Glycosylated Aroma Compound Responses in 'Riesling' Wine Grapes to Cluster Exposure and Vine Yield

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SUMMARY. Concentrations of aroma precursor compounds in 'Riesling' wine grapes (*Vitis vinifera*) are reported to correlate with fruit zone cluster exposure, although optimal cultural influences with respect to exposure timing and canopy assessment methods have not been established. To determine the impact of cluster exposure on concentrations of potential aroma compounds, correlations between light exposure metrics during the growing season and relative concentrations of eight representative aroma compounds at harvest were determined. The aroma compounds were carbon-13 (C13) norisoprenoids [1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), β -damascenone, and vitispirane], monoterpenes (linalool oxide, α -terpineol), and phenolics (4-vinylguaiacol, vanillin and eugenol). Cluster exposure was determined using metrics of varying spatial precision [percent interior cluster (PIC), cluster exposure layer (CEL), $\ln(\text{CEL})$, cluster exposure flux availability (CEFA), and the percent ambient photosynthetic photon flux (PPF)] at two sites and two phenological stages (fruit set and veraison) in two consecutive seasons (2008 and 2009). Pairwise combinations of cluster exposure metrics and compounds resulted in 360 permutations, of which 22 were significant. Response data suggested that none of the compounds studied respond to variable cluster exposure levels below 20% of ambient sunlight (CEFA < 0.2), and that low cluster exposure may be particularly effective in minimizing C13 norisoprenoid concentrations at harvest. Yield components were also tested but found to have lower R^2 values compared with cluster exposure metrics. Active canopy management, in which vine vigor and fruit exposure are independently controlled, is likely to be more effective in influencing potential aroma compounds than selectively harvesting for naturally occurring variation in cluster exposure. In comparing the relative predictive strength among metrics, CEFA $\equiv \ln(\text{CEL}) > \text{CEL} > \text{PIC} \equiv \text{percent PPF}$, suggesting that cluster exposure metrics with greater spatial sensitivity are more effective for establishing light response curves.

The distinguishing aroma characteristics of varietal wines produced from 'Riesling' are largely absent from its fresh berries (Sacks et al., 2012). The major compound classes thought to be responsible for 'Riesling' wine varietal character include monoterpenes [e.g., linalool (floral)] and carbon-13 norisoprenoids [especially 1,1,6-trimethyl-1,2-dihydronaphthalene (kerosene)]. These compound classes exist primarily as "bound," nonodorous glycosides in

wine grapes (Lee et al., 2007; Marais et al., 1992; Park et al., 1991), which can form free aroma compounds via hydrolysis during fermentation or aging. Several volatile phenols also exist as glycosides in 'Riesling' wine grapes (Ryona and Sacks, 2013), as well as in other wine grape varieties (Loscoco et al., 2009), but their contribution

to 'Riesling' varietal character is less clear.

Multiple studies have reported correlations between cluster exposure and either free aroma compounds or their bound glycosylated precursors. For example, the concentration of TDN precursors in 'Riesling' is correlated to preveraison fruit exposure (Kwasniewski et al., 2010; Marais et al., 1992). β -damascenone, another C13 norisoprenoid, enhances fruity aromas (Pineau et al., 2007) and is considered to correlate positively with wine quality. Although β -damascenone precursors are significantly affected by cluster exposure, the relationship can be either positive or negative depending on the degree of cluster exposure (Lee et al., 2007). Free monoterpene concentrations in 'Traminette' (*Vitis* sp.) and bound monoterpenes in 'Riesling' increased with cluster exposure (Marais et al., 1992; Skinkis et al., 2010), although no consistent trend was observed for bound monoterpenes in shaded and unshaded fruit of 'Muscat of Frontignan' (*Vitis vinifera*) (Bureau et al., 2000). The behavior of volatile phenol glycosides in response to shading is not as well studied, yet no consistent trend is apparent (Bureau et al., 2000). However, other nonodorous phenolics, such as anthocyanins, increase with increasing cluster sunlight exposure in red grape (*Vitis* sp.) varieties (reviewed by Downey et al., 2006), although experimental results have been inconsistent (Downey et al., 2004), and evidence suggests that the sunlight response plateaus well before exposure reaches ambient sunlight levels (Joscelyne et al., 2007).

Viticultural treatments such as leaf pulling and shoot thinning are often employed to manipulate fruit exposure. Although anecdotal reports suggest that most grape growers in the northeastern United States do not

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Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	μL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	micron	μm	1
28,350	oz	mg	3.5274×10^{-5}
1	ppb	$\mu\text{g}\cdot\text{L}^{-1}$	1
1	ppm	$\text{mg}\cdot\text{L}^{-1}$	1
$(^{\circ}\text{F} - 32) \div 1.8$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$(^{\circ}\text{C} \times 1.8) + 32$

manage their 'Riesling' canopies to obtain a specific cluster light environment, such practices could be useful for targeting specific sensory characteristics in finished wines (e.g., by decreasing TDN concentrations while increasing concentrations of monoterpenes). However, there is uncertainty about quantitative relationships between canopy architecture and resulting concentrations of flavor compounds or their precursors. In the specific case of managing fruit cluster exposure, quantitative knowledge of dose-response thresholds would improve the precision of models and the canopy management practices that they guide (Meyers et al., 2012). In particular, it is not known if naturally occurring differences in cluster exposure, as opposed to differences arising from imposed treatment, correlate with differences in bound volatiles. Knowledge of this relationship could guide differential harvest practices. The objective of this work was to evaluate the correlation of quantitative measures of naturally occurring cluster exposure to the concentrations of eight representative bound aroma compound precursors in 'Riesling'.

Materials and methods

VINE MATERIAL. Two 'Riesling' blocks were studied for naturally occurring microclimatic variation at two commercial vineyards (site A planted in 1995 and B planted in 1996) in Lodi, NY (Finger Lakes region, east side of Seneca Lake). At site A (lat. 42.57°N, long. 76.86°W), 72 Scott Henry trained 'Riesling' vines (18 four-vine panels) were selected for consistency (i.e., no missing vines or young replants) from a subplot of six rows. At site B (lat. 42.54°N, long. 76.87°W), 66 'Riesling' vines (22 three-vine panels) trained to two-tier flatbow with vertical shoot positioning (VSP) were selected for consistency (i.e., no missing vines or young replants) from a subplot of seven rows. Both sites were planted in north-south row orientation and managed according to standard viticultural practices for 'Riesling' canopies in the Finger Lakes region. Vine spacing was 2.0 × 2.8 m and 2.2 × 2.8 m at sites A and B, respectively. Exterior rows and panels were excluded. The experimental unit was one panel (i.e., four consecutive vines at site A and three consecutive vines at site B).

CANOPY CHARACTERIZATION. Enhanced point quadrat analysis [EPQA (Meyers and Vanden Heuvel, 2008)] was performed at fruit set (27 June 2008 and 5 July 2009) and veraison (15 Aug. 2008 and 25 Aug. 2009) by inserting a thin metal rod into the fruiting zone along the transverse axis of the canopy row. A tape measure was used as a guide for insertions, which were made at 20-cm intervals along the length of the panel at the height of the fruiting wire, resulting in 35 insertions per panel. A photosynthetically active radiation (PAR) sensor (AccuPAR LP-80; Decagon Devices, Pullman, WA) was used to measure PPF when sun was directly overhead. The sensor bar was placed in the fruiting zone and aligned with the longitudinal axis of the row and the sensors facing directly upward toward the sky as described by Meyers and Vanden Heuvel (2008). Point quadrat analysis [PQA (Smart and Robinson, 1991)] and EPQA metrics were computed for each vineyard panel using a spreadsheet program (Excel version 12.0.6514.5000 SP2; Microsoft, Redmond, WA) and EPQA-CEM Tools version 1.6.2 (J.M. Meyers, unpublished).

WEATHER DATA. Growing degree day [GDD (base 50 °F)] data were obtained from site-located weather stations (HOBO; Onset Computer Corp., Bourne, MA). Precipitation data were obtained from the Network for Environmental and Weather Applications (Cornell University, Ithaca, NY) weather station in Valois, NY (lat. 42.53°N, long. 76.88°W). Cumulative totals were calculated for the ranges between 15 April and fruit set (27 June 2008 and 4 July 2009), between fruit set and veraison (15 Aug. 2008 and 24 Aug. 2009), and between veraison and harvest. Harvest dates in 2008 were 13 Oct. and 9 Oct. for sites A and B, respectively. In 2009, fruit were harvested at both sites on 15 Oct.

ANALYSIS OF BOUND C13-NORISOPRENOIDS, MONOTERPENES, AND VOLATILE PHENOLS. Vines were individually hand harvested and yield was recorded. All vines from each panel (i.e., no cluster subsampling) were combined and whole-cluster pressed. Resulting musts were immediately treated with 50 mg·L⁻¹ sulfur dioxide and sampled. Samples (200 mL in 2008, 500 mL in 2009) were frozen

at -40 °C for later analysis. Glycosides were extracted from juice samples via a solid-phase extraction (SPE) protocol derived from Ibarz et al. (2006). Juice samples were thawed at room temperature for about 24 h and centrifuged before SPE processing.

SPE cartridges (4 mL) packed with 200 mg of sorbent (LiChrolut EN; Merck, Darmstadt, Germany) were preconditioned with sequential washings of dichloromethane [DCM (5 mL)], methanol (5 mL), and water (10 mL) and loaded with 50 mL of juice at a flow rate of ≈2 mL·min⁻¹ via an SPE processor (Cerex; Varian, Palo Alto, CA). The SPE cartridge was washed with water (4 mL) followed by a 2:1 v/v mixture of pentane and DCM (7.7 mL). The analytes were eluted with a 9:1 v/v mixture of ethyl acetate and methanol (4 mL) and dried under nitrogen to complete dryness.

Samples were reconstituted with 10 mL of 0.2 M citric acid buffer (adjusted with sodium hydroxide to a pH of 2.5) and incubated at 100 °C for 1 h to hydrolyze the glycosides. 2-Octanol was added to the cooled solution to serve as an internal standard at a target concentration of 250 µg·L⁻¹. A fresh SPE cartridge (4 mL) packed with 200 mg of sorbent was preconditioned with sequential washings of DCM (5 mL), methanol (5 mL), and water (5 mL) and the sample loaded at a flow rate of ≈2 mL·min⁻¹. The SPE column was dried under nitrogen for 15 min, volatiles eluted with 2.8 mL of DCM, concentrated under nitrogen to a volume of ≈300 µL, and 100 µL transferred to an autosampler vial before gas chromatography-mass spectrometry (GC-MS) analysis.

An ion trap mass spectrometer (Varian cp-3800/Saturn 2000) was used for GC-MS analyses (Varian, Palo Alto, CA). One microliter of sample was injected splitless onto a VF WAX MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The injector temperature was 250 °C. Helium was the carrier gas at a constant flow rate of 1 mL·min⁻¹. The temperature program was 50 °C for 2 min, raised to 170 °C at 5 °C per min, raised to 250 °C at 10 °C per min, and held for 3 min. The transfer line temperature was 250 °C, the manifold was set at 50 °C, and the ion trap was set at 170 °C. The mass spectrometer was operated in full scan mode over a mass range of *m/z* 25–220, scanned

at 5600 *m/z* per second. The effective sampling rate was 1 Hz.

Following analyses, data were exported to a different manufacturer's software (ChromaTOF version 4.22; Leco Corp., St. Joseph, MI) for peak identification and semiquantification. Analytes were identified via Kovats retention index and library spectra and quantified relative to the 2-octanol internal standard. Positive identifications were based on match values > 850 against a standard spectra database (version 5.0; National Institute of Standards and Technology, Gaithersburg, MD) and linear retention indices within 30 RI units of values listed for a C20M column in Flavornet (Flavornet.org, 2004). The quantification ions used were: 2-octanol, *m/z* = 55; TDN, *m/z* = 157; β -damascenone, *m/z* = 121; vitispirane, *m/z* = 134; linalool oxide, *m/z* = 59; α -terpineol, *m/z* = 59; 4-vinylguaiacol, *m/z* = 135; vanillin, *m/z* = 151; and eugenol, *m/z* = 164. Vitispirane peak area was calculated as the sum of peak areas for vitispirane A and B. Some monoterpenes were assumed to have been partially rearranged to α -terpineol during acid hydrolysis (Baxter et al., 1978), so α -terpineol was expected to serve as a proxy for the relative concentrations of the total monoterpene concentrations in the juice.

STATISTICAL ANALYSIS. Regression analyses were performed for each site-year-compound combination to test for responses to cluster exposure at both fruit set and veraison. Significant responses ($P \leq 0.05$) were plotted. Regression analyses were performed to test compound concentration responses to vine yield, average cluster weight, pruning weight, and crop load data (pruning weight data were not collected at site B in 2009). Significant responses ($P \leq 0.05$) were reported.

Relative efficacy of the five cluster exposure measures was tested as follows: each of the five cluster exposure metrics was assigned an ordinal value, between one and five, according to the expected order of precision (Meyers and Vanden Heuvel, 2008). Fruit zone percent *PPF*, which does not differentiate exposure levels among clusters, was assigned a value of one. Percent interior cluster, a ratio of fully exposed clusters to total clusters, was assigned a value of two. Cluster exposure layer, a measure of average cluster distance from the canopy exterior, was

assigned a value of three. The natural logarithm of *CEL*, *ln(CEL)*, which accounts for the exponential relationship between canopy light attenuation and canopy depth (Dokoozlian and Kliever, 1995; Smart 1985), was assigned a value of four. Cluster exposure flux availability, which improves upon the precision of *ln(CEL)* by using the difference between fruit zone *PPF* and ambient *PPF* to determine an attenuation constant for the canopy, was assigned a value of five. A regression analysis was performed comparing the assigned ordinals to the frequency of significant analyte responses for each metric. All regressions and significance tests were performed via statistical software (SAS version 9.1.3; SAS Institute, Cary, NC).

Results and discussion

VINEYARD VARIABILITY. Average vine yields, cluster weights, pruning weights, and crop load indices (Table 1) varied across site-year combinations. Value ranges of fruit zone microclimatic metrics (Table 2) and concentrations of bound volatiles (Table 3) varied across site-year combinations.

CLUSTER EXPOSURE AND COMPOUND CONCENTRATION. Correlation analysis of cluster exposure metrics and bound volatiles (Table 4) revealed 22 significant ($P \leq 0.05$) correlations representing 12 unique site-year-timing-compound combinations (i.e., some site-year-timing-compound combinations yielded significant responses for more than one cluster exposure metric). Among the bound volatiles, significant correlations of bound TDN and light

exposure metrics responses were most frequent (nine), followed by α -terpineol (three), vitispirane (three), linalool oxide (three), β -damascenone (two), eugenol (two), 4-vinylguaiacol (zero), and vanillin (zero). Regression analysis of all significant responses revealed generally higher predictive power at veraison compared with fruit set (mean R^2 values were 0.46 and 0.31). The analyte responses of the 12 unique site-year-timing-compound combinations are presented in Table 4. When more than one metric revealed a significant response for the site-year-timing-compound combination, only the metric with the most predictive power (usually CEFA) for that combination is presented. As a caveat, calibration curves were not run, and potentially nonlinear responses could have occurred for high concentration samples, which may have decreased the correlation values.

Bound C13 norisoprenoids (vitispirane, TDN, and β -damascenone) were best correlated to cluster exposure at site A in 2008 where a significant correlation was observed for four of the six timing-compound combinations (Table 4). Otherwise, C13 norisoprenoid responses were less consistent (only three significant responses among the remaining 19 site-timing-compound combinations in Table 4) and fitted curves had comparatively lower predictive power (R^2 values at or below 0.18). Specifically, TDN and vitispirane responded positively to cluster exposure measured at fruit set at site A in 2008, and both TDN and β -damascenone responded positively to cluster exposure measured at veraison. At site B, TDN and vitispirane

Table 1. Ranges (minimum–maximum), means, and standard deviations of vine yield, cluster weight, pruning weight, and crop load of New York Finger Lakes region 'Riesling' wine grape vines at harvest in 2008 and 2009.

		Vine yield (kg) ^y	Cluster wt (kg)	Pruning wt (kg)	Crop load (ratio) ^x
Site ^z	Yr	Min–max	Min–max	Min–max	Min–max
		Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
A	2008	8.15–13.59	0.079–0.120	0.53–1.58	7.97–15.38
		12.02 (1.53)	0.107 (0.011)	1.13 (0.26)	11.05 (2.29)
	2009	3.06–7.53	0.070–0.109	0.83–1.38	2.55–6.80
		5.15 (1.32)	0.087 (0.010)	1.20 (0.15)	4.43 (1.48)
B	2008	9.57–15.01	0.068–0.089	0.87–1.77	6.47–12.42
		11.62 (1.21)	0.079 (0.007)	1.34 (0.24)	8.68 (1.65)
	2009	7.17–12.90	0.057–0.107	No data	No data
		8.93 (1.61)	0.076 (0.013)		

^aVines at sites A and B were trained to Scott Henry and two-tier flatbow, respectively.

^b1 kg = 2.2046 lb.

^cCrop load is calculated as the ratio of vine yield to pruning weight.

Table 2. Ranges, means, and standard deviations of leaf layer number (LLN), occlusion layer number (OLN), percent interior clusters (PIC), cluster exposure layer (CEL), cluster exposure flux availability (CEFA), and photosynthetic photon flux (PPF) for New York Finger Lakes region ‘Riesling’ wine grape vines at fruit set and veraison in 2008 and 2009.

Site ^a	Yr	Timing	LLN (no.)	OLN (no.)	PIC (%)	CEL (no.)	CEFA (no.)	PPF (%)
			Min-max	Min-max	Min-max	Min-max	Min-max	Min-max
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
A	2008	Fruit set	1.40–2.50	2.00–3.18	50.0–84.5	0.50–0.95	0.13–0.40	6.20–48.6
			2.04 (0.28)	2.74 (0.34)	60.8 (10.6)	0.68 (0.13)	0.28 (0.07)	23.1 (9.38)
		Veraison	1.65–2.62	2.02–3.54	35.2–81.4	0.35–0.93	0.16–0.46	0.50–27.00
			2.08 (0.30)	2.83 (0.42)	54.7 (14.7)	0.61 (0.17)	0.31 (0.09)	5.11 (4.62)
	2009	Fruit set	1.84–2.64	2.14–3.19	66.0–94.1	0.80–1.53	0.03–0.21	0.78–28.4
			2.16 (0.23)	2.72 (0.29)	79.0 (9.5)	1.09 (0.26)	0.13 (0.06)	5.13 (5.09)
		Veraison	1.64–2.36	2.26–3.09	53.1–91.2	0.59–1.28	0.07–0.28	0.51–13.03
			2.05 (0.24)	2.72 (0.26)	76.0 (10.3)	0.99 (0.19)	0.14 (0.06)	1.59% (1.22)
B	2008	Fruit set	1.77–3.15	2.29–4.03	50.0–94.8	0.56–1.21	0.13–0.38	0.70–44.7
			2.42 (0.38)	3.00 (0.51)	70.3 (11.4)	0.83 (0.19)	0.24 (0.06)	9.46 (7.89)
		Veraison	2.90–4.33	4.03–5.70	65.5–100	1.00–1.75	0.06–0.20	0.40–11.2
			3.50 (0.43)	4.67 (0.50)	87.8 (8.27)	1.36 (0.24)	0.12 (0.04)	1.73 (1.62)
	2009	Fruit set	2.71–3.85	3.87–5.26	89.1–100	1.23–2.03	0.03–0.18	0.44–17.9
			3.35 (0.31)	4.52 (0.43)	96.1 (3.95)	1.69 (0.22)	0.08 (0.04)	3.97 (3.88)
		Veraison	2.26–3.44	3.32–4.88	87.9–97.6	1.26–1.81	0.01–0.06	0.51–5.05
			2.78 (0.38)	4.06 (0.43)	92.4 (3.54)	1.47 (0.17)	0.04 (0.02)	1.41 (0.86)

^aVines at sites A and B were trained to Scott Henry and two-tier flatbow, respectively.

Table 3. Relative ranges (minimum–maximum), means, and standard deviations of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), β -damascenone, vitispirane, α -terpineol, linalool oxide, 4-vinyl guaiacol, vanillin, and eugenol in New York Finger Lakes region ‘Riesling’ wine grape glycosidically bound analyte concentrations at harvest in 2008 and 2009.

Site ^a	Yr	TDN ^b	β -Damascenone	Vitispirane	α -Terpineol	Linalool oxide	4-Vinyl guaiacol	Vanillin	Eugenol
		Min-max	Min-max	Min-max	Min-max	Min-max	Min-max	Min-max	Min-max
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
A	2008	2.59–5.51	0.44–0.97	0.93–2.44	2.4–3.99	6.78–14.88	10.22–77.72	3.28–6.78	ND ^c
		3.56 (0.90)	0.61 (0.16)	1.52 (0.45)	3.30 (0.51)	9.63 (2.41)	31.32 (22.31)	4.62 (1.49)	
	2009	0.43–1.81	0.04–0.14	0.03–0.09	0.16–0.57	0.21–0.67	0.21–1.06	0.09–0.83	0.03–0.18
		1.07 (0.35)	0.08 (0.03)	0.06 (0.02)	0.34 (0.12)	0.41 (0.13)	0.54 (0.22)	0.28 (0.18)	0.09 (0.04)
B	2008	0.73–2.06	0.03–0.90	0.02–0.16	0.02–0.96	0.18–1.40	0.05–3.79	0.14–1.19	0.03–0.28
		1.53 (0.36)	0.33 (0.20)	0.08 (0.03)	0.41 (0.22)	0.99 (0.26)	1.02 (0.94)	0.44 (0.25)	0.11 (0.06)
	2009	0.75–2.42	0.07–0.14	0.04–0.11	0.21–0.68	0.17–1.04	0.04–2.95	0.11–0.96	ND
		1.35 (0.48)	0.09 (0.02)	0.07 (0.02)	0.42 (0.12)	0.54 (0.19)	0.71 (0.49)	0.35 (0.18)	

^aVines at sites A and B were trained to Scott Henry and two-tier flatbow, respectively.

^bValue ranges are unitless ratios derived from gas chromatography–mass spectrometry peak areas versus an internal standard of 2-octanol.

^cND = Not detected.

responded negatively to fruit set exposure while TDN responded negatively at veraison; however, low R^2 values suggest that the site B responses were not biologically predictive. These observations were surprising because the concentrations of C13 norisoprenoid precursors at harvest were reported to correlate with cluster exposure at both fruit set and veraison (Kwasniewski et al., 2010). However, previous work on the response of TDN to cluster exposure (Gerdes et al., 2002) suggested that TDN concentrations in ‘Riesling’ only respond to fruit zone sunlight exposures above 20% of ambient sunlight (study conducted in

Davis, CA). In this study, veraison CEFA only surpassed 0.20 at site A in 2008, the site that showed the most significant correlations between cluster exposure and C13 norisoprenoid concentrations. Thus, a possible explanation for the inconsistent correlation of bound C13 norisoprenoids and cluster light exposure is that a sufficient range in light exposure was only achieved at site A in 2008. If correct, this suggests that canopy management interventions intended to reduce TDN concentrations will not be economically beneficial if the high range of cluster exposure is already below 20% ambient light and,

conversely, any intervention intended to increase TDN will not be beneficial unless clusters are exposed above 20% ambient sunlight. However, we observed a 2- to 4-fold variation in TDN within sites and years in this study, comparable to variation observed as a result of leaf removal interventions in other studies (Gerdes et al., 2002; Kwasniewski et al., 2010). A possible explanation is that the factors that control natural variation in cluster shading within a vineyard could have confounding effects on TDN precursor accumulation. If correct, this suggests that deliberate canopy management, in which vine vigor and cluster exposure

Table 4. Regression equations and coefficient of determination (R^2) values for significant responses of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), β -damascenone, vitispirane, α -terpineol, linalool oxide, 4-vinyl guaiacol, vanillin, and eugenol in New York Finger Lakes region 'Riesling' wine grape glycosidically bound analyte concentrations to cluster exposure metrics in 2008 and 2009. Exposure metrics tested were leaf layer number (LLN), occlusion layer number (OLN), percent interior clusters (PIC), cluster exposure layer (CEL), cluster exposure flux availability (CEFA), and photosynthetic photon flux (PPF).

Analyte ^z	Timing	2008		2009	
		Site A	Site B	Site A	Site B
		Regression equation (R^2 values) ^y			
TDN	FS	$y = -34.1 \ln(\text{CEL}) + 22.36 (R^2 = 0.5)^*$	$y = -2.64 \text{ CEFA} + 2.16 (R^2 = 0.18)^*$	NS ^s	$y = -4.99 \text{ CEFA} + 1.75 (R^2 = 0.16)^*$
		$y = -46.46 \text{ CEL} + 67.57 (R^2 = 0.48)^*$			
	V	$y = 0.56 \text{ PIC} + 69.74 (R^2 = 0.48)^*$		NS	NS
		$y = 99.57 \text{ CEFA} + 7.56 (R^2 = 0.77)^{**}$	NS		
		$y = -28.42 \ln(\text{CEL}) + 23.48 (R^2 = 0.69)^{**}$			
		$y = -41.34 \text{ CEL} + 63.41 (R^2 = 0.64)^{**}$			
		$y = -0.50 \text{ PIC} + 65.42 (R^2 = 0.63)^{**}$			
β -Damascenone	FS	NS	NS	NS	NS
	V	$y = 14.38 \text{ CEFA} + 2.07 (R^2 = 0.54)^*$	NS	NS	NS
Vitispirane	FS	$y = -4.12 \ln(\text{CEL}) + 4.36 (R^2 = 0.49)^*$		NS	NS
		$y = 46.40 \text{ PPF} + 5.27 (R^2 = 0.51)^*$	$y = -0.19 \text{ CEFA} + 0.12 (R^2 = 0.18)^*$		
	V	$y = 48.02 \text{ CEFA} + 3.00 (R^2 = 0.47)^*$		NS	NS
		NS	NS		
α -Terpineol	FS	NS	$y = -0.02 \text{ PPF} + 0.58 (R^2 = 0.14)^*$	NS	$y = -1.39 \text{ CEFA} + 0.53 (R^2 = 0.19)^*$
				NS	$y = 0.41 \ln(\text{CEL}) + 0.20 (R^2 = 0.17)^*$
Linalool oxide	V	NS	NS	NS	NS
	FS	NS	NS	NS	NS
4-Vinylguaiacol	V	NS	$y = 0.53 \ln(\text{CEL}) + 0.87 (R^2 = 0.23)^*$	NS	$y = -1.76 \ln(\text{CEL}) + 2.00 (R^2 = 0.16)^*$
	FS	NS	$y = 0.37 \text{ CEL} + 0.053 (R^2 = 0.21)^*$	NS	
Vanillin	V	NS	NS	NS	NS
	FS	NS	NS	NS	NS
Eugenol	V	ND ^w	NS	NS	ND
	FS	ND	$y = 0.20 \ln(\text{CEL}) + 0.06 (R^2 = 0.45)^{**}$	NS	ND
			$y = 0.15 \text{ CEL} - 0.09 (R^2 = 0.46)^{**}$	NS	

^xValue ranges are unitless ratios derived from gas chromatography-mass spectrometry peak areas versus an internal standard of 2-octanol.

^yN = 9, 24, 33, and 30, for A-2008, A-2009, B-2008, and B-2009, respectively.

^sNS = Nonsignificant.

^wND = Not detected.

^zP ≤ 0.05, ^{**}P ≤ 0.01.

are independently controlled, may be more effective in influencing aroma compounds than selective harvesting of naturally occurring variability.

Total GDD accumulation and rainfall (Fig. 1) were similar in 2008 and 2009, although in 2008 accumulations were weighted toward the latter half of the growing season. It might seem reasonable to partially attribute the comparatively weak responses in 2009 compared with 2008 to lower postveraison GDD, but it has been reported that the rate of C13 norisoprenoid precursor accumulation in 'Riesling' plateaus well before typical harvest date and is independent of moderate seasonal GDD differences (Ryona and Sacks, 2013).

Monoterpenes were negatively correlated to cluster exposure in four site-year-timing combinations. α -terpineol responded negatively to fruit set cluster exposure measured as percent PPF in 2008 (Table 4) and as CEFA and $\ln(\text{CEL})$ in 2009 (Table 4), while linalool oxide responded negatively to veraison cluster exposure measured as CEL and $\ln(\text{CEL})$ in 2008 (Table 4) and as $\ln(\text{CEL})$ in 2009

(Table 4), albeit with low R^2 values (ranging from 0.14 to 0.23). Monoterpenes were reported to respond positively to sunlight between the range of 20% and 50% of ambient (Belancic et al., 1997); however, our responses derived from quantitative measures of light exposure (i.e., percent PPF and CEFA) exhibited a narrow range of low exposure values. This might suggest the existence of a dose-response threshold and that monoterpenes concentration in clusters receiving less than $\approx 20\%$ of ambient sunlight should be expected to be independent of dose. Also, as mentioned in the discussion of C13 norisoprenoids, the light exposure differences were not actively imposed, but a consequence of other environmental factors.

Of the bound volatile phenols analyzed, only one was correlated with cluster exposure, and only in a single site-year-timing combination. The specific response, a negative eugenol response to veraison cluster exposure at site B in 2008 (Table 4), was unexpected. Many volatile phenols in wine, including vanillin and eugenol,

originate primarily from the breakdown of oak-derived lignin (Chatonnet and Dubourdieu, 1998), although 4-vinylguaiacol can be formed by enzymatic decarboxylation of ferulic acid during fermentation. However, these volatile phenols can also exist as bound glycosides in grapes (Loscos et al., 2009). A consistent relationship between foliage shading of clusters and volatile phenol glycosides was reported in grapes (Bureau et al., 2000), but this research was limited. Evidence for a broad relationship between sunlight exposure and other grape polyphenols in wine (e.g., flavonoids such as anthocyanins) has been well documented (Downey et al., 2006), but it is not clear that volatile phenol glycosides have similar behavior. Specific evidence of a positive correlation between sunlight intensity and eugenol has been demonstrated in basil [*Ocimum basilicum* (Xianmin et al., 2008)]. However, the limited evidence presented here does not make a clear case for a relationship between sunlight intensity and any of the volatile phenol glycosides.

YIELD COMPONENTS AND COMPOUND CONCENTRATION. All of the compounds classes studied responded variably to yield factors (Table 5). Bound C13 norisoprenoids responses were found only at site B in 2008 where TDN responded negatively to average cluster weight; and β -damascenone responded positively to average pruning weight and negatively to crop load. Linear regression indicated that fruit set cluster exposure and average cluster weight correlate strongly and positively ($r = 0.65$). This correlation, combined with the insignificant correlation between crop load and TDN, suggests that the increased cluster weight may have led to increased berry size, thus reducing skin to pulp ratios and C13 concentrations; however, the absence of berry size data precludes drawing any firm conclusions.

Both linalool oxide and α -terpineol responded negatively to crop load at site B in 2008 and negatively to average cluster weight at site B in 2009; however, low R^2 values and insignificant response in other site-year combinations do not suggest a biologically significant response model. Similarly, inconsistent and generally weak responses in bound phenols

Fig. 1. Panel A: Growing degree day (GDD) accumulation [base 50 °F (10.0 °C)] from budbreak to fruit set (BB-FS), from fruit set to onset of veraison (FS-V), and from onset of veraison to harvest (V-H). Total GDD accumulation was typical for the region (New York Finger Lakes), and 'Riesling' is routinely ripened in regions with less than 2000 GDDs. Panel B: Rainfall accumulation from budbreak to fruit set (BB-FS), from fruit set to onset of veraison (FS-V), and from onset of veraison to harvest (V-H). Sites A and B share precipitation data from the same weather station, and no rainfall occurred during differential harvest dates, so sites A and B are reported with equivalent rainfall accumulations; 1 inch = 2.54 cm.

Table 5. Regression equations and coefficient of determination (R^2) values for significant responses of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), β -damascenone, vitispirane, α -terpineol, linalool oxide, 4-vinyl guaiacol, vanillin, and eugenol in New York Finger Lakes region ‘Riesling’ wine grape glycosidically bound analyte concentrations to yield components in 2008 and 2009. Yield components tested were average cluster weight (ACW), average pruning weight (APW), and average vine yield (AVY).

Analyte ^z	2008		2009	
	Site A	Site B	Site A	Site B
	Regression equation (R^2 values) ^y			
TDN	NS ^x	$y = 3.25-22.14 \text{ ACW } (R^2 = 0.20)^*$	NS	NS
β -Damascenone	NS	$y = -0.50 + 0.59 \text{ APW } (R^2 = 0.42)^{***}$	NS	NS
		$y = 0.84-0.06 \text{ CL } (R^2 = 0.19)^*$		
Vitispirane	NS	$y = -0.07 + 0.12 \text{ APW } (R^2 = 0.21)^*$	NS	NS
α -Terpineol	$y = -3.58 + 0.54 \text{ AVY } (R^2 = 0.63)^*$	$y = -0.41 + 0.58 \text{ APW } (R^2 = 0.32)^{**}$	NS	$y = 0.77-4.66$
		$y = 1.17-0.09 \text{ CL } (R^2 = 0.17)^*$		$\text{ACW } (R^2 = 0.25)^{**}$
Linalool oxide	NS	$y = 1.86-0.11 \text{ CL } (R^2 = 0.19)^*$	NS	$y = 2.54-15.70$
				$\text{ACW } (R^2 = 0.21)^*$
4-vinylguaiacol	$y = -230.36 + 20.50 \text{ AVY } (R^2 = 0.48)^*$	$y = 6.14-63.68 \text{ ACW } (R^2 = 0.22)^{**}$	NS	NS
		$y = -1.98 + 2.22 \text{ APW } (R^2 = 0.34)^{***}$		
		$y = 3.30-0.26 \text{ CL } (R^2 = 0.21)^*$		
Vanillin	$y = -12.79 + 164.51 \text{ ACW } (R^2 = 0.61)^*$		NS	NS
Eugenol	ND ^w	$y = -0.25 + 0.25 \text{ APW } (R^2 = 0.25)^*$	NS	ND

^zValue ranges are unitless ratios derived from gas chromatography–mass spectrometry peak areas versus an internal standard of 2-octanol.

^y $N = 9, 24, 33$, and 30 , for A-2008, A-2009, B-2008, and B-2009, respectively.

^xNS = Nonsignificant.

^wND = Not detected.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

preclude drawing conclusions. As a caveat, although the SPE material used here is reported to achieve good recoveries for a wide range of wine analytes (Lopez et al., 2002), our results could be affected by the use of a single nonpolar standard, especially for more polar compounds like vanillin.

BEST METRICS FOR PREDICTING BOUND VOLATILE CONCENTRATIONS. Different metrics could be used in quantifying cluster exposure for the purpose of building predictive models. Among the metrics we tested, those with higher spatial complexity, namely CEFA and $\ln(\text{CEL})$, offered better predictive power for bound volatile concentration than those using categorical assignments of cluster exposure, such as PIC, or that using a single measurement such as fruit zone *PPF*. More specifically, among the 22 significant site–year–timing–compound–metric responses, CEFA and $\ln(\text{CEL})$ appeared with the highest frequency (seven) followed by CEL (four), PIC (two), and percent *PPF* (two). These 22 responses represent 12 unique site–year–timing–compound combinations. Correlation analysis of relative metric efficacy resulted in a Pearson correlation coefficient of 0.94 [frequency = 1.5(ordinal) – 0.1; $P = 0.015$] suggesting that cluster exposure metrics with greater spatial sensitivity are more

effective for establishing light response curves [$\text{CEFA} \cong \ln(\text{CEL}) > \text{CEL} > \text{PIC} \cong \text{percent } PPF$].

The relatively low frequency of significant responses using categorical PQA metrics, such as PIC or a fixed-location percent *PPF* measurement to quantify cluster exposure, implies that subtleties in microclimatic spatial structure influence cluster exposure variability and corresponding biological responses. Thus, knowledge of spatial variability can potentially be used to guide vineyard operations, particularly when the variability has been determined to be nonrandom (Meyers et al., 2011). However, there were examples in this study where CEFA was not the best predictor. These scenarios could be explained by experimental error during canopy calibration because of operator error or limited ceptometer precision at near-zero *PPF* values, or because the bound volatile is poorly correlated with sun exposure but indirectly correlated with the other metrics.

Conclusions

The correlations among bound volatiles in ‘Riesling’ and cluster light exposure metrics, while significant in some cases, were generally weaker than that in studies where changes in the cluster light environment were imposed and yield component responses

were generally weaker than those for cluster exposure. Thus, active canopy management, in which vine vigor and fruit exposure are independently controlled, is likely to be more effective in influencing potential aroma compounds than selectively harvesting for naturally occurring variation in cluster exposure. However, even in the absence of imposed viticultural treatments, highly exposed fruit zone architectures may lead to higher bound C13 norisoprenoid concentrations. The low predictive power of the negative monoterpene responses at fruit set reported here precludes concluding that these responses are biologically significant, although the low range of cluster exposures studied suggests that additional research might be useful in establishing predictive responses. Similarly, the single yet strong negative response of eugenol to veraison cluster exposure suggests that a study over a larger range of exposure intensities might be revealing.

Although measures of cluster exposure often strongly correlate among themselves, they are not equivalent in their ability to quantitatively predict biological responses, suggesting that metrics such as CEFA and $\ln(\text{CEL})$, which capture subtle parametric variability, are superior predictors of biological response and may justify the required investment in equipment and labor.

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