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Effects of Cluster Light Exposure on 3-Isobutyl-2-methoxypyrazine Accumulation and Degradation Patterns in Red Wine Grapes (*Vitis vinifera* L. Cv. Cabernet Franc)

IMELDA RYONA,[†] BRUCE S. PAN,[†] DIEGO S. INTRIGLIOLO,[§] ALAN N. LAKSO,[#] AND GAVIN L. SACKS*,[†]

Department of Food Science and Technology and Department of Horticulture Science, Cornell University, 630 West North Street, Geneva, New York, and Instituto Valenciano Investigaciones Agrarias, Centro Desarrollo Agricultura Sostenible, Apartado Official 46113, Moncada (Valencia), Spain

The effects of light exposure on 3-isobutyl-2-methoxypyrazine (IBMP) accumulation and degradation in *Vitis vinifera* L. cv. Cabernet Franc berries were assessed by comparison of shaded and exposed clusters within the same vine throughout a growing season. Twenty-seven vines were shoot-thinned to create regions of high and low cluster-light exposure within each vine. Samples were collected at 10 time points starting from 5 to 130 days postbloom. The experimental design allowed for intravine comparison of IBMP levels between treatments at each time. Vine-to-vine variability of IBMP and the correlation of IBMP to malic acid were also evaluated. Cluster exposure reduced accumulation of IBMP at all preveraison time points by 21−44%, but did not increase postveraison degradation. Significant vine-to-vine variability in IBMP content was observed, with the highest level of IBMP in shaded berries in the most vigorous block of vines. Although IBMP concentration by weight decreased significantly due to dilution just prior to color change (veraison), no significant IBMP degradation per berry occurred until after color change (day 70 postbloom). By contrast, malic acid degradation began prior to color change, and malic acid concentrations were not affected by cluster exposure preveraison, but were affected postveraison. A survey of 13 sites in New York state (Seneca Lake) showed that IBMP concentrations at 2 weeks preveraison were highly correlated ($R^2 = 0.936$, $p < 0.0001$) to levels at harvest, whereas classic grape maturity indices at harvest were uncorrelated with IBMP at harvest. In summary, light exposure conditions critically influence IBMP accumulation but not IBMP degradation.

KEYWORDS: Cluster shading; GC×GC-TOF-MS; wine; grapes; herbaceousness; 3-isobutyl-2-methoxypyrazine; IBMP

INTRODUCTION

The 3-alkyl-2-methoxypyrazines (MPs) are a class of odorants possessing herbaceous, musty, and unripe aromas in wine (*1*). Although they are widely distributed in the plant kingdom (*2*), most reports on MPs in recent years have focused on their presence in wine grapes (*Vitis vinifera*), and especially the red and white Bordeaux varieties (e.g., Cabernet Sauvignon, Cabernet Franc, Merlot, and Sauvignon Blanc). The MP usually considered to be the most relevant to wine flavor, 3-isobutyl-2-methoxypyrazine (IBMP), is well-correlated with the intensity of the wine's "bell pepper" character (*3*). 3-*sec*-Butyl-2-methoxypyrazine (sBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) are present at lower concentrations in wine grapes (*4, 5*), although IPMP may be much higher in wines contaminated by the multicolored Asian ladybug (*6, 7*).

The sensory threshold of IBMP is variously reported as 0.5−2.0 ppt in water (*8−11*) and 10−16 ppt in red wine (*1, 3, 9*). At levels near this threshold, IBMP can contribute positively to the varietal character of some wines, but excessive levels are unpleasantly green and herbaceous (*12, 13*). Descriptive analyses of wines often show that vegetal and fruity characters are inversely correlated (*14−17*). As the latter is generally more desirable to consumers, controlling IBMP in wine is an important challenge for the wine industry. The IBMP content of finished red wines is largely dependent on their concentration in grapes at harvest (*12*). IBMP is quantitatively extracted early

* Author to whom correspondence should be addressed [e-mail gls9@cornell.edu; telephone (315) 787-2458; fax (315) 787-2397].
[†] Department of Food Science, Cornell University.
[§] Centro Desarrollo Agricultura Sostenible.
[#] Department of Horticulture Science, Cornell University.

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in fermentation using conventional red winemaking techniques (*18*). Common cellar practices such as bentonite fining (*6*), oak contact (*6, 19*), pectinases (*19*), and microoxygenation (*20*) do not affect IBMP levels. Other practices such as thermovinification (*21*) and activated charcoal (*6*) can reduce IBMP, but lack selectivity and thus may remove desirable components from the wine.

Because of the difficulty of removing IBMP in the winery, it is important to understand how environmental or physiological conditions influence IBMP levels in the vineyard. Several studies have reported that IBMP concentrations in Cabernet Sauvignon rise after fruit set, peak around or just before veraison, and then decline toward harvest (*18, 22, 23*). In general, light-exposed clusters are reported to have reduced IBMP levels at harvest, although there is some ambiguity in the literature. Several studies have reported that preveraison cluster exposure will result in decreased IBMP levels at harvest (*18, 21, 24, 25*). For example, Roujou de Boubee noted a 68% decrease in IBMP concentration in harvested grapes for vines subjected to leaf removal and shoot thinning prior to veraison (*21*), but leaf removal treatments after veraison had minimal impact (10% decrease). Marais et al. reported reductions in IBMP levels in Sauvignon Blanc by up to 50% in light-exposed berries for several sites in South Africa studied over two years, and careful inspection of their data show reduced IBMP at both veraison and harvest (*24*). IBMP concentrations at both veraison and harvest have been reported to correlate with cluster shading, reported as leaf layer number (LLN) (*4*). Well-exposed berries with a LLN = 0 had ~58% lower IBMP than berries with LLN ≥ 3 at both veraison and harvest. By contrast, no significant decrease in IBMP at harvest has been observed when exposure treatments are implemented during or after veraison. For example, cluster shading after veraison resulted in no significant difference in IBMP at harvest, and in a slight increase (~2 pg/g) in IBMP in finished wines (*22*). Thus, preveraison cluster exposure appears to be critical to reducing IBMP levels at harvest. Grape berries detached preveraison and exposed to artificial light are reported to accumulate higher levels of MPs than preveraison berries stored in the dark (*23*), although it is possible that the physiology of these harvested berries is not comparable to that of unharvested berries still attached to the vine.

Assuming that cluster light exposure reduces IBMP levels, the mechanism of this effect is unclear. Many authors have attempted to explain these differences as resulting from degradation induced by either light or temperature. Because IBMP will decompose in aqueous solution in the presence of light, cluster light exposure may result in direct photodegradation of IBMP (*1, 26*). Alternatively, reduction in IBMP may be due to thermal degradation, as exposed clusters typically have much higher temperatures than shaded clusters (*27*). Studies on Cabernet Sauvignon (*5*) and Sauvignon Blanc (*13*) have noted an inverse correlation between growing degree days and IBMP concentration. A similar pattern between temperature extremes and IBMP concentration was observed in Brazilian Cabernet Sauvignon (*15*). Furthermore, several authors have commented on the similarity between the patterns of IBMP and malic acid concentrations during the growing season, and the latter is known to be respired more quickly at higher temperatures during berry maturation (*15, 28*−*31*).

As an alternative explanation to either light- or heat-induced degradation, cluster exposure may decrease IBMP at harvest by reducing IBMP accumulation. Higher IBMP levels have been observed preveraison at more vigorous sites (*25*), and higher IBMP at harvest has been associated with conditions that stimulate vine vigor, such as high soil−water availability (*32*) or low shoot number (*12, 33*). One suggested mechanism is that IBMP is translocated from the leaves to the berries via the xylem. According to this hypothesis, increased vigor results in more leaf area and thus more sources of IBMP for the berries (*21*). However, increased vine growth can also lead to greater cluster shading, and thus vigor and exposure effects may be confounded.

In summary, it is evident that cluster light exposure preveraison affects IBMP levels at harvest, but it is unclear if this results from changes in IBMP accumulation or IBMP degradation. Previous time course studies have not compared differences in IBMP within shaded and exposed clusters throughout the growing season. Furthermore, comparisons were made between different vines. Changes in MP levels may thus be obscured by vine-to-vine variability or physiological consequences such as vine growth (vigor).

To assess whether light exposure affects IBMP accumulation or IBMP degradation, we performed intravine comparisons of IBMP levels in shaded and exposed grapes during the growing season. Cane-pruned vines were shoot-thinned to create regions of high and low light exposure along the canes, and similar numbers of shoots were retained along each vine. We evaluated vine-to-vine variability of IBMP within a single vineyard and their correlations to pruning weight and canopy density. We further evaluated correlation of pre- and postveraison IBMP concentrations among vineyards in comparison to classic chemical measures of berry maturity. Finally, we compared IBMP and malic acid concentration profiles to evaluate if the accumulation and degradation of these compounds are correlated.

MATERIALS AND METHODS

Experimental Vineyard. *V. vinifera* L. cv. Cabernet Franc (clone CL327) vines were grafted on rootstock 101-14 and planted in 2005 in Cornell University's experimental vineyard at New York State Agriculture Experimental Station, Geneva, NY (43° N, 77° W). The vineyard soils were officially classified by the USDA as a Lima series (http://www2.ftw.nrcs.usda.gov/osd/dat/L/LIMA.html) with a fine silt loam structure, moderately well-drained structure, and >2 m depth. The experimental plot consisted of 9 rows with 27 vines per row planted on a north/south orientation at a spacing of 2.7 m/row × 2.1 m/vine. Standard pest-control practices were applied, and no disease was observed. Drip irrigation was applied to replace vine water requirements from the sixth week at 5 days postbloom until the end of the season with a total of 135 mm. Irrigation was scheduled according to the soil−water balance approach (*34*) using the crop coefficient values reported by Williams and Ayars (*35*), corresponding to the average leaf area values of the vines used in the experiment. Frequency of water application was high and varied from 3 to 5 days per week. Total rainfall during the growing season was 308 mm. From April 1 to Oct 31, 2007, cumulative growing degree days were 1522 GDD based on 10 °C, which correlates to region II by Winkler's classification system (*36*). More weather data details can be accessed at http://www.nysaes.cornell.edu/weather/.

In the experimental vineyard, an internal row was selected as the '*experimental row*'. These vines were cane-pruned in April 2007 for a vertical-shoot-positioning trellis system with catch wires leaving four canes per vine. Two days before the beginning of bloom (June 16, 2007), vines were shoot-thinned, leaving 9−13 shoots on each side of the vine. The spacing of the shoots was varied to create shaded and exposed regions on each side of the vine (**Figure 1**). The shoots of adjacent vines overlapped and effectively developed well-shaded regions. On August 2 (preveraison) and August 30 (postveraison) at 3 p.m., photosynthetically active radiation (PAR) and cluster temperature were measured on 10 vines × 2 treatments. PAR was measured by a light meter (LI-COR-LI-250; LI-COR Inc., Lincoln, NE) positioned within the fruiting zone. Surface temperatures of shaded and exposed

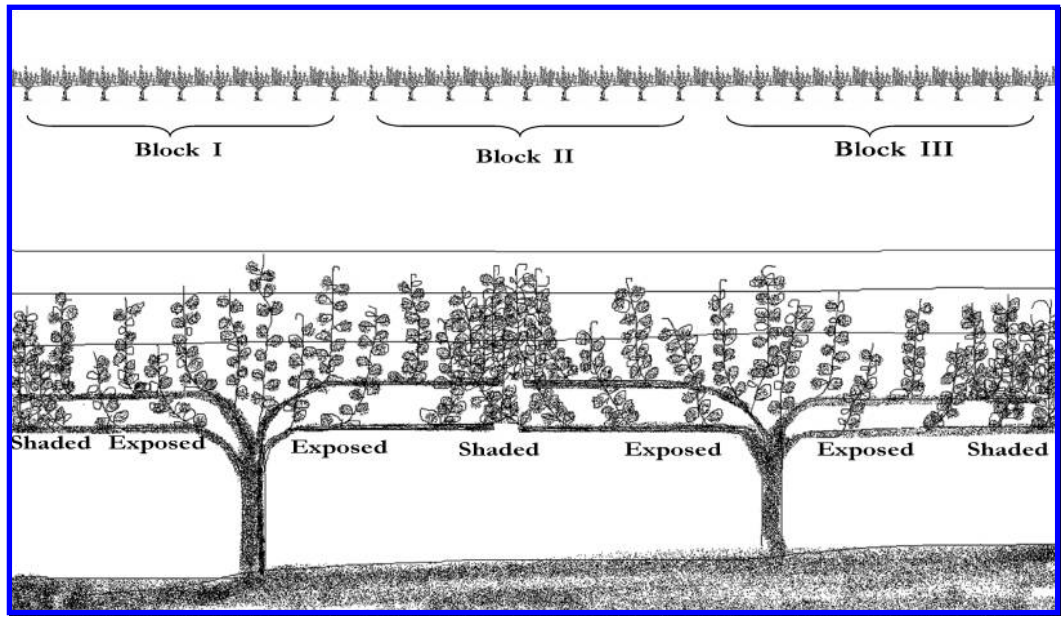

Figure 1. Schematic of the experimental design. Vines were differentially shoot-thinned to create shaded and exposed clusters within the same vine. The shoots of adjacent vines overlapped and efficiently facilitated the architecture of well-shaded regions.

clusters were measured by an infrared thermometer M120E (Micron Infrared, Inc., Oakland, NJ). During the growing season shoots were repositioned weekly to maintain the desired exposures.

Sampling Protocols. The experimental row of 27 vines (**Figure 1**) was divided into three blocks (9 vines per block). There were a total of 10 sampling dates between 5 and 130 days postbloom. The first seven sampling dates were done weekly from 5 days postbloom (June 28) to 47 days postbloom (August 9). The last three sampling dates were scheduled on 70 days postbloom (veraison, September 1, 2007), 100 days postbloom, and 130 days postbloom. The initial color change of berries was noted on August 24. The veraison sample was collected on September 1 (70 days postbloom) at the midpoint of color change.

On the first nine sampling dates, four exposed and four shaded clusters were collected from one vine of each block. Thus, *no vine was repeatedly sampled*. This experimental design minimized biological variability by comparing shaded and exposed clusters within a single vine. Also, because each vine was sampled only once, harvesting would not perturb future measurements. The exception to the sampling protocol was the final sampling date (130 days postbloom), when clusters were collected from two of the previously sampled vines from each block. This change was necessary because all vines in the study had been sampled once before, and insufficient clusters remained to sample exclusively from one vine.

At the end of canopy growth, September 13, we performed point quadrant analysis (PQA) according to the method of Richard Smart (*37*) to evaluate canopy density within the three blocks. Insertions were performed at intervals of 20 cm along the experimental row, for a total of 93 insertions per block. The data collected permitted calculation of leaf layer number, percent interior clusters, percent interior leaves, and percent gaps. In addition, the pruning weight of each vine was determined in April 2008 and expressed on a fresh weight basis.

Intervineyard Comparison. IBMP concentrations of Cabernet Franc grape were measured pre- and postveraison at 13 Seneca Lake sites (11 grower cooperators and our 2 treatments within our experimental vineyard) during the summer of 2007. The sites were selected on the basis of grower cooperation and reflection of a diverse range of soil types. Although the timing of specific practices varied from site to site, all but one site used vertical-shoot positioning and early leaf removal to ensure open canopies, and only well-exposed clusters were sampled. Berries were sampled at day ~47 postbloom on either August 8 or 9, 2007, which was expected to be near the peak value of IBMP concentration on the basis of the results of our experimental plot. Berries were sampled again at harvest between October 6 and 24, 2007, on the basis of grower cooperator evaluations of maturity. Despite the range of harvest dates and maturities, we observed excellent correlation between preveraison and harvest IBMP levels.

Analytical Reagents. NaCl, NaOH, $CaCl_2$, glycine, hydrazine sulfite, and EDTA reagents were purchased from Fisher Scientific (Atlanta, GA). IBMP and SPME fiber (2 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane) were purchased from Supelco (Sigma Aldrich, Bellefonte, PA). Water was purified by a Milli-Q system from Millipore (Bedford, MA). NAD II and MDH enzyme were from Sigma-Aldrich (St. Louis, MO). [$^2H_2$]-IBMP was synthesized in our laboratory according to the method of Kotseridis (*38*).

Berry Analyses: Total Soluble Solid (TSS), Titratable Acidity (TA), pH, and Malic Acid. Immediately after harvesting, berries were detached from the clusters and kept at −80 °C until analysis. Frozen berries (80−100) were selected from random locations throughout the clusters, thawed, and pressed by hand through cheesecloth, and the juice was collected for immediate analyses. Soluble solids were measured by refractometer (Leica Auto ABBE; AO Scientific Instruments, Buffalo, NY). pH was measured by a Thermo Orion Star pH-meter (Walthman, MA). TA was measured by titration against 0.1 N NaOH (Digital Buret III; BrandTech Scientific, Inc., Essex, CT) to pH 8.1. Malic acid was quantified enzymatically, adopted from the method of Mayer and Busch (*39*). The analysis results in conversion of NAD^+ to NADH, which can be monitored spectrophotometrically at 340 nm (Turner spectrophotometer SP-830; Barnstead International, Dubuque, IA).

IBMP Analyses: Extraction and Quantification. Three sample preparation replicates were performed for each sampling point. For each replicate, 30 frozen berries were pulverized at 1650 strokes/min for 2 min using a 2000 Geno/Grinder (SPEX Certiprep, Metuchen, NJ). The homogenized berry paste was diluted by 50% with EDTA/NaOH (pH 7.5). Subsequently, to prevent further enzymatic activity, 5% of $CaCl_2$ was added to the diluted slurry in a beaker with magnetic stirring bar and mixed for 1 min. Ten grams of the berry slurry was transferred to a brown 20 mL SPME vial along with 3 g of NaCl. An internal standard, [$^2H_2$]-IBMP was also added to yield a final concentration of 10 pg/g. The SPME vial was agitated offline for 10 min at 80 °C in a heated ultrasonic bath to ensure quantitative extraction of IBMP from the berry tissue.

The berry sample was extracted by headspace−solid phase microextraction (HS-SPME) using a LEAP CombiPAL autosampler (Carrboro, NC). Optimal sensitivity was achieved with a 10 min online agitation at 650 rpm agitation rate and an incubation temperature of 80 °C prior to fiber insertion. A three-phase fiber (DVB/CAR/PDMS) was then

Table 1. IBMP Concentration (Picograms per Gram of Fresh Weight) of Shaded and Exposed Berries from Fruit Growth to Ripening

	block 1		block 2		block 3		mean % reduction in IBMP in exposed berries[a]
sampling day (postbloom)	exposed	shaded	exposed	shaded	exposed	shaded	blocks 1, 2, and 3
5	3 ± 1	6 ± 1	4 ± 1	7 ± 1	2 ± 1	4 ± 1	44.4 *
12	8 ± 1	11 ± 2	6 ± 1	9 ± 1	7 ± 2	11 ± 3	32.9 *
19	47 ± 8	84 ± 7	32 ± 4	52 ± 11	39 ± 4	60 ± 7	39.7 *
26	75 ± 4	106 ± 7	69 ± 9	83 ± 6	79 ± 13	90 ± 11	21.3 NS
33	99 ± 6	147 ± 8	90 ± 10	103 ± 9	96 ± 11	113 ± 8	21.4 NS
40	174 ± 7	211 ± 8	130 ± 11	213 ± 7	139 ± 16	175 ± 7	25.8 *
47	97 ± 9	180 ± 6	111 ± 12	159 ± 8	103 ± 16	218 ± 9	44.1 *
70	59 ± 25	68 ± 22	69 ± 14	86 ± 38	79 ± 11	90 ± 25	15.3 *
100	14 ± 2	25 ± 7	11 ± 2	22 ± 1	11 ± 2	16 ± 3	40.6 *
130	11 ± 2	16 ± 2	9 ± 1	10 ± 1	16 ± 1	14 ± 1	12.1 NS

[a] An asterisk indicates significant reduction on $p < 0.05$; NS indicates nonsignificant reduction on $p < 0.05$. Days 26 and 33 have significant reduction at $p < 0.08$.

inserted, and the vial was agitated at 100 rpm for 30 min at 80 °C. The extraction time and fiber choice were similar to those reported by other authors (*33, 40*), although our optimized extraction temperature was higher.

Quantification was performed by two-dimensional comprehensive gas chromatography coupled to a time-of-flight mass spectrometer (GC×GC-TOF-MS) (LECO Pegasus 4D, Leco Corp., St. Joseph, MI). GC×GC provides roughly an order of magnitude improvement in peak height and separation space compared to conventional one-dimensional gas chromatography, resulting in lower detection thresholds and fewer overlaps. The utility of GC×GC-TOF-MS in measuring IBMP in wine was recently described (*41*). SPME injections were splitless with a desorption temperature of 270 °C. The first capillary column (30m × 0.25 mm × 0.50 μm) was an RTX5 (Restek, Bellefonte, PA), and the second column (2.5m × 0.10 mm × 0.10 μm) was a VF-WAXms (Varian, Palo Alto, CA). Helium was used as a carrier gas at a flow rate of 1 mL/min. The temperature program was as follows: initial hold for 5 min at 40 °C, followed by a 5 °C/min ramp to 120 °C; then, 2 °C/min to 150 °C, no hold; then 10 °C/min to 250 °C, 15 min hold. The GC×GC modulation time was 3 s. The MS transfer line temperature was 230 °C. The TOF-MS was operated in EI mode with an ionization energy of 70 eV. The electron multiplier was set to 1680 V. The TOF-MS data were stored at an effective acquisition rate of 120 Hz over a mass range of *m/z* 20−400.

Data processing was carried out by ChromaTOF software. The qualifier ions were *m/z* 124, 151, and 166 for IBMP and *m/z* 126, 153, and 168 for [$^2H_2$]-IBMP. The quantifier ions were *m/z* 124 and 126. The total run time per analysis lasted 75 min, including 10 min of online agitation. Calibration standards ($n = 5$) were prepared in EDTA/NaOH (pH 7.5) over a concentration range of 0.5−200 pg/g. A $1/x$ weighted linear regression of areas of 124/126 ions against IBMP concentration resulted in an $R^2 = 0.999$, $p < 0.0001$. The limit of quantification (LOQ) was 1 pg/g, calculated as 10 times the background noise by using the method of Pallesen (*42*).

Statistical Analysis. Statistical analysis was performed by JMP version 7 (SAS Institute, Cary, NC) using ANOVA; comparison of means was analyzed by matched pair *t* test, Student's *t* test, and Tukey HSD. R Development Core Team 2008, ISBN 3-900051-07-0, http://www.r-project.org (R Foundation for Statistical Computing, Vienna, Austria), was used to perform "adjacent pair difference test" with a Bonferroni adjusted 5% significant level to analyze significant differences reflected in **Figure 4**.

RESULTS AND DISCUSSION

Cluster Exposure and Temperatures. Based on pre- and postveraison measurements, clusters in the shaded region along the canopy received 3 ± 2% of full light (100%), whereas those in exposed region received 84 ± 7%. Shaded berries were also significantly cooler under sunny conditions, with differences between shaded and exposed berries of 5.3 ± 1.7 °C for preveraison (August 2) and 7.8 ± 1.5 °C for postveraison (August 30). Because of canopy growth, shoots were repositioned on a weekly basis.

IBMP Accumulation and Degradation during Berry Growth. The vineyard row was divided into blocks 1, 2, and 3, as described under Experimental Design. IBMP concentrations in exposed and shaded clusters for each block and time point are reported in **Table 1**. Quantifiable levels of IBMP (2−7 pg/g) are detected at the first time point, 5 days postbloom. To our knowledge, these are the earliest measurements of IBMP during the growing season and the first confirmation that berries accumulate IBMP immediately upon setting. Maximum values of IBMP for exposed and shaded clusters were in the range of 130−218 pg/g, which is comparable to values observed in Cabernet Sauvignon in other regions (*3, 22*). In both treatments, IBMP concentrations fell markedly over the last three time points (70, 100, and 130 days postbloom). A preveraison peak value in IBMP followed by degradation during ripening has also been described by other authors (*3, 43, 44*). Final values at 130 days postbloom were in the range of 9−16 pg/g.

Intravine Comparison of IBMP Concentration on Shaded and Exposed Berries. Our experimental design allowed us to compare IBMP levels in shaded and exposed berries within the same vine. For preveraison time points, exposed clusters had significantly lower IBMP ($p < 0.05$, matched pair *t* test) at days 5, 12, 19, 40, and 47 postbloom. In addition, days 26 and 33 were significantly lower at $p < 0.08$ (**Table 1**). The reduction at preveraison time points ranged from 21 to 44%, with a mean reduction of 33%.

A comparison of mean IBMP concentration (pg/g of fresh weight) for shaded and exposed berries during the growing season clearly demonstrated that cluster shading results in greater IBMP accumulation prior to veraison (**Figure 2**). A significant reduction of IBMP accumulation in exposed clusters was observed from day 5, indicating that cluster light exposure impacts IBMP accumulation early in berry development. The maximum difference was reached at day 47, prior to veraison. The mean percentage difference in IBMP content between shaded and exposed fruit did not further increase postveraison.

Most strikingly, for all 21 comparisons (3 blocks × 7 preveraison time points), the exposed clusters always had lower IBMP concentrations than their shaded counterparts on the same vine. A significant difference in IBMP levels ($p < 0.05$) was also observed at 70 days (15.3%) and 100 days (40.6%) postbloom. Curiously, no significant difference was observed for the last measurement at 130 days postbloom. It appears that the exposed clusters reached a final low value of 10−15 pg/g at day 100 as there was little change after that date, which is

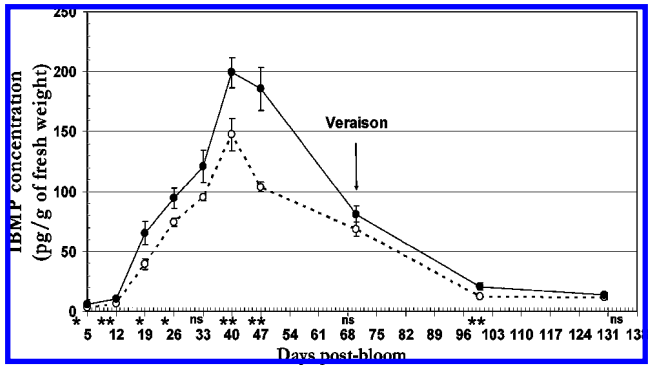

Figure 2. IBMP concentrations of shaded (●—) and exposed berries (○- - -) during the growing season. The error bars reflect standard error for the three replicates (three vines per treatment). Significant differences between treatments were evaluated by a paired *t* test (∗∗, $p < 0.05$; ∗, $p < 0.08$; ns, not significant).

comparable to the reported threshold for IBMP in red wine (*3*). The shaded clusters eventually declined to that value at day 130. It is not clear if there is a physiological "set-point" for final IBMP levels or if IBMP can continue to degrade indefinitely given the right conditions. Preveraison cluster exposure may be less important for reducing IBMP in grapes harvested after extended hang times. Nonetheless, the practical goal is to reduce the concentrations to values below the sensory threshold of 10−16 ppt (*1, 3, 9*) by harvest.

In summary, shading resulted in higher levels of IBMP due to preveraison processes, and not postveraison processes. Whereas previous papers have demonstrated that early cluster light exposure will result in lower IBMP at harvest (*21, 24*), this is the first clear demonstration that cluster exposure affects IBMP accumulation and not degradation. Interestingly, no significant effect of shading was observed at the last sampling day.

Interpretation of Impact of Cluster Exposure on IBMP during the Growing Season. Previous viticultural studies support our observation that preveraison cluster light exposure is more critical than postveraison exposure by reducing IBMP accumulation. Reports that have observed a decrease in IBMP levels as a result of cluster exposure have invariably imposed the treatment preveraison, whereas postveraison treatments have had minimal effects (*21, 22, 24*). Thus, our study's conclusion that IBMP accumulation preveraison is decreased by cluster light exposure is well-corroborated by earlier results. In summary, cultural practices aimed at increasing cluster exposure and reducing the IBMP level at harvest, such as leaf removal or shoot thinning/shoot positioning, must be carried out preveraison to reduce IBMP accumulation. The mechanism by which cluster light exposure mediates IBMP concentration at a molecular level is unknown. We believe it is unlikely that a direct light or thermal degradation mechanism can explain the preveraison differences, as we would expect to see this phenomenon persist after veraison when the berries darken and thus are relatively warmer.

Vine-to-Vine Variation of IBMP Concentration within the Same Treatment. Our experimental design allowed us to compare the variability of IBMP within the same vineyard site. At each time point, clusters were sampled from one vine of each block (**Figure 1**), which allows evaluation of vine-to-vine variability for both shaded and exposed clusters among blocks. Between days 19 and 40, we observed mean vine-to-vine relative standard deviations (%RSD) of 18 and 12% for shaded and exposed clusters respectively. The higher degree of vine-to-vine variability is reflected by larger standard error for the shaded clusters (**Figure 2**). This is likely because the shaded canopies were more heterogeneous in their nature. After veraison (days 100 and 130), the mean %RSD values were 25 and 22% for shaded and exposed clusters, respectively. For the shaded clusters, significant differences were noted among blocks ($p < 0.05$) for all time points after day 12 with the exception of days 70 and 100 postbloom. The exposed treatment showed significant differences among vines only on days 19, 40, and 130 postbloom. As expected, the highest level of variation was observed around veraison (day 70), the period of maximal change in IBMP content.

Table 2. IBMP Concentrations, Pruning Weights and Point Quadrant Analysis (PQA) Results from Block 1, 2, and 3 Canopies

	block 1	block 2	block 3
IBMP, mean difference (shaded) (%)[a]	+10 A	−5.7 B	−4.3 B
IBMP, mean difference (exposed) (%)[a]	+4.9 A	−5.3 A	+0.4 A
mean pruning weight (g/vine)	1096 A	607B	665 B
leaf layer number	1.64	1.28	1.38
% of interior cluster	43.5	28.0	31.6
% of interior leaf	31.4	26.1	28.1
% gap	4.3	8.6	9.7

[a] For each sampling date and treatment, mean values of IBMP were calculated. The percent difference between the concentration in each block and the mean value was then calculated for each sampling date. Within a row, different letters indicate that the blocks were significantly different by a *t*-test comparison ($p < 0.05$). Mean differences in IBMP are reported for each block and each treatment.

To our knowledge, this represents the first explicit measurement of IBMP variability between vines within a vineyard. The observed level of variability for the last two sampling dates (>20%) is interesting. First, the vines were selected to be relatively homogeneous (same clone, rootstock, and viticultural practices) and were along the same short row. Second, the mean variation in IBMP among vines was comparable to or greater than the decreases observed from light exposure, underscoring the importance of comparing the treatment effect within a vine.

It was evident during the course of the experiment that vines in blocks 2 and 3 were less vigorous than vines in block 1. Differences in vine vigor were evaluated by point quadrant analysis (PQA) and pruning weight (**Table 2**). The PQA data indicated block 1 had the highest vigor, followed by block 3 as indicated by the highest leaf layer number (LLN), percent of interior clusters (PIC), and percent of interior leaves (PIL). Pruning weights were also significantly higher for block 1 than for either block 2 or 3 (Tukey comparison, $p < 0.05$).

To permit comparison of the blocks over different sampling dates, IBMP concentrations were normalized. Mean values of IBMP were calculated for each sampling date and treatment. The percent differences between the concentration in each block and the mean value for all three blocks were then calculated for each sampling date. Mean differences in IBMP are reported for each block and each treatment (**Table 2**). For shaded clusters, samples from block 1 averaged 10% higher than the mean at each time point ($p < 0.05$ by Tukey comparison of means), whereas blocks 2 and 3 averaged 5% lower. Exposed clusters in block 1 averaged 5% higher than the mean, but this difference was not significant.

Thus, the highest vigor block also had the highest levels of IBMP in shaded clusters over the growing season. We see two possible explanations for this observation. First, the more vigorous vines may have had a greater degree of shading of the shaded clusters. Second, IBMP accumulation may be stimulated by vegetative growth. For both cases, an unknown

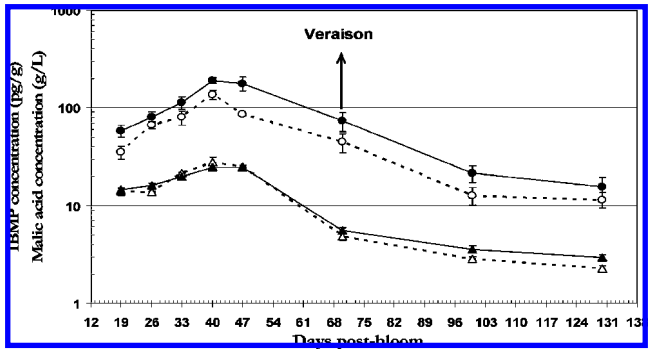

Figure 3. Semilog plot of levels of IBMP (●—, shaded; ○- - -, exposed) and malic acid (▲—, shaded; △- - -, exposed) in grape berries from day 19 to day 130 postbloom. In contrast to IBMP, malic acid concentrations were not affected by the shading treatment preveraison, but they were significantly lower in exposed berries at days 100 and 130 ($p < 0.1$, standard errors).

signal transduction pathway may exist, for example, a cryptochrome photoreceptor-mediated pathway similar to those found to regulate flavonoid production in other plants (*45*).

Comparison of Malic Acid and IBMP Dynamics in Response to Light Exposure. Because of the similarity between malic acid and IBMP concentration profiles during the growing season, and especially because of their steep decline postveraison, several authors have proposed that malic acid and IBMP degradation are controlled by similar mechanisms (*3, 12, 44, 46*). This hypothesis has been further supported by the observation that cooler climates tend to have higher levels of both IBMP and malic acid than warmer climates (*4*).

We quantified malic acid concentrations in shaded and exposed berries over the growing season. Superficially, the patterns seem quite similar in both shaded and exposed clusters (**Figure 3**). The concentrations of malic acid and IBMP accumulate during the first few weeks, peak just prior to veraison (days 40−47), and drop precipitously through veraison. However, the preveraison impact of light on malic acid and IBMP was dramatically different. Malic acid levels are not significantly different between shaded and exposed clusters at any time point prior to veraison, but the levels of malic acid are lower ($p < 0.1$) in exposed clusters at 100 and 130 days postbloom, likely via rapid respiration due to higher berry temperatures (*28, 30, 47*).

We also observed that the onset times of IBMP and malic acid degradation were not the same, independent of shading effects. To decouple the diluting effects of berry enlargement from actual degradation, mean berry weights were determined and IBMP and malic acid concentrations were calculated on a per berry basis. There was no significant difference in berry weight due to the treatments as determined by a matched pair t test ($p > 0.05$). For each sample (time × block), per berry and by weight concentrations of IBMP and malic acid were expressed as a percentage of the mean maximum values for the three blocks. Normalized data for exposed and shaded treatments were pooled at each time point to improve the statistical power of the analysis. Before pooling the data, we used a paired t test to verify that there were no significant differences in normalized IBMP concentration as a result of treatment, that is, the treatment did not affect the onset of IBMP degradation. The normalized IBMP concentrations (per berry and per weight) accumulate slowly during initial cell division (**Figure 4**), with the majority of IBMP synthesis occurring between days 20 and 40. Although IBMP decreases significantly on a per weight basis from days 40 to 70, no significant decrease is observed on a per berry basis. Over the next 30 days (days 70−100), we observe a

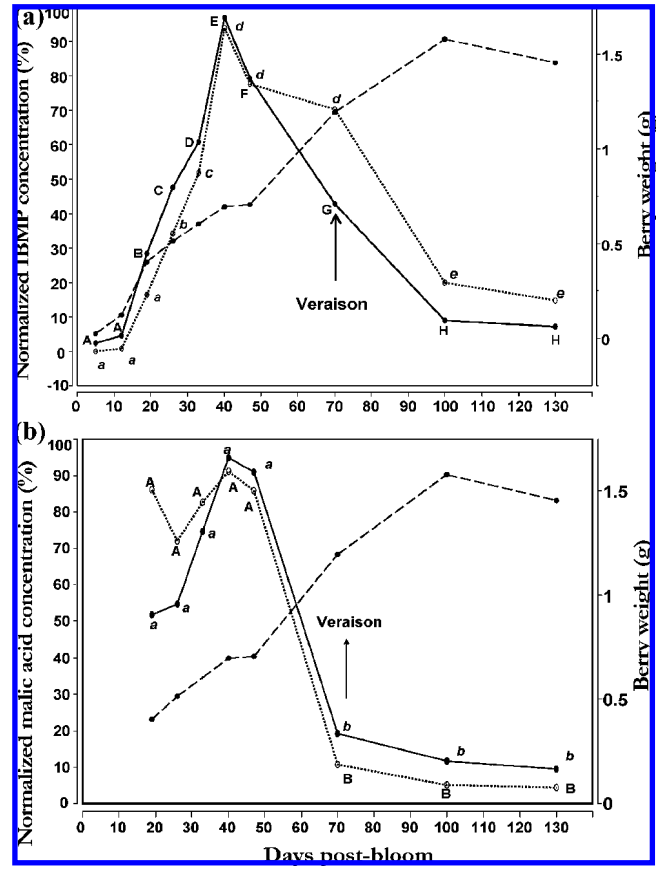

Figure 4. Normalized mean concentrations of (**a**) IBMP and (**b**) malic acid during the growing season, expressed in units of per gram (●—) and per berry (○···). The normalization methodology is described in the text. Mean berry weights ($n = 10$) at each time point are also shown (●- - -). Time points with significantly different IBMP/malic acid concentrations ($p < 0.05$, adjacent pair difference test) are indicated by different letters (upper case = by weight, lower case = per berry).

significant decrease in IBMP per berry, down to 20% of the maximum value. Thus, dilution is more important in decreasing IBMP concentration by weight just before color change, whereas the majority of degradation happens only after color change (day 70).

On the contrary, normalized malic acid concentration significantly decreased between days 47 and 70 on both per berry and per weight bases, due to its use as a substrate in primary metabolism (*48*). No significant difference was observed in malic acid concentration on a per weight or per berry basis between days 70 and 130. We conclude that although IBMP and malic acid are highest preveraison and lowest at harvest, they differ in two important respects. First, they respond to cluster exposure in very different manners, with malic acid decreasing more with postveraison exposure and IBMP accumulating less with preveraison exposure. Second, malic acid degradation begins before color change, whereas IBMP degradation starts later, around the time of color change. Because regions that are warm and sunny during the summer are often warm and sunny during the fall, IBMP and malic acid will often be positively correlated (*4, 15*). However, because different mechanisms influence malic acid and IBMP dynamics, the two metrics may not always be correlated. For example, hot and sunny preveraison conditions followed by a cool and cloudy ripening period are expected to yield low IBMP accumulation and high malic acid levels.

Table 3. IBMP Levels Preveraison (~Day 47 Postbloom) and at Harvest versus Classic Grape Chemical Parameters and Maturity Indices of Cabernet Franc Grapes across 13 Seneca Lake Sites

	IBMP concentration (ppt)		grape chemical parameters and classic maturity indices					
site	preveraison	harvest[a]	TSS (°Brix)	pH	TA (g/L)	weight (g)	TSS (g/L)/TA	TSS (°Brix)[a] × pH^2
1	185.7 ± 30.1	20.9 ± 4.8	21.9	3.29	7.5	1.63	29.2	237
2	103.8 ± 6.9	12.4 ± 1.7	21.9	3.33	7.1	1.53	30.8	243
3	41.4 ± 3.5	3.8 ± 0.1	22.3	3.24	7.6	0.86	29.3	234
4	24.9 ± 5.4	3.6 ± 0.2	22.8	3.45	7.3	0.96	31.4	271
5	129.9 ± 1.8	11.9 ± 0.7	23.0	3.16	9.3	1.42	24.7	229
6	217.7 ± 13.9	23.7 ± 1.4	20.7	3.21	7.7	1.34	27.1	213
7	115.8 ± 12.0	11.1 ± 1.0	21.0	3.17	8.2	1.71	25.4	211
8	100.2 ± 4.4	6.7 ± 0.8	21.9	3.31	7.1	1.77	31.0	240
9	78.0 ± 7.1	8.2 ± 0.2	22.0	3.35	7.0	1.70	31.4	247
10	68.8 ± 1.3	5.2 ± 0.9	20.9	3.40	6.4	1.43	32.7	24 1
11	61.4 ± 6.1	6.9 ± 0.4	20.4	3.40	6.2	1.35	33.0	236
12	63.5 ± 3.5	7.8 ± 1.7	20.6	3.42	5.8	1.52	35.3	240
13	75.5 ± 6.2	7.6 ± 0.3	20.0	3.41	6.0	1.68	33.2	233
mean	97.4	10.0	21.5	3.32	7.2	1.45	30.4	237
SD	54.9	6.1	0.9	0.10	1.0	0.28	3.1	15

[a] Harvest date: sites 1 and 2, Oct 6; sites 3−7, Oct 10; sites 8 and 9, Oct 17; sites 10−13, Oct 24.

Intervineyard, Veraison−Harvest IBMP Comparisons. IBMP concentration of Cabernet Franc was measured pre- and postveraison at 13 Seneca Lake sites (11 grower cooperators plus shaded and exposed samples from our experimental vineyard). Whereas preveraison measurements were performed within a few days of each other (~47 days postbloom), postveraison measurements were based on each grower cooperator's definition of maturity. Thus, we intentionally captured a range of fruit maturities to determine if basic fruit chemistry correlated with IBMP at harvest.

IBMP levels (preveraison and harvest) and basic grape chemistry parameters of Cabernet Franc were determined on grapes collected from 13 sites. IBMP concentrations ranged from 24.9 to 217.8 pg/g preveraison and from 3.6 to 23.7 pg/g at harvest (**Table 3**). The high variation of IBMP concentration among sites may reflect differences in growing conditions, clones, rootstock, and viticultural treatments. The mean TSS, TA, and pH values were 21.5 ± 0.9 °Brix, 7.2 ± 1.0 g/L, 3.32 ± 0.10, respectively. The latest harvested grapes (sites 10−13) had lower TA, lower TSS, and higher pH. Mean berry weight (n = 10) ranged from 0.86 to 1.77 g with a mean value of 1.45 ± 0.28 g. We also calculated and report classic maturity indices, TSS/TA ratio and TSS × pH^2 for each site (**Table 3**). Typical target values for these indices in red wine grapes are 35 and 260, respectively (*49*). The ratio of TSS/TA ranged from 24.7 to 35.3 with a mean value of 30.4. The TSS × pH^2 ranged from 211 to 271 with a mean value of 237. Whereas these values are lower than the common targets for maturity, they are typical of red wine grapes harvested in the shorter New York state growing season.

Regression analyses were performed between harvest IBMP versus chemical parameters and maturity indices (**Table 4**). No significant correlation was observed between any of the classic harvest parameters and IBMP levels at harvest. However, a strong correlation (R^2 = 0.936, p < 0.0001) was observed between preveraison IBMP and harvest IBMP levels (**Figure 5**). Harvest values of IBMP were about 10% of the preveraison value, regardless of vineyard site or practice. We note that a similar relationship (90% degradation) between veraison and harvest was observed for Sauvignon Blanc in warmer and sunnier Australia (*44*). These results suggest that final values at harvest can be predicted early in the season and underscore the importance of good preveraison viticultural practices. These observations support our conclusion that IBMP levels at harvest

Table 4. Correlation Coefficients of Harvest IBMP Concentrations versus Grape Chemical Parameters at the 13 Sites[a]

correlation	correlation coefficient, R	P value
IBMP × TSS (g/L)	0.095	0.752
IBMP × pH	0.509	0.075
IBMP × TA (g/L)	0.359	0.227
IBMP × berry weight (g)	0.283	0.347
IBMP × TSS/TA	0.493	0.087
IBMP × TSS (°Brix) × pH^2	0.544	0.055
IBMP × IBMP preveraison	0.967	<0.0001

[a] All parameters were measured at harvest with the exception of IBMP preveraison.

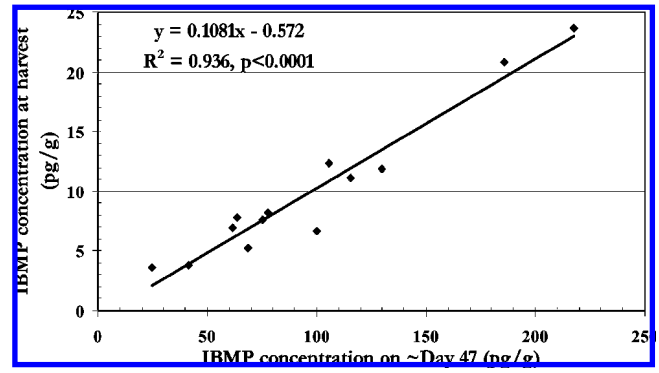

Figure 5. IBMP concentration at 2 weeks preveraison versus IBMP concentration at maturity for 13 sites of Seneca Lake.

are more strongly influenced by preveraison as opposed to postveraison conditions. Understanding why differences in IBMP accumulation occurred at the different sites is not straightforward, as this may reflect differences in water availability, vine balance, clone, cluster light exposure, canopy architecture, and other factors. Current work by our group is addressing these issues.

In conclusion, cluster light exposure significantly reduced IBMP (21−44%) on exposed clusters throughout berry growth. Preveraison cluster light exposure was more critical than postveraison exposure by reducing IBMP accumulation, although it is not clear if this was a result of higher berry temperatures or increased light interception. The importance of early-season conditions was further demonstrated by the high correlation of harvest IBMP concentrations to preveraison

concentrations at multiple vineyard sites. We also observed a high degree of variability in IBMP content between vines with similar treatments (>20% RSD for postveraison time points). Although this experiment focused on the effects of light, some of the variation could be explained by variation in vine vigor within the vineyard row. Less vigorous vines, as evaluated by point quadrant analysis (PQA) and pruning weights, accumulated lower levels of IBMP. IBMP and malic acid both accumulate early in the season and decrease toward harvest, but their dynamics and responses to light exposure are distinct. Finally, we note that the factors governing IBMP degradation are still poorly characterized. Our work demonstrates only that light and temperature will not accelerate degradation of IBMP either preveraison or postveraison. Thus, further research on the pathways associated with IBMP degradation as well as synthesis is well warranted.

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