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Comparison of Odor-Active Compounds in Grapes and Wines from *Vitis vinifera* and Non-Foxy American Grape Species

Qun Sun, Matthew J. Gates, Edward H. Lavin, Terry E. Acree, and Gavin L. Sacks*

Department of Food Science, NYSAES, Cornell University, 630 West North Street, Geneva, New York 14456, United States

ABSTRACT: Native American grape (*Vitis*) species have many desirable properties for winegrape breeding, but hybrids of these non-*vinifera* wild grapes with *Vitis vinifera* often have undesirable aromas. Other than the foxy-smelling compounds in *Vitis labrusca* and *Vitis rotundifolia*, the aromas inherent to American *Vitis* species are not well characterized. In this paper, the key odorants in wine produced from the American grape species *Vitis riparia* and *Vitis cinerea* were characterized in comparison to wine produced from European winegrapes (*V. vinifera*). Volatile compounds were extracted by solid-phase microextraction (SPME) and identified by gas chromatography−olfactometry/mass spectrometry (GC-O/MS). On the basis of flavor dilution values, most grape-derived compounds with fruity and floral aromas were at similar potency, but non-*vinifera* wines had higher concentrations of odorants with vegetative and earthy aromas: eugenol, *cis*-3-hexenol, 1,8-cineole, 3-isobutyl-2-methoxypyrazine (IBMP), and 3-isopropyl-2-methoxypyrazine (IPMP). Elevated concentrations of these compounds in non-*vinifera* wines were confirmed by quantitative GC-MS. Concentrations of IBMP and IPMP were well above sensory threshold in both non-*vinifera* wines. In a follow-up study, IBMP and IPMP were surveyed in 31 accessions of *V. riparia*, *V. rupestris*, and *V. cinerea*. Some accessions had concentrations of >350 pg/g IBMP or >30 pg/g IPMP, well above concentrations reported in previous studies of harvest-ripe *vinifera* grapes. Methyl anthranilate and 2-aminoacetophenone, key odorants responsible for the foxiness of *V. labrusca* grapes, were undetectable in both the *V. riparia* and *V. cinerea* wines (<10 μg/L).

KEYWORDS: non-*vinifera*, hybrid grape, SPME, methoxypyrazines, GC-O/MS, GC×GC-TOF-MS

INTRODUCTION

At least 60 species of grapes (*Vitis*) are reported worldwide.[1] One species, the European wine grape (*Vitis vinifera*), accounts for the majority of world wine production, but it can be challenging to grow due to high susceptibility to diseases (e.g., powdery mildew) and poor cold hardiness. Native American species and interspecific hybrids of non-*vinifera* grape species and *vinifera* generally have better resistance to both abiotic and biotic stresses and, as a result, are popular in areas with continental and humid climates such as midwestern and eastern North America.[2]

The flavor chemistry of some wild American species, notably those that demonstrate "foxy" aromas such as *Vitis labrusca* and *Vitis rotundifolia*, are relatively well studied. Methyl anthranilate (MA) has long been known to be an impact odorant in Concord (*Vitis labruscana* Bailey cv. 'Concord') and several related *labrusca*-containing cultivars.[3] 2-Aminoacetophenone (2AAP) has also been implicated as critical to the perception of foxiness, especially because many "foxy-smelling" grapes have negligible MA concentrations.[4] Furaneol ("caramel", "strawberry") is also found in concentrations well over threshold in many *V. labruscana* grapes,[5] and Furaneol and 2AAP are also suggested to be the characteristic odorants of Muscadine (*V. rotundifolia*) juice.[4,6] In the wild, *V. labrusca*, *V. rotundifolia*, and related species are consumed primarily by small mammals, and the observed increase in 2AAP and MA in ripening fruit may serve as deterrent to birds.[7]

Although "American grape aroma" and "foxy" are often used interchangeably,[8] the aroma chemistry of many of the American species are still poorly characterized, and it is not evident that all American species should be described as foxy. Species heavily used in breeding, such as *Vitis riparia*, *Vitis aestivalis*, and *Vitis rupestris*, are perhaps best known for their importance to breeding phylloxera-resistant rootstocks (e.g., 'Riparia gloire') but are also in the parentage of hybrid winegrape cultivars such as the classic French−American hybrids Marechal Foch and Chambourcin[9] and in newer releases such as Frontenac and Corot noir.[10] These interspecific hybrids reportedly do not possess the foxy aromas inherent to grapes with *V. labrusca* parentage[9] but are often considered to have inferior aroma qualities as compared to *V. vinifera*.[11] However, odorants responsible for the negative characteristics of these cultivars or their wild parents are still not well-defined, which serves as a hindrance to enologists, viticulturalists, and grape breeders. For example, grape breeders interested in selecting progeny without acceptable aroma may wait 2−4 years after making a cross to have sufficient fruit available for evaluation.

The most potent volatiles in some interspecific hybrids without *labrusca* parentage, including Frontenac, Vidal blanc, and Seyval blanc, have been determined by GC-O/MS,[12,13] although key odorants were not quantified. (Semi)quantitative studies of volatiles in wines produced from interspecific hybrids without *labrusca* parentage have been reported,[14−16] but these reports did not specifically identify odorants that contribute to the hybrid off-aroma. *V. riparia* volatile composition analysis by GC-MS has been reported,[17] but the focus of this earlier work was on juice and profiled the quantitatively dominant volatiles rather than determining the most odor-active ones.

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Table 1. Basic Juice Chemistry for *Vitis* Species Harvested in 2009

Vitis species	soluble solids (°Brix)	titratable acidity (g/L as tartaric)	pH
V. vinifera	20.8	10.7	3.32
V. riparia	20.6	34.7	3.11
V. cinerea	21.0	36.6	3.03

In this work, we report the key odorants in wines produced from *V. riparia* and *Vitis cinerea* in comparison to a *V. vinifera* wine. As stated earlier, *V. riparia* is widely used by breeders due to its good cold hardiness, and it is in the pedigree of several, well-known interspecific hybrids. Although *V. cinerea* has good disease resistance, it is usually avoided in breeding winegrapes due to poor flavor quality. Knowledge of the key odorants in these wild species should facilitate the breeding of interspecific hybrids with desirable aroma characteristics and provide targets for viticultural and enological studies intending to improve winegrape qualities.

MATERIALS AND METHODS

Reagents, Samples, and Standards. Ethyl butanoate, 99%; ethyl hexanoate, 99%; ethyl octanoate, 99+%; ethyl valerate, 99%; octanoic acid, 99%; and phenethyl acetate, 98+%, were purchased from Acros Organics (Geel, Belgium). Butanoic acid, 99+%; β-citronellol, 95%; ethyl isobutanoate, 99%; ethyl 2-methylbutanoate, 99%; ethyl *trans*-cinnamate, 99%; eugenol, 99%; nerol, 97%; 1-octen-3-ol, 98%; 2-phenylethanol, 99+%; 3-isobutyl-2-methoxypyrazine, 99%; and 3-isopropyl-2-methoxypyrazine, 97%, were purchased from Sigma-Aldrich (St. Louis, MO) (+)-*cis*-Rose-oxide, 99%; geraniol, 99%; and 1-hexanol, 99%, were purchased from Fluka (Sigma-Aldrich). Acetic acid; β-damascenone; decanoic acid; guaiacol; (*Z*)-2-hexen-1-ol, 95%; *cis*-3-hexenol, 98%; hexanoic acid; isoamyl acetate; isoamyl alcohol, 98.5%; isobutyl alcohol, 99%; isovaleric acid; linalool, 97+%; methionol; δ-nonalactone, 98%; γ-nonalactone; 2-octanol, 97%; α-terpineol; and *p*-vinylguaiacol, 98%, were purchased from SAFC Supply Solutions (Sigma-Aldrich). A C_7-C_{30} hydrocarbon mixture for determination of linear retention indices (RI) was obtained from Supelco (Bellefonte, PA). Water was purified through a Milli-Q Water System (Millipore, Billerica, MA). Absolute ethanol (100%) was purchased from Pharmco-AAPER (Shelbyville, KY). Dichloromethane, L-tartaric acid (99%), and sodium chloride (NaCl) were purchased from Fisher Scientific (Fair Lawn, NJ). [$^2H_2$]-3-Isobutyl-2-methoxypyrazine (IBMP) was synthesized in our laboratory according to the method of Kotseridis et al.[18]

Grape Sampling. *V. vinifera* (cv. Cabernet franc and Lemberger) grapes were harvested from Sawmill Creek Vineyards (Hector, NY) on October 10, 2009, and October 5, 2010. *V. rupestris* (7 accessions, 2010 only) *V. riparia* (9 accessions, 2009 and 2010), and *V. cinerea* (10 accessions, 2009 and 2010) grapes were harvested from the USDA–ARS Cold Hardy Grape Germplasm Collection vineyard (Geneva, NY). Samples from 2009 were pooled and used to produce wines for GC-O/MS studies. Basic juice chemistry for 2009 samples is reported in Table 1 (soluble solids, pH, titratable acidity). Soluble solids in 2010 samples were measured by refractometry and used for quantification of methoxypyrazines (MPs) in individual accessions.

Winemaking. Accessions of the same species were combined, manually destemmed, and crushed. Musts were supplemented with 1 g/L diammonium hydrogen phosphate (Presque Isle Wine Cellars, PA); 0.1 g/L Fermaid K (Lallemand, Rexdale, ON, Canada) and 0.15 g/L Goferm (Lallemand, Rexdale, ON, Canada) were added prior to inoculation with EC1118 yeast (Lallemand, Montréal, QC, Canada) at a rate of 0.26 g/L. Skin fermentations were performed in 4 L glass fermenters fitted with airlocks and took place at 20 °C. The fermenter was shaken two or three times per day to submerge the cap. Primary fermentation was determined to be complete when residual sugar was measured to be lower than 0.5% using Clinitest tablets (Bayer, Etobicoke, ON, Canada). All fermentations reached dryness within a 24 h period. Wine was pressed by hand with cheesecloth, and sulfur dioxide was added to maintain 40 mg/L free sulfur dioxide. Wines were cold stabilized at 2 °C, screened for faults by a trained panel, and bottled.

Volatile Extraction for GC-O. Three extraction techniques were initially compared. Solid-phase microextraction (SPME) was eventually selected for use in quantitative GC-O analyses. SPME was performed with a 50/30 μm fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; Supelco, Bellefonte, PA). Fibers were thermally conditioned for 1 h at 270 °C before their first use. For sampling of volatiles, 5 mL of wine and 5 mL of water were added to a 20 mL SPME glass vial (Supelco) containing 3 g of NaCl. The vial was tightly capped with a Teflon/silicone septum (Supelco) and incubated at 40 °C for 10 min. The SPME fiber was exposed to the sample for 50 min at 40 °C, and the vial was sonicated throughout the extraction.

Solid-phase extraction (SPE) sorbent and liquid–liquid extraction (LLE) were also investigated. SPE was adapted from ref 19. Wines were extracted on 200 mg LiChrolut EN SPE cartridges (Merck, Darmstadt, Germany) fitted with 15 mL reservoirs preconditioned with 4 mL of dichloromethane, 4 mL of methanol, and 4 mL of model wine (10% ethanol, 5 g/L tartaric acid, adjusted to pH 3.5 with 2 M NaOH), and then 50 mL of wine was loaded onto the cartridges at 2 mL/min and dried (N_2, 1.7 bar, 20 min) on a Varian Cerex SPE processer (Walnut Creek, CA). The analytes were recovered by elution with 1.3 mL of dichloromethane. LLE was adapted from ref 14. A 50 mL wine sample was sequentially extracted with 3 × 15 mL of Freon 113. The extracts were pooled, dried with anhydrous magnesium sulfate, and concentrated to 1.0 mL by rotary evaporator (BUCHI Corp., New Castle, DE).

Gas Chromatography–Olfactometry/Mass Spectrometry Analysis (GC-O/MS). GC-O analyses were performed on a CharmAnalysis system (Datu, Inc., Geneva, NY) equipped with either an Agilent DB-5 (30 m × 0.25 mm × 0.25 μm) or a Varian CP-WAX 58 FFAP (25 m × 0.25 mm × 0.20 μm) column. Following extraction, the SPME fiber was inserted into the split/splitless injection port (held at 250 °C) for 5 min. Dilutions were performed by adjusting the split to 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256. The GC was operated in constant pressure mode at a pressure of 10 psi. The oven temperature was held at 35 °C for 3 min, ramped at 6 °C/min to 250 °C, and then held for 5 min. The GC effluent was combined with a humidified air stream at 7 L/min before entering the sniff port. Two sniffer panelists were used for the initial evaluation of extracts to generate aroma descriptors. The odor descriptor reported is the consensus of the panelists. The panelists were first screened for anosmias by GC-O using an odorant mixture.[20] The dilution analyses were performed by a single sniffer. Sniffing of all extract dilutions was repeated twice until no odor was detected. To determine retention indices for each column, the column outlet was manually switched to an FID detector, and a C_7-C_{30} *n*-alkane standard run. Linear retention indices were then calculated using standard approaches. The FD value was geometrically averaged from the data of two replicates using the equation $FD = 2^{(a+b)/2}$.

Compound identification was performed by GC-MS using a HP6890 coupled to a HP model 5970 mass selective detector (Agilent Technologies, Palo Alto, CA) fitted with the same columns used for GC-O. During GC-MS analyses, the SPME fiber was inserted, splitless, onto an injector set at 250 °C. The purge was activated at 2 min. The carrier gas was helium (1 mL/min). The oven temperature was held at 35 °C for 3 min, programmed at 6 °C/min to 250 °C, and held for 5 min isothermally. Mass spectra were acquired over m/z 33–250. Chemstation software version G1701EA E.02.00.493.33 was used for data acquisition. Compounds were tentatively identified by matching the

retention index (RI) of the unknown compound with the RI of standard compounds as well as odor character and mass spectral data. Where possible, identification was confirmed by comparison against authentic standards.

Quantification of Aroma Compounds in Wines. Eugenol, 1,8-cineole, and *cis*-3-hexenol were extracted from wine by LiChrolut EN SPE as described in the earlier under Volatile Extraction for GC-O/MS. Quantification was performed by GC-TOF-MS (Pegasus IV, Leco Corp., St. Joseph, MI). The injector temperature was 250 °C, and the injection size was 1 μL. The GC column was a Varian CP-WAX (30 m × 0.25 mm × 0.25 μm). The oven temperature was held at 40 °C for 3 min, then increased to 200 °C at 5 °C/min, ramped to 240 °C at 10 °C/min, and held at 240 °C for 15 min. The TOF-MS was operated in EI mode with an ionization energy of 70 eV. The voltage of the electron multiplier was 1700 V. The data acquisition rate of the TOF-MS was set to 3 Hz, and a mass range of m/z 35−400 was stored. The native Leco ChromaTof software was used for data processing. The quantifier ions for eugenol, 1,8-cineole, and *cis*-3-hexenol were m/z 164,154, and 67, respectively. Qualifier ions were m/z 131, 149 (eugenol), 108, 111 (1,8-cineole), and 55, 82 (*cis*-3-hexenol). Concentrations were determined with respect to four-point calibration curves prepared in model wine, and calibration curves for all three analytes had $r^2 > 0.99$. Limits of detection were calculated as 3× the root mean squared noise on either side of the peak. All extractions and subsequent quantifications were performed in duplicate.

IBMP and IPMP were quantified in wine by SPME-GC×GC-TOF-MS, using a previously described method.[21] In brief, HS-SPME was performed by a LEAP CombiPAL Autosampler (Carrboro, NC) using a three-phase fiber (DVB/CAR/PDMS). A 5 mL sample was combined with 5 mL of EDTA buffer (pH 7.5) and weighed into a 20 mL amber SPME vial (Supelco) along with 3 g of NaCl and 20 μL of internal standard (2.5 ng/mL [$^2H_2$]-IBMP in H_2O). The vial was incubated online with a 650 rpm agitation rate under 80 °C for 10 min before fiber insertion. After fiber insertion, the vial was agitated at 100 rpm for 30 min at 80 °C. Quantification was performed by GC×GC-TOF-MS (Pegasus IV, Leco Corp.) using two columns. The first column (30 m × 0.25 mm × 0.50 μm) was an RTX5 (Restek, Bellefonte, PA), and the second column (2.5 m × 0.10 mm × 0.10 μm) was a VF-WAXms (Varian, Palo Alto, CA). High-purity helium was used as a carrier gas with flow rate of 1 mL/min. The injector was held at 270 °C. The temperature program for the column oven was as follows: 40 °C for 5 min, ramping to 120 °C at a rate of 5 °C/min, then increasing from 120 to 150 °C at a rate of 2 °C/min, ramping to 250 °C at 10 °C/min, and then held at 15 min at 250 °C. The GC×GC modulation time and the MS transfer line temperature were set to 3 s and 230 °C, respectively. The TOF-MS was operated in EI mode with an ionization energy of 70 eV. The voltage of the electron multiplier was 1680 V. The data acquisition rate of the TOF-MS was set to 120 Hz in a mass range of m/z 20−400. The qualifier ions were m/z 124, 151, and 166 for IBMP and m/z 126, 153, and 168 for [$^2H_2$]-IBMP, respectively. The quantifier ions were m/z 124 and 126, respectively. For 3-isopropyl-2-methoxypyrazine (IPMP), the qualifier ions were m/z 137, 124, and 152 and the quantifier ion was m/z 137.

Quantification of IBMP and IPMP in Grapes. Sample preparation was adopted from a previously described protocol.[21] For each accession, 50 g of frozen whole berries was homogenized by bead milling (2000 Geno/Grinder, SPEX Certiprep, Metuchen, NJ). The homogenate was diluted 50% w/w with 0.1 M EDTA (adjusted to pH 7.5 with NaOH) to facilitate sample handling. The diluted homogenate (10 g) was weighed into a 20 mL amber SPME vial (Supelco) along with 3 g of NaCl and 20 μL of internal standard (2.5 ng/mL [$^2H_2$]-IBMP in H_2O). Quantification was performed by SPME-GC×GC-TOF-MS. The SPME extraction occurred at 80 °C but was otherwise identical to the protocol described for wine analyses of IBMP and IPMP.

Detection of MA and 2AAP in Non-*vinifera* Wines. To determine if 2AAP and MA were present in non-*vinifera* wines, 50 mL each of *riparia* and *cinerea* wine were spiked with 50 μL of MA and 2AAP standard solutions. The standard solutions were prepared in acetonitrile and contained both standards at concentrations of 0.1 or 1 g/L, resulting in wines spiked with 0.1 mg/L MA + 0.1 mg/L 2AAP or 1 mg/L MA + 1 mg/L 2AAP. Unspiked control wines were also prepared. An internal standard solution (0.5 g/L of 2-octanol in acetonitrile, 25 μL) was added to each sample. All samples were prepared in duplicate.

SPE was performed as described in under Volatile Extraction for GC-O/MS. GC-MS analyses were performed on a Varian CP-3800 gas chromatograph coupled to a Saturn 2000 ion trap mass spectrometer, fitted with a Varian VF-WAX MS column (30 m × 0.25 mm × 0.25 μm). For analysis, 1 μL of sample was injected, splitless, onto a Varian PTV 1079 injector set at 250 °C. The purge was activated at 1 min. The carrier gas was helium (1 mL/min). The oven was held initially at 40 °C for 5 min, ramped to 170 °C at 5 °C/min, ramped to 250 °C at 10 °C/min, and then held for 3 min. The transfer line, manifold, and ion trap temperatures were set at 250, 50, and 170 °C, respectively. The instrumental scan rate was 5600 amu/s, the prescan time was 100 μs, and the scan rate was 10 μscans/s, resulting in an effective sampling rate of 1 Hz. A mass range of m/z 25−220 was stored and detected. Data files were converted to netCDF format using VxCapture (Adron Systems, Laporte, MN) and imported into Leco ChromaTOF v 4.33 software for visualization and analysis. Selected ion chromatograms of m/z 119 + 151 (MA) and m/z 120 (2AAP) were plotted, and ChromaTOF software was used to calculate signal-to-noise ratios for the 0.1 mg/L spikes. Limits of detection were calculated as the concentration necessary to achieve S/N = 3.

RESULTS AND DISCUSSION

Comparison of Extraction Techniques for GC-O Analyses. We evaluated three extraction techniques previously used for GC-O studies of wine volatiles: SPE, SPME, and LLE. Two compounds were detectable in SPE and LLE extracts that were not detectable in SPME extracts, namely, the products of carbohydrate degradation, sotolon and Furaneol (data not shown). These compounds were previously reported in SPE and LLE extracts[22,23] that use solvents and sorbents with intermediate polarity, but they are frequently absent in GC-O studies employing SPME, stir-bar sorption extraction, or apolar LLE. However, these odorants had similar intensity by GC-O across all three wine samples, and because the techniques yielded otherwise similar results, SPME was selected due to its convenience.

Detection and Identification of Odor-Active Compounds by Quantitative GC-O. Forty odor-active aroma compounds were detected by GC-O using two different columns (nonpolar DB-5 and polar FFAP). The compound identities, flavor dilution (FD) values, and identification criteria are listed in Table 2. These compounds are subdivided into three categories: fermentation-derived compounds, grape-derived compounds, and unknowns. Fermentation-derived compounds include ethyl esters, acetate esters, fatty acids, and fusel alcohols produced de novo via yeast metabolism from sugars and amino acids.[24] Grape-derived compounds include those primary odorants initially present in the grape as well as compounds likely to have been released during fermentation from nonodorous precursors.[24]

Fermentation-Derived Compounds. The majority of compounds (24 of 40) detected and identified by GC-O/MS included esters, fusel alcohols, and fatty acids likely derived solely from fermentation. Similar results have been observed in other GC-O/MS studies of wines. For example, 14 of the 26 most potent compounds (FD ≥ 16) in a Grenache rosé wine[22] were fermentation derived, and comparable results have been observed for Gewurztraminer.[23] The most potent fermentation

Table 2. Odor-Active Compounds Found in Wines by GC-O[a]

		RI		FD value				
no.	volatile compound	DB-5	CP-WAX	*Vitis vinifera*	*Vitis riparia*	*Vitis cinerea*	descriptor	basis of identification[b]
				Grape-Derived Compounds				
1	β-damascenone	1385	1767	128	256	256	cooked apple	MS, RI
2	ethyl cinnamate	1467	2141	32	16	32	floral	MS, RI
3	linalool	1098	1548	16	32	8	floral	MS, RI
4	β-ionone	1452	1890	16	16	32	sweet	MS, RIL
5	α-terpineol		1725	8	8	16	floral	MS, RI
6	3-isobutyl-2-methoxypyrazine	1180	1527	8	4	16	bell pepper	MS, RI
7	guaiacol	1092	1873	4	8	32	smoky	MS, RI
8	octen-3-ol	973	1404	4	8	2	mushroom	MS, RI
9	(+)-*cis*-rose oxide	1109		2	0	0	floral	MS, RI
10	3-isopropyl-2-methoxypyrazine		1424	1	64	64	earthy	MS, RI
11	eugenol	1357	2183	1	4	64	clove	MS, RI
12	citronellol	1313		1	4	1	floral	MS, RI
13	(*Z*)-linalool oxide	1065		1	2	1	floral	MS, RI
14	*cis*-3-hexenol	853	1390	0	2	16	green	MS, RI
15	1,8-cineole	1029	1192	0	2	4	minty	MS, RI
				Fermentation-Derived Compounds				
1	ethyl isobutanoate	750	947	128	128	128	apple	MS, RI
2	isoamyl alcohol	726	1209	128	128	64	chocolate	MS, RI
3	ethyl hexanoate	998	1224	128	64	16	fruity	MS, RI
4	methyl furanthiol	862	1316	32	32	8	potato	RIL
5	ethyl 3-methylbutanoate	852	1058	32	16	16	fruity	MS, RI
6	phenyl ethanol	1113	1922	32	16	16	floral	MS, RI
7	ethyl phenylacetate	1242		32	16	16	floral	MS, RI
8	ethyl 2-methylbutanoate	846	1048	16	128	32	fruity	MS, RI
9	dimethyl trisulfide	965	1376	8	64	128	dirty	RIL
10	butanoic acid	821		8	128	64	fruity	RI
11	isobutyl acetate		1013	8	32	32	fruity	MS, RI
12	ethyl acetate	608	907	8	8	16	solvent	MS, RI
13	isoamyl acetate	899	1118	8	4	2	banana	MS, RI
14	isovaleric acid		1671	4	32	128	potato	RI
15	ethyl butanoate	796	1031	32	64	64	fruity	MS, RI
16	isobutanol	654	1093	4	8	8	coca	MS, RI
17	diacetyl	636	960	4	2	8	butter	MS, RI
18	ethyl propionate	665	985	4	2	4	fruity	MS, RI
19	ethyl octanoate	1228	1436	4	2	1	floral	MS, RI
20	1-hexanol	874	1362	1	2	2	green	MS, RI
21	(*Z*)-2-penten-1-ol	770		0	1	1	rubber	MS, RIL
22	ethyl lactate		1345	0	0	2	floral	MS, RI
				Unknown Compounds				
1	unknown	1363		32	64	1	sweet	RIL
2	unknown	668		32	64	16	dirty	RIL
3	unknown	1049		1	4	1	fruity	RIL

[a] Initial evaluation of extracts and generation of descriptors was performed by two sniffer panelists, and dilution analyses were performed by a single sniffer. [b] MS, compounds were identified by the MS spectra; RI, compounds were identified by comparison with retention indices of standards; RIL, compounds were identified by comparison with retention indices from www.flavornet.org.

aroma compounds, with FD values >16 for all wines, were ethyl isobutanoate, ethyl hexanoate, ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, isoamyl alcohol, and phenylethyl acetate (Table 2, bottom). Again, these compounds have been reported to have high FD values not only in wines[25] but also in a wide range of fermented beverages, including spirits.[26]

Table 3. Mean Concentrations of Aroma Compounds in Duplicate Wines[a]

volatile	odor threshold	concentration range from the literature	*vinifera*	*riparia*	*cinerea*
eugenol (μg/L)	6	4−73[19]	4 a	16 b	328 c
1,8-cineole (μg/L)	1.1	for untainted wines, <0.8 (white wines), ∼1.7 (red wines)[32]	nd	1 a	4 b
cis-3-hexenol (μg/L)	400	40−240[36]	75 a	205 b	3990 c
IBMP (ng/L)	10−15	5−20 in Bordeaux cultivars (Cabernet Sauvignon, Sauvignon blanc)[42]	16 a	56 b	57 b
IPMP (ng/L)	0.2−1.5	nd−2 in Bordeaux cultivars[42]	nd	3 a	6 b

[a] Different letters in the same row indicate significantly different concentrations for the volatile among wines. References for sensory thresholds are reported in the text. nd, not detected, <0.5 μg/L for 1,8-cineole.

The concentrations of fermentation-derived compounds are well-known to vary with initial sugar concentration, oxygen availability, must lipid composition, levels of yeast assimilable nitrogen, and fermentation temperature.[27] Because we attempted to standardize fermentation conditions in this work, only modest differences in FD among fermentation-derived compounds, generally less than a factor of 4, were observed in our study for nearly all compounds.

Notable exceptions include isovaleric acid, butanoic acid, and ethyl butanoate, where FD values in *vinifera* were an order of magnitude less than those of the wild species wines. We performed a semiquantitative analysis of these three compounds by SPE-GC-MS (data not shown) and observed only minor differences (<12%), even though larger semiquantitative differences were apparent in SPME-GC-MS during compound identification. Thus, we suspect that differences in FD values among wines for short-chain fatty acids were an artifact of the SPME procedure. SPME is poor at extracting semipolar compounds and thus is susceptible to differences in matrix composition for these analytes, possibly as a result of decreased volatility in different matrices.[28]

Grape-Derived Compounds. Several classes of grape-derived compounds are commonly reported in wines: methoxypyrazines (MPs), volatile thiols, volatile phenols, C_{13} norisoprenoids, and monoterpenes.[24] Of these compound classes, only volatile thiols were not detected (Table 2), with the exception of 2-methyl-3-furanthiol, which was detected in all wines. Volatile thiols such as 3-mercaptohexanol are readily oxidized,[29] and it is possible that the small-scale winemaking or extraction conditions we used resulted in the loss of some key aroma compounds.

Of the grape-derived compounds detected by GC-O, five of these odorants had FD values greater than two dilution steps (>4-fold) in non-*vinifera* wines relative to *vinifera*: eugenol, IPMP, IBMP, *cis*-3-hexenol, and 1,8-cineole.

All of these odorants have aroma characteristics that fall into the "vegetal, earthy, minty" family (Table 3). Conversely, we observed little or no differences in FD for compounds with a fruity/floral character, including β-damascenone, ethyl cinnamate, linalool, β-ionone, α-terpineol, *cis*-rose oxide, citronellol, and (*Z*)-linalool oxide. Differences in fruity versus vegetative aromas are often the most important characteristic for distinguishing wines by sensory descriptive analysis.[30] Thus, on the basis of GC-O data, the major difference between non-*vinifera* and *vinifera* wines appears to be the presence of higher concentrations of vegetative odorants in non-*vinifera* wines rather than other differences such as the absence of fruity aroma compounds.

As a caveat, these compounds identified by GC-O are only candidate odorants for explaining differences in wines produced from wild American species or their offspring. Determining if these odorants individually or collectively contribute to the characteristic aroma of native American species would demand other techniques such as reconstitution studies,[25] but this was outside the scope of the current study.

Quantification of High-Potency Odorants in Non-*vinifera* Wines. We utilized SPE-GC-TOF-MS and SPME-GC×GC-TOF-MS to quantify the five compounds identified as uniquely high in non-*vinifera* wines by GC-O/MS analyses. Concentrations in the studied wines, concentrations from literature studies on *V. vinifera* wines, sensory thresholds, and sensory descriptors are summarized in Table 3.

Eugenol is reported to have a "clove"-like aroma, and its concentrations were significantly higher in *V. riparia* (18 μg/L) and *V. cinerea* (328 μg/L) than in the *vinifera* wine (5 μg/L) and greater than the sensory threshold of eugenol in a 12% ethanol/water matrix.[31] Eugenol was also previously detected in the *V. riparia*-containing hybrids Frontenac[13] and Marechal Foch,[16] although exact quantification was not performed. Eugenol can be detected as a bound, glycosylated precursor in grapes, but high concentrations in wines are usually associated with contact with oak.[24] The mean concentration in Spanish red wines is reportedly 29 μg/L (range = 4−73 μg/L),[19] and the upper end of this range is below the concentration observed in our *V. cinerea* wine. To our knowledge, eugenol aromas are not generally considered a defect in wine, but their presence in unoaked red wines may be undesirable.

1,8-Cineole, also known as eucalyptol, has been reported to contribute a "eucalyptus" aroma, and it has a threshold of 1.1 μg/L in red wine.[32] 1,8-Cineole was undetectable in our *vinifera* wine (<0.5 μg/L), but it was above threshold in both *V. riparia* (1.3 μg/L) and *V. cinerea* (4 μg/L) (Table 3), consistent with observed differences in FD values in Table 2. The presence of 1,8-cineole at concentrations up to 20 μg/L has been reported in red wines, potentially due to exogenous contamination of grapes by eucalyptus tree emission, known as "eucalyptus taint",[32] although this phenomenon seems unlikely in upstate New York. Endogenous formation of 1,8-cineole has been reported to occur preveraison before decreasing during berry maturation,[33] but it is not possible to infer 1,8-cineole behavior in our study because only a single time point was sampled. Alternatively, Farina et al.[34] suggested that other monoterpenes (e.g., terpineol, limonene) could serve as precursors of 1,8-cineole in Tannat. We did not attempt to quantify these potential precursors in our current work.

cis-3-Hexenol ("leafy-grassy" aroma) is one of several 6-carbon alcohols and aldehydes formed by enzymatic oxidation of lipids following mechanical damage to grapes, especially underripe grapes or green tissue.[33,35] Whereas *cis*-3-hexenol is detectable immediately following crushing, it is also putatively formed during fermentation by reduction of *cis*-3-hexenal.[35] Concentrations of *cis*-3-hexenol in *vinifera* wines are reported to range from 40 to 240 μg/L.[36] Although this is below the reported sensory threshold for *cis*-3-hexenol in 10% ethanol (400 μg/L), it is

Table 4. Methoxypyrazine Concentrations in Different Wild Species Accessions

species	accession	IBMP (ng/L)	IPMP (ng/L)	total soluble solids (°Brix)
cinerea	1	13 ± 1	10 ± 3	20.5
	2	110 ± 9	7 ± 2	20.3
	3	143 ± 8	17 ± 3	18.0
	4	17 ± 1	1 ± 0.4	15.0
	5	18 ± 2	nd[a]	20.1
	6	286 ± 12	16 ± 4	23.5
	7	251 ± 10	31 ± 5	20.6
	8	52 ± 6	nd	15.3
	9	353 ± 16	nd	20.4
	10	33 ± 5	8 ± 2	21.2
riparia	1	50 ± 5	nd	17.9
	2	50 ± 3	nd	23.9
	3	166 ± 10	nd	24.8
	4	109 ± 9	4 ± 1	17.8
	5	79 ± 8	nd	20.6
	6	310 ± 13	13 ± 3	25.7
	7	82 ± 8	nd	19.5
	8	65 ± 6	nd	23.4
	9	89 ± 6	nd	22.3
	10	36 ± 5	nd	20.4
	11	33 ± 5	nd	18.4
	12	43 ± 6	nd	19.2
	13	13 ± 1	nd	20.1
	14	36 ± 5	nd	22.3
rupestris	1	24 ± 6	1 ± 0.5	19.5
	2	29 ± 6	1 ± 0.5	20.1
	3	14 ± 2	nd	17.8
	4	14 ± 2	nd	19.6
	5	16 ± 0	nd	18.2
	6	13 ± 1	nd	19.8
	7	11 ± 1	nd	18.4

[a] nd means below the limit of detection (<1 ng/L).

suggested that perithreshold concentrations could increase or modify the perception of herbaceousness caused by methoxypyrazines.[37] In our work, we found much greater *cis*-3-hexenol concentrations in *V. cinerea* wine (3990 μg/L) than in either the *V. riparia* wine (205 μg/L) or the *vinifera* wine (75 μg/L) of the aforementioned studies. Although we did not perform sensory experiments, it seems very likely that *cis*-3-hexenol would have a noticeable impact on *cinerea* wine aroma at a concentration 10-fold over threshold. Potentially, the higher concentrations of *cis*-3-hexenol in *V. riparia* or *V. cinerea* wines could be due either to higher concentrations of linolenic acid, the likely precursor of *cis*-3-hexenol, or due to higher activity of key enzymes associated with *cis*-3-hexenol formation (e.g., hydroperoxylyase, lipoxygenase).

Two MPs, IBMP and IPMP, were determined to have higher FD values by GC-O in one or both of the non-*vinifera* wines compared to the *vinifera*, and these differences were confirmed by quantitative analysis. IBMP and IPMP are generally described as having "herbaceous" and "earthy" aromas and thresholds of 10−15 ng/L[38,39] and 0.2−1.5 ng/L[40] in wine, respectively. Whereas MPs are not observed in all grape cultivars at harvest, the *vinifera* wine in this study contained Cabernet franc, a cultivar known to have detectable IBMP. MP concentrations in the *vinifera* wine were consistent with previous studies: the concentration of IBMP in the *vinifera* wine (15 ng/L) was within the reported range of values and that of IPMP was less than the instrumental limits of detection, ∼1 ng/L.[21] By comparison, the concentration of IBMP was nearly 60 ng/L in both the *V. riparia* and *V. cinerea* wines, comparable to the highest values reported in *V. vinifera*.[41] Similarly, IPMP is usually undetectable in wines,[42] but it was present at levels well above its reported sensory thresholds in *V. riparia* (3 ng/L) and *V. cinerea* (6 ng/L) wines.

Concentrations of MPs in *V. cinerea*, *V. rupestris*, and *V. riparia* Accessions. Whereas MPs can contribute to varietal character in some wines, they are considered to be undesirable at concentrations well in excess of threshold, especially in red wines.[41] Because MP concentrations in grapes and their corresponding wines are well correlated,[21] reducing MPs early in the selection process would seem to be a logical target for grape breeders interested in eliminating selections with poor flavor potential. The 2009 wines used in the GC-O/MS studies were blends of multiple accessions from the USDA−ARS Cold Hardy Grape Germplasm Collection, and it was not possible to determine if MPs were uniformly high in all *V. riparia* and *V. cinerea* accessions. In 2010, we performed a survey of the 10 *cinerea* and 14 *riparia* accessions we used for wine production in 2009. We also included 7 accessions of *V. rupestris* because this cultivar is widely used in grape breeding for cool and humid climates. Accessions were sampled on the same day. Although a wide range in soluble solids was observed, the mean value (20 °Brix) is within the range commonly observed for *vinifera* in the Finger Lakes area at harvest. The IBMP concentration in some *V. cinerea* and *V. riparia* accessions was remarkably high (Table 4). We observed IBMP ranging from 13 to 353 pg/g in *V. cinerea* and from 13 to 310 pg/g in *V. riparia*. IBMP concentrations were less variable in *V. rupestris* accessions (11−29 pg/g), a range more comparable to that reported in Cabernet Sauvignon and related cultivars. The highest IBMP concentrations detected (>300 pg/g) in the non-*vinifera* accessions are well above any concentrations reported in *vinifera* at harvest, but they are comparable to concentrations observed in *vinifera* preveraison.[43] In *vinifera*, high IBMP at harvest can either arise from greater accumulation of IBMP preveraison or slower degradation postveraison, but because only a single time point was sampled, it is not clear if IBMP dynamics in non-*vinifera* species are similar to those in *vinifera*. No correlation was observed between Brix and IBMP ($p > 0.05$), so differences in maturity seem unlikely to explain observed differences in IBMP. IPMP was found in all non-*vinifera* species, ranging from undetectable to 31 pg/g in *V. cinerea*, from undetectable to 13 pg/g in *V. riparia*, and from undetectable to 1 pg/g in *V. rupestris* (Table 4). In all accessions, IBMP concentration was greater than IPMP concentration, as has been observed in *vinifera*. The concentrations of the two MPs were positively correlated, although the correlation was modest ($r = 0.55$, $p < 0.05$).

Attempt To Detect "Foxy" Aroma Compounds in Non-*vinifera* Wines. The "foxy, grapey"-smelling MA and 2AAP were not detected by GC-O in *V. riparia* or *V. cinerea* wines. These compounds are present at concentrations >100 ng/mL in *V. labruscana* grapes,[4] and they are readily detectable with high dilution values in *V. labruscana* and *V. rotundifolia* juices or wines using GC-O.[6] To confirm that MA and 2AAP were not present in

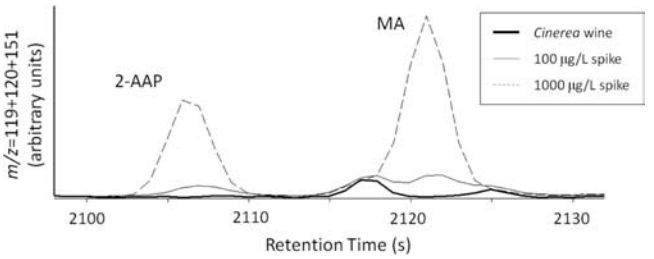

Figure 1. Selected ion chromatograms, m/z 119 + 120 + 151, for *cinerea* wine (bold line) and the same wine spiked with 100 μg/L MA + 100 μg/L 2AAP (thin line) or 1000 μg/L MA + 1000 μg/L 2AAP (dashed line).

supratheshold concentrations in the *V. riparia* and *V. cinerea* wines, we attempted to detect the compounds by Lichrolut EN SPE followed by GC−ion trap−MS. The GC-TOF-MS was not used due to the unavailability of the instrument. The detection threshold (S/N > 3) was 9 μg/L for MA and 7 μg/L for 2AAP, and neither compound was detectable in the wines under study. Representative chromatograms of *V. cinerea* wine and the same wine spiked with MA and 2AAP are shown in Figure 1. Similar results were observed with *V. riparia* wine (data not shown). The detection thresholds of the analytical method are well below the 300 μg/L sensory threshold reported for MA in wine[44] but not below the 1 μg/L sensory threshold reported for 2AAP.[8] Thus, the contribution of 2AAP to the aroma of these non-*vinifera* wines cannot be excluded. However, it is evident that 2AAP and MA are present at concentrations at least 1−2 orders of magnitude lower than concentrations reported in *V. labruscana* wines. MA and 2AAP are bird deterrents,[7] and their presence in *V. labrusca* and *V. rotundifolia* is likely indicative of strategy to encourage consumption by mammals rather than birds.[45] Conversely, *V. riparia* and *V. cinerea* are more similar to *vinifera* (e.g., dark color, small berries, upward growth habit), and birds are well-known to consume *vinifera* berries.[45] The observation that MA and 2AAP are not detectable in *V. riparia* and *V. cinerea* is not surprising, but it is also clear evidence that "foxy" and "American grape aroma" should not be used synonymously.

In summary, we have used GC-O/MS to characterize the aroma profile of wines produced from non-*vinifera* (*V. riparia* and *V. cinerea*) grape species without "foxy" characteristics in comparison to wine produced from European winegrapes (*V. vinifera*). In agreement with previous studies, most compounds with high FD values were derived solely from fermentation (e.g., ethyl esters, acetate esters, fatty acids, and fusel alcohols) and did not differ among wines. Grape-derived aroma compounds with floral and fruity characteristics (e.g., linalool and β-damascenone) also did not differ in FD value. Key odorants associated with "foxiness" were also not detected by GC-O or GC-MS. However, on the basis of cumulative FD values, non-*vinifera* wines had more aroma compounds with vegetative and earthy aromas, and this was confirmed by quantitative GC-MS studies. A survey of MP concentrations in *V. rupestris*, *V. riparia*, and *V. cinerea* from a diverse germplasm collection revealed accessions with >350 pg/g IBMP and 30 pg/g IPMP, well above concentrations reported in previous studies of mature *V. vinifera* grapes. Because neither MP was detected in previous GC-O/MS studies of *V. riparia*-containing hybrids,[12,13] and because MPs are sensorially detectable in the grape berries, it seems plausible that breeders have selected against this trait in the development of these winegrape cultivars. However, this still requires time to produce fruit for evaluation after a new cross is made. Identifying genetic markers for high MP concentrations in wild species should allow early selection of low MP offspring without the need to wait two growing seasons for fruit production, similar to approaches proposed for selecting other desirable fruit traits such as seedlessness in table grapes.[46]

AUTHOR INFORMATION

Corresponding Author

*E-mail: gls9@cornell.edu. Phone: (315) 787-2458. Fax: (315) 787-2397.

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REFERENCES

(1) USDA−Agricultural Research Service. Grin species records of *Vitis*; http://www.ars-grin.gov/cgi-bin/npgs/html/splist.pl?12735 (accessed May 11, 2011).

(2) Pinney, T. *A History of Wine in America: From Prohibition to the Present*; University of California Press: Berkeley, CA, 2005; pp xvi, 532.

(3) Sale, J. W.; Wilson, J. B. Distribution of volatile flavor in grapes and grape juices. *J. Agric. Res.* **1926**, *33*, 0301–0310.

(4) Acree, T. E.; Lavin, E. H.; Nishida, R.; Watanabe, S. In *O-Amino Acetophenone the "Foxy" Smelling Component of Labruscana Grapes*; Flavour Science and Technology − 6th Weurmann Symposium; Bessiere, Y., Thomas, A. F., Eds.; Wiley: Hoboken, NJ, USA; 1990; pp 49−52.

(5) Rapp, A.; Knipser, W.; Engel, L.; Ullemeyer, H.; Heimann, W. Off-flavor compounds in the berry and wine aroma of grapevine hybrids. 1. The strawberry-like flavor. *Vitis* **1980**, *19* (1), 13–23.

(6) Baek, H. H.; Cadwallader, K. R.; Marroquin, E.; Silva, J. L. Identification of predominant aroma compounds in Muscadine grape juice. *J. Food Sci.* **1997**, *62* (2), 249–252.

(7) Mason, J. R.; Clark, L.; Shah, P. S. Ortho-aminoacetophenone repellency to birds − similarities to methyl anthranilate. *J. Wildlife Manag.* **1991**, *55* (2), 334–340.

(8) Rapp, A. Volatile flavour of wine: correlation between instrumental analysis and sensory perception. *Nahrung−Food* **1998**, *42* (6), 351–363.

(9) Reisch, B. I.; Pool, R. M.; Peterson, D. V.; Martens, M. H.; Henick-Kling, T. Wine and juice grape varieties for cool climates. In *Information Bulletin 233* [online] Cornell Cooperative Extension, 1993; http://ecommons.cornell.edu/bitstream/1813/3558/2/Wine%20and%20Juice%20Grape%20Varieties%20for%20Cool%20Climates.pdf (accessed May 11, 2011).

(10) Smiley, L. A.; Domoto, P.; Nonnecke, G.; Miller, W. W. Cold climate cultivars 2011; http://viticulture.hort.iastate.edu/cultivars/cultivars.html (accessed May 11, 2011).

(11) Robinson, J. *The Oxford Companion to Wine*, 3rd ed.; Oxford University Press: Oxford, U.K., 2006.

(12) Chisholm, M. G.; Guiher, L. A.; Vonah, T. M.; Beaumont, J. L. Comparison of some French−American hybrid wines with White Riesling using gas-chromatography olfactometry. *Am. J. Enol. Vitic.* **1994**, *45* (2), 201–212.

(13) Mansfield, A. K.; Vickers, Z. M. Characterization of the aroma of red Frontenac table wines by descriptive analysis. *Am. J. Enol. Vitic.* **2009**, *60* (4), 435–441.

(14) Ong, P. K. C.; Acree, T. E. Similarities in the aroma chemistry of Gewurztraminer variety wines and lychee (*Litchi chinesis* Sonn.) fruit. *J. Agric. Food Chem.* **1999**, *47* (2), 665–670.

(15) Skinkis, P. A.; Bordelon, B. P.; Wood, K. V. Comparison of monoterpene constituents in Traminette, Gewurztraminer, and Riesling winegrapes. *Am. J. Enol. Vitic.* **2008**, *59* (4), 440–445.

(16) Sun, Q.; Sacks, G.; Lerch, S.; Heuvel, J. E. V. Impact of shoot thinning and harvest date on yield components, fruit composition, and wine quality of Marechal Foch. *Am. J. Enol. Vitic.* **2011**, *62* (1), 32–41.

(17) Schreier, P.; Paroschy, J. H. Volatile components of wild grapes, *Vitis riparia*, m. *Can. Inst. Food Sci. Technol. J.* **1980**, *13* (3), 118–121.

(18) Kotseridis, Y.; Baumes, R.; Skouroumounis, G. K. Synthesis of labelled [2H4]β-damascenone, [2H2]2-methoxy-3-isobutylpyrazine, [2H3]α-ionone, and [2H3]β-ionone, for quantification in grapes, juice and wines. *J. Chromatogr., A* **1998**, *824*, 71–78.

(19) Lopez, R.; Aznar, M.; Cacho, J.; Ferreira, V. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *J. Chromatogr., A* **2002**, *966* (1−2), 167–177.

(20) Marin, A. B.; Acree, T. E.; Barnard, J. Variation in odor detection thresholds determined by Charm analysis. *Chem. Senses* **1988**, *13* (3), 435–444.

(21) Ryona, I.; Pan, B. S.; Sacks, G. L. Rapid measurement of 3-alkyl-2-methoxypyrazine content of winegrapes to predict levels in resultant wines. *J. Agric. Food Chem.* **2009**, *57* (18), 8250–8257.

(22) Ferreira, V.; Ortin, N.; Escudero, A.; Lopez, R.; Cacho, J. Chemical characterization of the aroma of Grenache rose wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50* (14), 4048–4054.

(23) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45* (8), 3022–3026.

(24) Ebeler, S. E.; Thorngate, J. H. Wine chemistry and flavor: looking into the crystal glass. *J. Agric. Food Chem.* **2009**, *57* (18), 8098–8108.

(25) Ferreira, V.; Cacho, J. Identification of impact odorants of wines. In *Wine Chemistry and Biochemistry*; Moreno-Arribas, M. V., Polo, M. C., Eds.; Springer: New York, 2009; pp xv, 735.

(26) Poisson, L.; Schieberle, P. Characterization of the most odor-active compounds in an American Bourbon whisky by application of the aroma extract dilution analysis. *J. Agric. Food Chem.* **2008**, *56* (14), 5813–5819.

(27) Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11* (2), 139–173.

(28) Castro, R.; Natera, R.; Duran, E.; Garcia-Barroso, C. Application of solid phase extraction techniques to analyse volatile compounds in wines and other enological products. *Eur. Food Res. Technol.* **2008**, *228* (1), 1–18.

(29) Blanchard, L.; Darriet, P.; Dubourdieu, D. Reactivity of 3-mercaptohexanol in red wine: impact of oxygen, phenolic fractions, and sulfur dioxide. *Am. J. Enol. Vitic.* **2004**, *55* (2), 115–120.

(30) Hein, K.; Ebeler, S. E.; Heymann, H. Perception of fruity and vegetative aromas in red wine. *J. Sensory Stud.* **2009**, *24* (3), 441–455.

(31) Ferreira, V.; Lopez, R.; Cacho, J. F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80* (11), 1659–1667.

(32) Capone, D. L.; Van Leeuwen, K.; Taylor, D. K.; Jeffery, D. W.; Pardon, K. H.; Elsey, G. M.; Sefton, M. A. Evolution and occurrence of 1,8-cineole (eucalyptol) in Australian wine. *J. Agric. Food Chem.* **2011**, *59* (3), 953–959.

(33) Kalua, C. M.; Boss, P. K. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2009**, *57* (9), 3818–3830.

(34) Farina, L.; Boido, E.; Carrau, F.; Versini, G.; Dellacassa, E. Terpene compounds as possible precursors of 1,8-cineole in red grapes and wines. *J. Agric. Food Chem.* **2005**, *53* (5), 1633–1636.

(35) Joslin, W. S.; Ough, C. S. Cause and fate of certain C6 compounds formed enzymatically in macerated grape leaves during harvest and wine fermentation. *Am. J. Enol. Vitic.* **1978**, *29* (1), 11–17.

(36) Aleixandre, J. L.; Lizama, V.; Alvarez, I.; Garcia, M. J. Varietal differentiation of red wines in the Valencian region (Spain). *J. Agric. Food Chem.* **2002**, *50* (4), 751–755.

(37) Escudero, A.; Campo, E.; Farina, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **2007**, *55* (11), 4501–4510.

(38) de Boubee, D. R.; Van Leeuwen, C.; Dubourdieu, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48* (10), 4830–4834.

(39) Maga, J. A. Sensory and stability properties of added methoxypyrazines to model and authentic wines. In *6th International Flavor Conference*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1989; pp 61−70.

(40) Pickering, G. J.; Karthik, A.; Inglis, D.; Sears, M.; Ker, K. Determination of ortho- and retronasal detection thresholds for 2-isopropyl-3-methoxypyrazine in wine. *J. Food Sci.* **2007**, *72* (7), S468–S472.

(41) Allen, M. S.; Lacey, M. J.; Boyd, S. Determination of methoxypyrazines in red wines by stable-isotope dilution gas-chromatography mass-spectrometry. *J. Agric. Food Chem.* **1994**, *42* (8), 1734–1738.

(42) Alberts, P.; Stander, M. A.; Paul, S. O.; de Villiers, A. Survey of 3-alkyl-2-methoxypyrazine content of South African Sauvignon blanc wines using a novel LC-APCI-MS/MS method. *J. Agric. Food Chem.* **2009**, *57* (20), 9347–9355.

(43) Ryona, I.; Pan, B. S.; Intrigliolo, D. S.; Lakso, A. N.; Sacks, G. L. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet franc). *J. Agric. Food Chem.* **2008**, *56* (22), 10838–10846.

(44) Rapp, A.; Versini, G. Methylanthranilate ("foxy taint") concentrations of hybrid and *Vitis vinifera* wines. *Vitis* **1996**, *35* (4), 215–216.

(45) Keller, M. *The Science of Grapevines: Anatomy and Physiology*; Academic Press: Burlington, MA, 2010.

(46) Young, P. R.; Vivier, M. A. Genetics and genomics approaches to improve grape quality for winemaking. In *Managaing Wine Quality. Vol. 1: Viticulture and Wine Quality*; Reynolds, A. G., Ed.; CRC Press: Boca Raton, FL, 2010; pp 316−364.