours and slowly decreasing prior to the lights turning off. Berries were sampled at 22°Brix, approximately 100 days after anthesis. Berries were immediately frozen after harvest and stored at −65°C until thawed and analyzed for MIBP. Data from the growth chamber study were analyzed via a one way ANOVA. Each individual vine was considered replicate. Means were compared using Duncans Multiple Range Test and considered significantly different at $P < 0.05$.

Analysis of MIBP

MIBP concentrations were measured in berries using the method of standard addition as described by Koch et al. (2010). Briefly, 36 g of frozen whole berries were homogenized on ice till smooth (Omni Homogenizer GLH 80 equipped with a 20 × 195 mm Saw Tooth Generator Probe Model #G29-195@T; Omni International, Marietta, GA) with 10 ml of an aqueous 2 m*M* NaF solution containing 200 ng l^{-1} of internal standard, 2-($^2D_3$)-methoxy-3-isobutylpyrazine (dMIBP; CDN Isotopes, Pointe-Claire, Quebec, Canada, 98% atom % D) and either 0, 4.0, 8.0, 20, 40, 60, 120 or 200 ng l^{-1} of MIBP standard (Sigma Chemical Co., St. Louis, MO). The homogenate was then centrifuged (Eppendorf Model 5403, Westbury, NY) at 5000 rpm for 5 min at 4°C. Replicate 36 g samples of each treatment were analyzed.

The homogenate from 36 g of berries was split into three 10-ml aliquots; each aliquot was transferred to a 20-ml glass, round-bottom, amber, screw cap headspace sampling vial (Supelco, Bellefonte, PA) containing 3.0 g NaCl. Extractions were performed using a 23 gauge, 2 cm divinylbenzene/Carboxen™/polydimethylsiloxane (PDMS/DVB/CARB) solid-phase microextraction fiber, that was exposed to the headspace of each sample

Table 1. Dates of anthesis, berry set, veraison and harvest. DOY is given in parentheses following each calendar date.

Year	Anthesis	Berry set	Veraison	Harvest
2005	May 24 (144)	May 31 (151)	July 30 (211)	September 20 (263)
2006	May 18 (138)	May 24 (144)	August 10 (222)	September 27 (270)
2007	May 30 (150)	June 4 (155)	July 25 (206)	August 19 (231)

vial and the sample extracted for 30 min at 40°C with continuous agitation. The fiber was then removed from the vial, placed into the GC-MS inlet (0.7 mm straight glass liner), held in the inlet at 260°C in splitless mode for 5 min for the analytes to desorb from the fiber, and finally the inlet flow was switched on at 50 ml min^{-1} with the fiber in the inlet for an additional 5 min.

Analyses were performed using an Agilent 6890 GC with a 5973MSD (Agilent, Santa Clara, CA) and Gerstel MPS2 autosampler (Gerstel Inc., Columbia, MD). The column was an HP 5MS capillary column (30 m length × 0.25 mm ID; 0.25 µm film thickness; Agilent). The GC oven temperature was maintained at a constant temperature of 40°C for 5 min, then increased 2.5°C min^{-1} to 80°C, 5°C min^{-1} to 110°C and 25°C min^{-1} to 230°C before holding steady for 5 min. The mass selective detector (MSD) interface was held at 280°C and the carrier gas was helium at a constant pressure of 4.77 psi with a nominal initial flow of 0.8 ml min^{-1} and average linear velocity of 32 cm s^{-1}. Selected ion monitoring was used at mass channels of m/z = 94 and 124 for MIBP and m/z = 127 and 154 for dMIBP. Peak areas of the ions m/z 124 and 127 were used for quantification and ions m/z 94 and 154 were used for qualification. Retention times for dMIBP and MIBP were ~26.17 and ~26.23 min respectively.

A standard addition calibration curve was prepared for each sample and a linear regression equation was calculated using the peak area ratio of MIBP relative to dMIBP after correcting the standard concentration for the dilution by the grapes. The concentration of MIBP in the sample supernatant was calculated at the point of the linear regression equation where the y-intercept was equal to zero. MIBP concentrations originally in the fruit were corrected for the dilution of the original grape sample during homogenization and expressed as pg MIBP per g fruit (i.e. 36 g berries diluted with 10.0 ml of aqueous dMIBP/MIBP solution and assuming a density of 1.0 g ml^{-1} for the standard solution).

Results

Field measurements

Of the 3 years in this study, the weather and vine phenology were similar in 2005 and 2007, but differed in several important ways in 2006. The season began earliest and had the longest time between bloom and veraison in 2006 (Table 1). Dates of bloom and berry set were 1–2 weeks later in 2005 and 2007 compared to 2006; and veraison was 1–2 weeks earlier in 2005 and 2007 compared to 2006 (Table 1). The duration from veraison to harvest was similar in 2005 and 2006, but was unusually short in 2007 (Table 1). The number of hot days between bloom and veraison was also greater in 2006 than in 2005 and 2007 (Table 2). Although accumulated DD up to set was low, accumulated DD up to veraison was greatest in 2006, and was therefore greatest between set and veraison in 2006 (Table 2). The number of hot days and accumulated DD up to harvest were also greatest in 2006 (Table 2). The warmer season in 2006 may have contributed to earlier fruit development and higher soluble solids at harvest, which were 24.4, 26.6 and 22.7°Brix in 2005, 2006 and 2007, respectively.

The daily patterns of PFD and cluster temperature measured in August of 2007 (Fig. 1) were similar to most dates in previous years, 2005 and 2006, and during the 2007-growing season. In this particular vineyard, the ambient PFD measured in the fruiting zone increased rapidly once the sun was south of the east/west row axis (approximately 1000), remained high until approximately 1400 and then decreased rapidly as the sun moved north of the row axis (Fig. 1A). The nominal-shading treatments (% ambient) corresponded to the midday light intensities. The daily integrated PFD for the nominal 10, 25 and 50% shading treatments on four different dates in 2007 were 2, 18 and 51% of the non-shaded control, respectively (Table 3). In general, cluster temperature was within 1°C of the unshaded controls (Table 3), although the 0% treatment was slightly

Table 2. Accumulated DD from April 1 to berry set (S), veraison (V) and harvest (H), and accumulated days with maximum daily temperature 30°C or greater from berry set-to-veraison (S–V) and berry set-to-harvest (S–H).

Year	DD April 1 to S	DD April 1 to V	No. of days >30°C S–V	DD April 1 to H	No. days >30°C S–H
2005	335	911	21	1381	42
2006	293	1129	37	1553	52
2007	410	911	20	1156	31

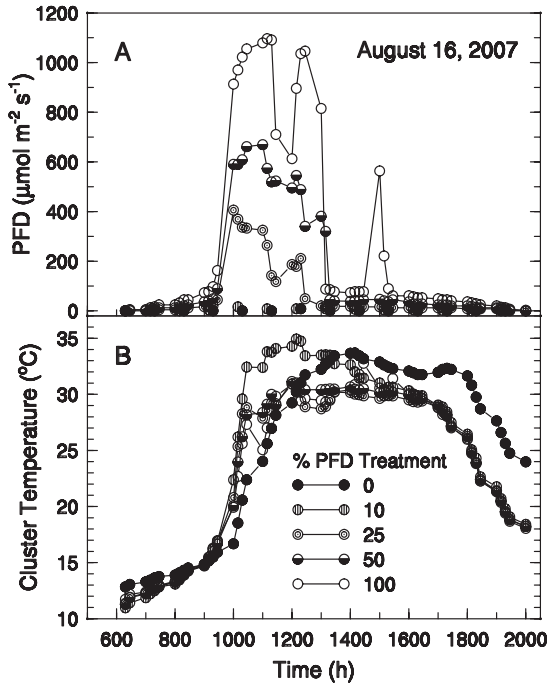

Fig. 1. The diurnal time-course of PFD (A) and cluster temperature (B) measured at the surface of clusters exposed to various shade treatments on August 16, 2007. Integrated PFD values for the entire day for this date and four others in 2007 are given in Table 3.

Table 3. Integrated PFD and mean cluster temperature measured on selected days during the 2007 growing season. Light was totally excluded for the 0% treatment with the use of shade cloth while that of the 100% treatment was not covered with shade cloth. Mean cluster temperature was calculated from measurements taken during the same time frame as used for the measurement of PFD. For reference, integrated ambient PFD (measured parallel to the soil surface) would average from 45 to 50 mol photons m^{-2} day^{-1} on a cloudless day at this location across dates.

	Cluster shade treatments (% of ambient control)				
Date	0	10	23	50	100
	PFD (mol photons m^{-2} day^{-1})				
June 27 (178)	0	0.18	1.16	5.19	11.3
July 26 (207)	0	0.30	2.10	4.72	12.8
August 26 (283)	0	0.20	3.07	7.52	13.5
September 9 (252)	0	0.20	2.27	6.28	8.94
Mean	0	–	–	–	11.6
	Cluster temperature (°C)				
June 27	28.2	27.7	27.0	27.0	27.2
July 26	24.8	24.0	23.3	23.3	23.7
August 26	26.0	25.7	24.3	24.5	24.3
September 9	25.5	25.8	24.4	24.5	24.6
Mean	26.1	25.8	24.8	24.8	25.0

greater compared to the other treatments in the afternoon (Fig. 1B).

The water potential of shaded clusters located in shaded part of canopy was approximately 0.05 and 0.2 MPa higher than sunlit clusters or shade cloth covered clusters at veraison and harvest, respectively (Table 4). Cluster water potential decreased approximately 0.6 MPa between veraison and harvest (Table 4). The water potential of shaded leaves was approximately 0.15 and 0.3 MPa higher than in sunlit leaves.

Table 4. Leaf and cluster water potentials measured at midday (12:45 to 13:45 h) on July 24 and August 22, 2007. Measurements were taken on leaves and clusters either exposed to direct sunlight or in the vines' shade. Cluster water potential was also measured on clusters in direct sunlight covered with shade cloth to exclude all light. Ambient temperature and vapor pressure deficit at the time of measurements on July 24th were 31.1°C and 2.95 kPa, respectively. Ambient temperature and vapor pressure deficit at the time of measurements on August 22nd were 33.9°C and 3.71 kPa, respectively. Values are means + SE (n = 8 for July and 6 for August).

Date	Organ	Exposure	Water potential (MPa)
July 24	Leaves	Sunlit	−0.89 +0.02
		Shaded	−0.73 +0.03
	Clusters	Sunlit	−0.74 +0.02
		Shaded	−0.68 +0.01
		Sunlit w/shade cloth	−0.73 +0.02
August 22	Leaves	Sunlit	−1.33 +0.01
		Shaded	−1.03 +0.02
	Clusters	Sunlit	−1.45 +0.02
		Shaded	−1.26 +0.01
		Sunlit w/shade cloth	−1.42 +0.02

MIBP measurements in shading treatments

From a similar initial concentration of about 60 pg g^{-1} fresh wt the concentration of MIBP increased about fourfold in the shaded treatment and about threefold in the exposed fruit (Fig. 2). The maximum concentration occurred at Day of Year (DOY) 190 in exposed fruit, but continued to increase until DOY 204 in shaded fruit (Fig. 2). At the final sample date, the concentration of MIBP was 50 ± 5 and 17 ± 2 pg g^{-1} fresh wt in shaded and exposed fruit, respectively.

The concentration of MIBP in harvested fruit was dependent upon the shading treatments, although the concentration of MIBP in all fruits in 2006 was so low such that the differences were not detectable (Fig. 3B). For fruit exposed to treatments from set-to-veraison, the concentration of MIBP was inversely to light intensity from 0% to about 50% of full sunlight in each season (Fig. 3A–C). Thus, clusters exposed to the lowest light intensity both between berry set and veraison and between berry set and harvest had the highest concentrations of MIBP in the berries at harvest, and the concentration of MIBP was less with each increase in light intensity down to a value that was about 50% of the maximum. In contrast to the halving of MIBP

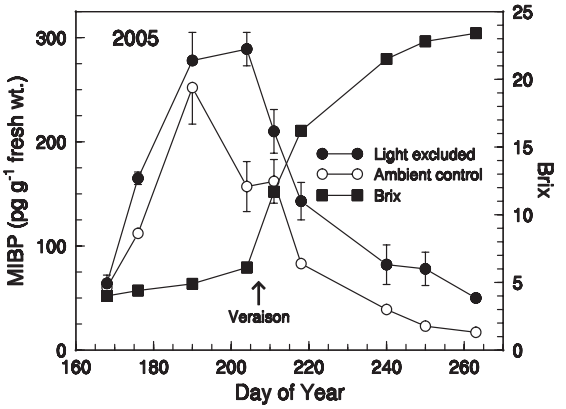

Fig. 2. The concentration of MIBP in berries of Cabernet Sauvignon at various times during the 2005 season for clusters that were either shaded to exclude all light or not shaded at any time during the growing season. DOY for the highest values of MIBP for the no light and ambient treatments were 204 and 190, respectively. Each data point is the mean of at least four replicates, and the bars represent one SE. The seasonal pattern for other treatments was similar.

concentration caused by light treatments prior to veraison, there was no light response of MIBP concentration to light treatments after veraison (Fig. 3B, C). The light response of MIBP in the various treatments was very similar in 2005 and 2007 (Fig. 3A, C), and the concentration of MIBP in all fruits was very low in 2006 (Fig. 3B).

The effects of light on the concentration of MIBP earlier in the season were similar to those observed at harvest. The highest concentration of MIBP before veraison was about 250 and 175 pg g^{-1} FW at the lowest light intensity in 2005 and 2006, respectively (Fig. 4A). The concentration of MIBP decreased with increasing light exposure down to about 50% full sunlight. The light response curves showed no further response as light was increased from 50 to 100% of ambient sunlight (Fig. 4A). Similarly at veraison, the concentration of MIBP decreased from high values of 200 and 100 pg g^{-1} FW at the lowest light intensity in 2005 and 2006, respectively, to values approximately 50% of the high values at light intensities that were 50% of full sunlight (Fig. 4B).

Growth chamber measurements

In order to evaluate the relative effects of temperature and light on MIBP in grape berries, potted Cabernet Sauvignon grapevines were placed in identical growth chambers just prior to berry set, and the same shade treatments were imposed under two air temperature regimes. The temperature regime in one of the chambers was programmed to simulate Davis, CA (warm), for the months of June, July and August, while the other chamber was programmed to simulate Santa Maria, CA (cool),

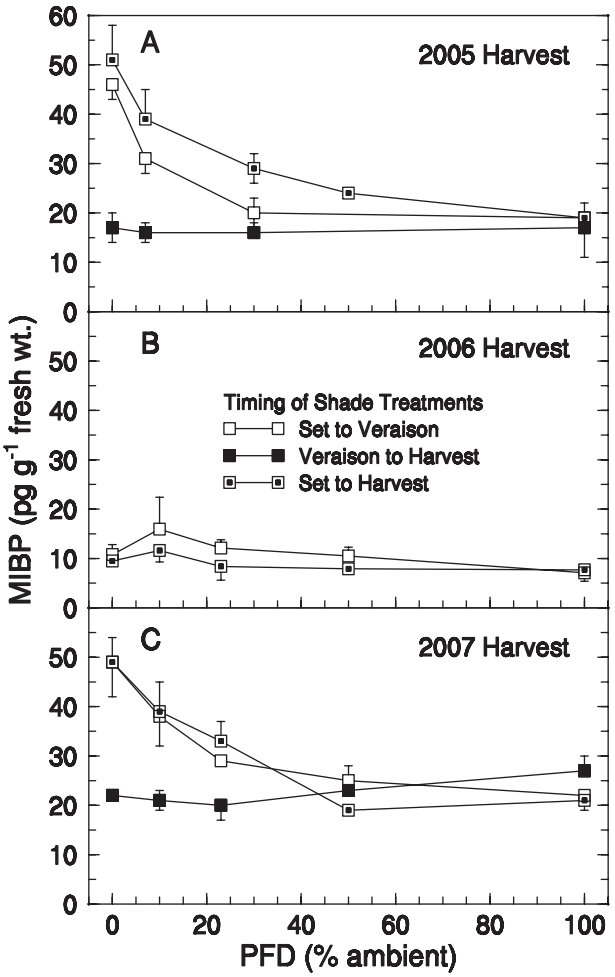

Fig. 3. The concentration of MIBP in Cabernet Sauvignon berries sampled at harvest from clusters exposed to various shading treatments during different stages of berry development in (A) 2005, (B) 2006 and (C) 2007. Berries were sampled on September 20, 2005; September 27, 2006 and August 19, 2007. Each data point is the mean of four replicates, and the bars represent one SE.

during the same months. The concentration of MIBP in the fruit at harvest from the low temperature/low light regime (LTLL) was about sixfold greater than that in the high temperature/high light (HTHL) treatment (Fig. 5). Decreasing either the temperature or the light, increased the concentration of MIBP about threefold over the low value obtained in HTHL, but the means were not significantly different at the conventional $P < 0.05$.

Discussion

The results showed that the cluster light environment and ambient temperature are both important in determining the concentration of MIBP in grape berries. Complete shading of clusters in the field increased the

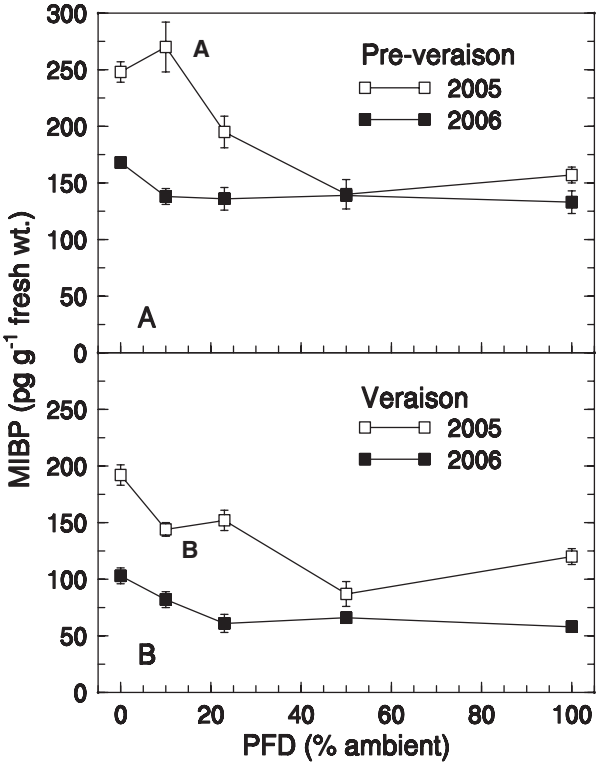

Fig. 4. The concentration of MIBP in Cabernet Sauvignon berries from clusters exposed to different shading treatments in 2005 and 2006 growing seasons for fruit sampled (A) before veraison and (B) at veraison. Pre-veraison berries were sampled on July 9 and July 18 in 2005 and 2006, and post-veraison berries were sampled on July 30, 2005 and August 10, 2006, respectively. Each data point is the mean of eight replicates, and the bars represent one SE. The data points were generated using samples from the set-to-veraison and set-to-harvest treatments, which before veraison had received the same amount of light.

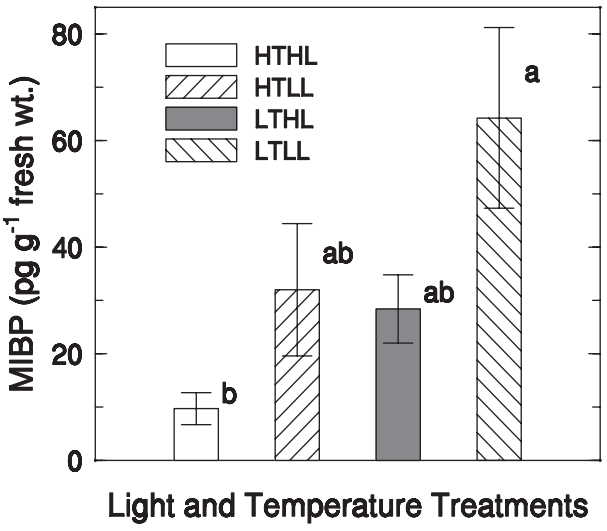

Fig. 5. The concentration of MIBP in Cabernet Sauvignon berries sampled at harvest from potted grapevines grown in two identical growth chambers with different air temperature and cluster-shading treatments. HT and LT refer to daytime maximum of 29–32 and 22–25°C, respectively. HL and LL refer to daytime ambient light and complete light exclusion, respectively. Values represent the means of four replicates, error bars represent one SE, and different letters indicate significant differences $P < 0.05$.

concentration of MIBP more than 100% compared to unshaded controls in 2 out of 3 years. Varying light early and late in fruit development showed that the role of light was important only when varied before ripening commences; the concentration of MIBP in berries at harvest was unaffected by shading treatments after veraison. By varying the light intensity on fruit at different stages of development, light response curves were developed for the inhibition of MIBP accumulation. These curves showed that only light early in fruit development affected MIBP in harvested fruit, and that the (inhibitory) light effect saturated at 25–50% of full sunlight. Canopy management to manipulate light penetration after veraison may be effective for controlling fruit color or for other reasons, but not for controlling the concentration of MIBP.

The results of the various shading treatments allow some insight into the role of light in fruit MIBP concentrations. The concentration of MIBP increased several fold, reaching a peak about 2 weeks before veraison, and then decreased at a decreasing rate throughout the rest of the season. In the only other study to monitor MIBP in fruit from early in the season, a very similar pattern was observed (Ryona et al. 2008). Light might inhibit synthesis, increase rate of loss, or possibly accelerate development to cause the peak and subsequent decrease to begin sooner than in clusters not exposed to light. If MIBP in grapes is controlled by the environment during ripening (Roujou de Boubée et al. 2000), we would have expected to see higher MIBP concentration in the clusters shaded only from veraison to harvest, but we found the opposite. Shading the clusters at any fraction of ambient light after veraison did not significantly affect the MIBP concentration in the berries at harvest compared to the unshaded control treatment. All early season treatments that increased light above nil, reduced the accumulation of MIBP. Altering the light environment pre-veraison and continuously through the whole season produced similar results, but altering light during ripening was ineffective. The light treatments before veraison, after veraison and all season had no effect on the decline of MIBP during ripening. The rate of loss of MIBP from the peak concentration was the same in shaded and exposed fruit (0.014 pg MIBP g^{-1} fresh weight day^{-1}; $P > 0.05$). These data indicate that it is the light environment experienced by the clusters from berry set-to veraison that

determines the MIBP concentration in the fruit at harvest. Although Hashizume and Samuta (1999) observed a small increase in MIBP when excised berries were exposed to light (compared to dark), it is clear from the results of this study, in which MIBP was present in all berries whether exposed to light or not and the highest concentrations of MIBP were present in fruit exposed to no light, that light is not required for biosynthesis. There are no other studies in which the response of berry MIBP to light was evaluated by perturbing incident light at different stages of development.

Ryona et al. (2008) reported a similar decline during ripening for clusters shaded and exposed the whole season, and, accordingly, show the excellent predictive potential of final (MIBP) from peak (MIBP) (see Fig. 5 in Ryona et al. 2008). The peak of MIBP was later in the 0% light treatment than in the 100% light treatment in this study. Thus, it is possible that light exclusion delays slightly the transition from MIBP accumulation to MIBP loss. However, veraison is not delayed by light exclusion in Shiraz (Downey et al. 2004) and only slightly if at all in Cabernet Sauvignon (Smart et al. 1988, Dokoozlian and Kliewer 1996). Thus, the evidence indicates that the inhibition of MIBP accumulation early in berry development is the primary effect of light on MIBP in harvested fruit.

That the light effect on MIBP was negative and saturated at 25–50% of ambient light suggests a different mechanism than that involved in light stimulation of anthocyanin biosynthesis, where light saturation occurred at about 10% of full sunlight (Dokoozlian and Kliewer 1996). Several studies have reported differences in MIBP concentrations associated with aspects of grapevine canopy structure or putative 'exposure' (Sala et al. 2004). Failure to measure and report light intensities renders experiments unrepeatable for purposes of testing the role of light in fruit development. Light intensities, inferred from counts of leaf layers necessarily involve approximately 90% decreases in light for each layer (providing limited resolution of the light response for physiological processes that are light sensitive), are accurate only for direct beam radiation along the single axis of measurement, and require large numbers of measurements for accurate estimates of cluster light environments (Wilson 1960). Furthermore, highly accurate light sensitive diodes are readily available at low cost. The shade treatments used here, various layers of neutral density cloth, maintained the relative shade effect throughout the day within the normal diurnal increase and decrease in ambient light intensity. These shade treatments are necessarily different than relying on the shade of leaves, which are not neutral density and are not uniformly displayed between cluster and sun during the day.

The seasonal pattern of MIBP in grapes, with a significant decrease in concentration during ripening, is similar to other fruits (Luning et al. 1994). Although light has had no effect on the rate of decrease (this study, Ryona et al. 2008), other factors such as air temperature (Allen et al. 1991), vine water status (Chapman et al. 2005) and crop load (Chapman et al. 2004a, 2004b) may. The midday water potential of clusters exposed and clusters under shade cloth were not different. Thus, the effect of shade cloth treatments on MIBP is not attributable to an indirect effect on cluster water status. However, both the shade cloth covered and fully exposed clusters were significantly different from clusters grown naturally in the shade. Unfortunately we did not analyze MIBP in the naturally shaded clusters in this study, but this difference in cluster water status may have played a role in other 'naturally' shaded studies (Ryona et al. 2008). Therefore, the effect of shading on MIBP in this study was probably not via altered cluster water status. Chapman et al. (2004a) showed that wines made from vines pruned to carry higher crop loads had lower concentrations of MIBP and lower vegetal intensity in aroma and flavor. Cluster thinning at veraison did not produce a similar response (Chapman et al. 2004b). Thus, early crop load may affect MIBP accumulation, but there were no differences in crop load among the shading treatments in this study.

We attribute the failure to realize similar shading effects on MIBP in one of three seasons to the very low MIBP in all light treatments at all sample dates in 2006. The low MIBP concentrations at harvest were all below the sensory threshold for wines (ca 8 ng l^{-1}, Allen et al. 1991). We further attribute the low MIBP in 2006 fruit to high temperatures early in fruit development, during the period of MIBP accumulation. Despite the cluster light environment being frequently invoked in the control of MIBP, there are several reports that MIBP in fruit or wines is inversely related to the fruit growth temperatures (Allen et al. 1991, Hashizume and Umeda 1996, Becnic and Agosin 2007, Falcao et al. 2007). Falcao et al. (2007) found a strong negative correlation between growing regime temperature and the concentration of MIBP in commercial wines made from fruit grown in those environments. Wine TA and pH did not show a dependence upon the temperatures, suggesting that MIBP is more sensitive to air temperature than organic acid metabolism. The 2006 season in this study was populated with more hot days and heat accumulation than in the other 2 years of the study. The last berry sample date in 2006 took place 1553 DD after berry set compared to 1381 and 1156 DD in 2005 and 2007, respectively. The 2006-growing season had more days between set and veraison in which the maximum

temperature was greater than 30°C than did the other two growing seasons. The growth chamber experiments indicated that warmer temperatures reduce the MIBP concentration probably as much as light exposure in the same light environment, although it is not clear if the temperature sensitivity is dependent on the berry developmental stage as well. The temperature effects are probably due to a low temperature optimum for MIBP biosynthesis rather than increased vapor pressure of MIBP in berries. Although MIBP is not present as a glucoside, and should be subject to temperature regulated volatilization, it has a high boiling point (210°C). Finally, interactions of light and temperature are essentially inevitable in the field (Spayd et al. 2002), and at higher intensities, the light energy converted to heat may itself be important in reducing MIBP in the berry. Thus, negative correlations of MIBP with air temperatures in the vineyard and in separate controlled environment studies showed that high temperatures early in fruit development were probably responsible for the low concentration of MIBP in the one season that shading treatments were ineffective (Allen and Lacey 1993).

A vegetal component has traditionally been part of Cabernet Sauvignon varietal character (Noble et al. 1995), and related varieties can have very high MIBP concentrations (Belancic and Agosin 2007, Carmenere; Lacey et al. 1991, Sauvignon blanc). However, recently preferences have moved away from any vegetal character in Cabernet-type wines (John Thorngate, personal communication). The industry consensus aversion to vegetal Cabernet-type wines has increased interest in MIBP and means to reduce its concentration, and may contribute to the sense that herbaceous character represents unripe fruit. Although there is good evidence that MIBP, when present at sufficiently high concentrations is responsible for the vegetal taste and aroma of wine made from some varieties such Sauvignon blanc (Allen et al. 1991), this is not the case when MIBP is present at concentrations near the sensory threshold in, e.g. Cabernet Sauvignon (Preston et al. 2008), where other solutes can contribute to or mask vegetal aromas (Chapman et al. 2005, Hein et al. 2009).

Finally, the rapid and early (pre-veraison) decrease in MIBP may indicate its role in fruit development as a deterrent to early fruit feeding. Plants need protection from not-helpful herbivores and attraction for seed dispersers. Feeding on berries before seeds are viable would be counterproductive for the grapevine. MIBP is the pyrazine found frequently in toxic insects and plants (Rothschild et al. 1984), and is thought to signal a warning like red color (Rothschild and Moore 1987, Kaye et al. 1989). Thus, high MIBP may provide a volatile signal that the fruit is not good to feed on. The rapid decrease to lowered concentrations of MIBP soon after veraison coincides with fruit (seed) maturity. Although the factors determining bird feeding on grapes are not understood (Saxton et al. 2004a, 2004b), bird feeding is much higher in ripening fruit than in ripe winegrapes at harvest (Herrmann and Anderson 2007). And crop loss to June beetle was highest (95%) when veraison coincided with high June beetle populations, and less when beetle populations peaked later (Hammons et al. 2010). Thus, MIBP may be synthesized early to ward off feeding until the seed maturity at the onset of ripening, and the extended ripening in domesticated varieties that interests humans may be but an afterthought for grapevines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Daily temperature (°C) regimes in the two growth chambers based upon a 5-year average at Davis and Santa Maria, California, for June, July and August.

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