TARGETED TRACE LEVEL VOLATILE ANALYSIS OF AROMA COMPOUNDS TYPICALLY FOUND IN GRAPES

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science in Chemistry

by

Brittany Alexis Stewart

December 2022

ABSTRACT

Despite nearly two centuries of grape vine propagation and cultivar breeding, little attention has been given to what chemical compounds work harmoniously within this complex matrix to produce the aromas and flavors consumers and producers alike have come to know and expect from each species. In this work, the analyses of seven common cultivars – *V. acerifolia, V. aestavlis, V. amurensis, V. labrusca, V. riparia, V. rupestris,* and *V. vulpine* - for sixteen volatile compounds are presented. Headspace solid-phase microextraction coupled with gas chromatography – mass spectrometry (HS-SPME-GC-MS) was utilized for the determination of analyte concentrations, which highlight the compounds at the foundation of grape smell and taste.

BIOGRAPHICAL SKETCH

Brittany Stewart was born in Portsmouth, Virginia on July 17, 1999. She was raised in Virginia Beach by her parents John and Penny Stewart. She was an avid learner throughout school, participating in a Spanish immersion program for the duration of elementary and middle school. She quickly realized she loved math and science, and decided she wanted to be "un científico." She loved baking so much that she annoyed her relatives watching cooking shows on every family vacation. Upon taking chemistry courses at Landstown High School with Megan Wong, she realized the precise measurements she had been doing to make cakes could be translated to a career in science. In the summer of 2017, she became a midshipman at the U.S. Naval Academy. After two years of travel, exercise, and adventure, she shifted her goal of being a Naval Aviator in favor of returning to her love for the lab. She enrolled at The Pennsylvania State University in the spring of 2020. Her chemistry degree was finished in 2021 with a physical chemistry research project. She studied the crosslinking efficiency of polyethylene-poly (vinyl alcohol) using differential scanning calorimetry (DSC) with Dr. Bratoljub Milosavljevic. She became a graduate student at Cornell University in the fall of 2021, joining the master's degree program. While looking for a research group to enter, she was given a tip that there was a "wine guy" that was putting analytical chemistry into action. Inevitably, she became a member of the Sacks Lab where she analyzed a mountain of GC-MS data, convinced everyone she was a viticulture and enology major, and took frequent trips to the Dairy Bar downstairs. She was also an involved member of PCM, the rock-climbing club, and CHAARG while in Ithaca.

This work is dedicated to the many wonderful friends that have helped make each day of this challenging year a little less stressful. You have given me so many wonderful memories.

ACKNOWLEDGMENTS

Thank you to Commander David Durkin, my first scientific mentor, for fostering my love for being in a chemistry lab. Your course brought me extreme joy, a lot of frustration, and admittedly a few tears. Struggling under your watchful eye gave me confidence that I chose the right field of study.

Thank you to Dr. Bratoljub Milosavljevic, the best professor I had while at Penn State, for helping me take the steps to get to where I am today. You taught me so much about research, scientific writing, and presenting my work. I look back on the lengthy nights in Whitmore Lab with extreme fondness.

Thank you to Terry Bates, the greatest mentor and friend, for taking me on this year. With my never-ending stream of questions and concerns delivered in texts, emails, and scribbled sticky notes left on your desk, you were always unbelievably patient, kind, and ready to help. This experience would not have been a fraction of what it was without you there believing in and supporting me.

Thank you to Dr. Gavin Sacks, my research advisor, for helping me achieve so much during a single year. I, like so many, am immensely lucky to have taken your wine chemistry course. Being exposed to your insight helped me transform an idea of what I'd like to do after this program into the start of a career. You pushed me in a way that created so much growth. I am so grateful for your endless patience, willingness to meet on short notice, and kindness as I figured it all out. Thank you for welcoming me into the Sacks Lab so openly.

TABLE OF CONTENTS

INTRODUCTION 1
INSTRUMENTATION 7
MATERIALS AND METHODS 9
STANDARDS AND SAMPLES 9
PROCEDURE 9
RESULTS AND DISCUSSION 13
CONCLUSIONS 25
REFERENCES 27
APPENDIX 1 34
APPENDIX 2 35

LIST OF FIGURES

FIGURE 1: Structures of Compounds of Interest and Deuterated Standards 4
FIGURE 2: MA, EA, and d_3-MA Chromatogram and Spectra 14
FIGURE 3: Chromatogram and Spectrum of Hexanal 14
FIGURE 4: Chromatogram and Spectrum of *trans*-2-Hexenal 15
FIGURE 5: Chromatogram and Spectrum of Hexanol 15
FIGURE 6: Chromatogram and Spectrum of Eugenol 15
FIGURE 7: Chromatogram and Spectrum of d_3-Guaiacol 16
FIGURE 8: Chromatogram and Spectrum of Benzaldehyde 16
FIGURE 9: Chromatogram and Spectrum of Vanillin, d_3-Vanillin 16
FIGURE 10: Chromatogram and Spectrum of Linalool, d_3-Linalool 17
FIGURE 11: Chromatogram and Spectrum of Geraniol 17
FIGURE 12: Chromatogram and Spectrum of Nerol 17
FIGURE 13: Chromatogram and Spectrum of Myrcene 18
FIGURE 14: Chromatogram and Spectrum of Eucalyptol 18
FIGURE 15: Chromatogram and Spectrum of α-Terpineol 18
FIGURE 16: Chromatogram and Spectrum of 1,4-Cineole 19
FIGURE 17: Chromatogram and Spectrum of β-Ionone 19
FIGURE 18: Heat Map 21

LIST OF TABLES

TABLE 1: Quantifier and Qualifier Ions..... 11

TABLE 2: Concentrations of Calibration Curves 12

LIST OF ABBREVIATIONS

Methyl Anthranilate ..... MA

Ethyl Anthranilate ..... EA

ortho-Aminoacetophenone ..... o-AAP

United States Department of Agriculture ..... USDA

Gas-Chromatography - Mass Spectrometry ..... GCMS

Solid Phase Microextraction ..... SPME

Head Space ..... HS

Polydimethylsiloxane ..... PDMS

Divinylbenzene ..... DVB

Carboxen® ..... CAR

Limit of Detection ..... LOD

Electron Ionization ..... EI

Relative Standard Deviation ..... %RSD

LIST OF SYMBOLS

Alpha .... α

Beta.. β

INTRODUCTION

Grape breeding has a long history extending back at least into the nineteenth century. Records show that an American horticulturist by the name of Edward Staniford Rogers released crossbred varieties of common European and American grapes as early as the 1860s [1]. Many believe, however, that intentional grape breeding truly began in the 18th and 19th centuries. During this period, the French began importing grapes from the Americas that differed from those found in Europe as a means of distinguishing themselves from competitors. These vines brought phylloxera along as well. Phylloxera is a pest native to North America that feeds on the roots and leaves of the *Vitis* species [36]. The insects destroyed vineyards of French vines, while leaving the American vines undamaged. This motivated the first of the well-known instances of French-American hybridization involving grafting French vines onto American rootstocks [2].

Breeding has typically been oriented towards creating heartier, more disease resistant varieties to make growing grape vines easier and more efficient, to achieve more berries with fewer pesticides and chemical treatments and create cultivars that better handle shipping and storage involved in modern food networks. Recently, breeding has instead been focusing on the flavor and odor aspects of varieties to make either a more desirable table grape or wine grape. The most notable of recent breeding for table grapes is the cultivation of Cotton Candy™ grapes by David Cain [3], a horticulturist in California. These grapes are not genetically modified in any way as many suspect; they are bred to have a high concentration of a compound called furaneol that is perceived as cotton candy. After tasting a proprietary varietal at a trade show, Cain worked for over a decade to crossbreed this grape with a Californian

variety with a thinner skin, better crunch, and larger size [37]. Consumers have proven to be much more receptive to new characteristics and names when shopping for table grapes than are wine buyers who seek specific grape varieties when purchasing products.

In wine, consumers are beginning to look for innovative products demonstrating bold flavors [4] which may deviate from "classic" profiles [5]. To meet the demand of current consumers companies have begun looking to produce new flavors and aromas with well-known varieties. The requirements for a successful new breed of wine grapes are somewhat harsher; for wine grape breeding to be successful, the desired odorants must not only be present at supra-threshold levels but also survive the severe conditions of fermentation so that the end wine product incorporates the desired effect.

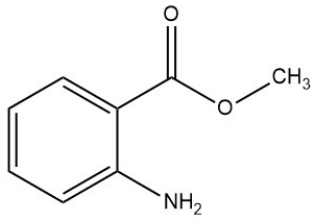
While the overarching profile of common grape varieties is well known from a sensory standpoint – typical flavor, color, size, and aroma – there has been very little work done to measure concentrations of odor compounds within specific species. In this work we attempt to base the often-subjective effort to improve wines with an approach based in science – using the tools of modern analytical chemistry to establish the actual chemical profile(s) of the volatiles found in grape species. Volatile profiling assists grape breeders in their continuous efforts to breed grapes with the ideal flavor for consumers, whether they are purchasing produce to eat fresh or to ferment them into wines, by revealing which compounds work in conjunction to create the unique taste of a certain species. This work opens the door for grapes like those bred by Cain, highlighting specific flavors to give a new experience while eating grapes.

While completing this process by taste and smell has its benefits, it also has limitations. The primary difficulty is the phenomenon of masking. A human sensor will only pick up what is the most potent; there is no way to detect what good qualities

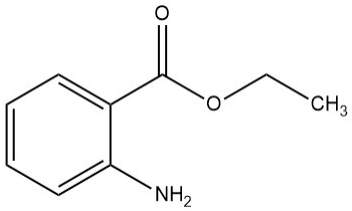
are hidden by that flavor or aroma. The ability for a person to evaluate intensity eventually saturates, and the difference between five-fold and ten-fold concentration is indistinguishable. Additionally, grape breeders are space and time limited. With a process that can take more than a decade to get to a production-ready vine, not every cross of interest can be brought through to the final stages. Using analytical techniques is more efficient and avoids the natural limitations of humans as analytical detectors. This is not to say people are removed entirely from the process; they simply hold a more specific role. In the scientific approach to producing new grapes or wine, things always start with a consumer sensory panel that indicates what specific characteristics the population is looking for. Scientists then work backwards from those features to figure out how to incorporate them. If it can be imparted by the grape itself, the next step could be to breed grapes with extravagantly high levels of that compound to blend in. People are also used where there is no good proxy for the desired traits, or to validate results, and evaluate consumer tastes, or "liking" – a factor that cannot be studied with instrumentation.

Aroma compounds typically found in grapes are methyl anthranilate (MA), ethyl anthranilate (EA), hexanal, *trans*-2-hexenal, hexanol, eugenol, benzaldehyde, vanillin, linalool, geraniol, nerol, myrcene, 1,8-cineole (eucalyptol), α-terpineol, 1,4-cineole, and β-ionone. These can be categorized as having either positive or negative aromas due to common preference.

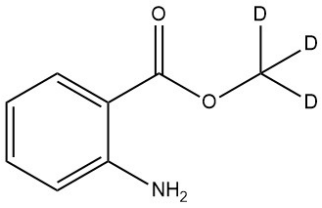
Since the goal of this study is to discover varieties with enjoyable taste, positive aromas are most of the analytes. The phenylpropanoids (MA, EA) are biologically produced esters that have an orange-flower and fruity odor. MA specifically has been credited to be the source of the "grapey" smell. Recent work has shown that the origin is instead the phenylpropanoid o-AAP [6]. *trans*-2-hexenal is an aldehyde that is considered to be beneficial because it has notes of almond, apple, and



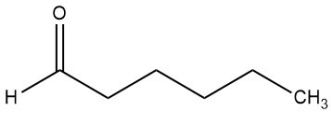
MA



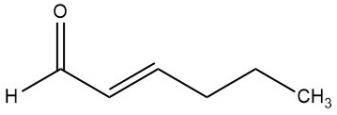
EA



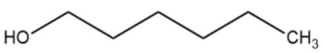
d3-MA



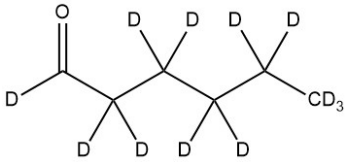
Hexanal



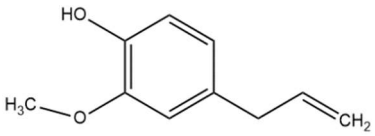
trans-2-Hexenal



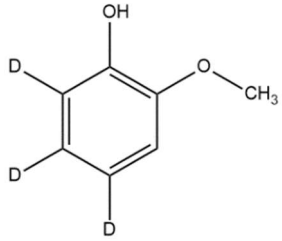
Hexanol



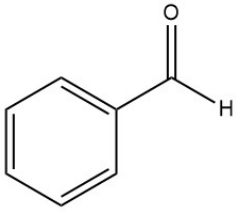
d12-Hexanal



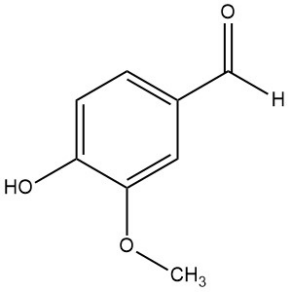
Eugenol



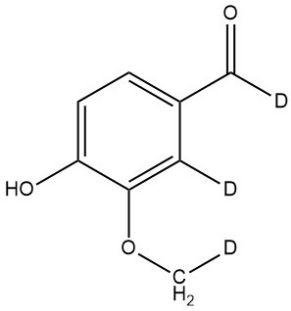
d3-Guaiacol



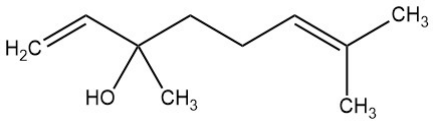
Benzaldehyde



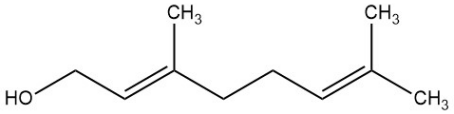
Vanillin



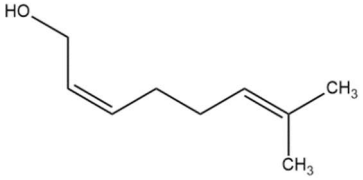
d3-Vanillin



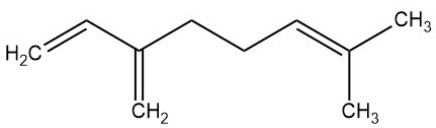
Linalool



Geraniol



Nerol



Myrcene

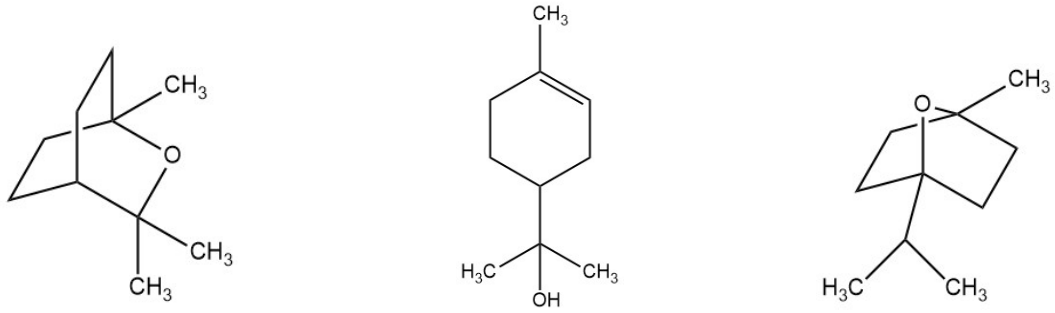


Figure 1: Structures of compounds of interest and deuterated standards.

plum. Eugenol and vanillin are volatile phenols reminiscent of spice, clove, and vanilla [7]. Eugenol and vanillin create highly prized flavors that are rarely found in high quantities in grapes or unaged wines, so these compounds are most often introduced by purposeful barrel aging and oak contact. Terpenes, both monoterpenes and sesquiterpenes, produce the signature smell and taste of most plants. Those commonly found in wine grapes are responsible for floral (linalool), rose (geraniol), sweet (nerol), balsamic (myrcene), eucalyptus (eucalyptol), lilac (α-terpineol), green (1,4-cineole), and violet (β-ionone) features [7]. Eucalyptol is the exception in that studies have suggested it is influenced predominantly by the presence and distance of Eucalyptus trees to the grape vines [6].

Negative compounds are also considered to get a complete understanding of the grape profile. These are odorants that are found in grape berries but are unwanted at typical levels. Viticulturists attempt to harvest berries when these compounds are at

low levels and minimize their influence overall. C6 Aldehydes / C6 Alcohols like hexanal and hexanol can be pleasant if diluted; otherwise, they are often associated with strong, green, or grassy notes. These are considered to be negative attributes. Benzaldehyde is an aromatic aldehyde that is perceived as bitter almond. It is rarely found above its 2000 µg/L detection threshold unless a wine has been contaminated by epoxy-resin coating used to prevent direct contact with concrete tanks [6].

Vitis vinifera is the leader in modern grape cultivation. A majority of the leading cultivated wine grapes are *vinifera* varieties, including most of those that the average consumer would readily recognize: Chardonnay, White Riesling, Gewürztraminer, Sauvignon blanc, Pinot noir, Cabernet Sauvignon, and Merlot. This European grape poses numerous challenges to its commercial cultivation due to its inability to endure cold temperatures, common pests, diseases, or acidic soil [8]. Despite these challenges the desire to cultivate these grapes is strong as these same vines provide high yields, excellent wine quality, and perfect flowers - meaning it can pollinate itself. Other species are preferred for their resistance to fungal diseases, tolerance of phylloxera, and ability to grow in less ideal conditions. For example, *Vitis riparia* has the advantage of tolerating much lower temperatures than *Vitis vinifera*; these grapes can continue to grow in conditions as low as -40˚C. *Vitis labrusca* has higher resistance to insects that would cripple *Vitis vinifera* [6].

The aim of this work is to characterize the aforementioned compounds contribution to the profile viticulturists and enologists have come to know in a handful of cultivars: *Vitis acerifolia* – maple leaf grapes, *Vitis aestivalis* – summer grapes, *Vitis amurensis* – amur grapes, *Vitis labrusca* – fox grapes, *Vitis riparia* – riverbank grapes, *Vitis rupestris* – sand grapes, and *Vitis vulpine* – frost grapes. All data acquired for grapes from the Geneva USDA germplasm will be compared to typical *Vinifera* concentrations, as it is seen as the standard within the industry. Using Gas

Chromatography Mass Spectrometry (GC-MS), the concentrations of the compounds of interest will be determined in each variety. Then those concentrations are evaluated in comparison to those of *Vitis vinifera* to reveal which of these varieties could be bred to highlight an unconventional flavor. With additional studies and collective contribution, a library of this information for the grape varieties being grown within the United States could be culminated for the entire industry to utilize.

INSTRUMENTATION

Volatile analysis by GC-MS involves extraction, transfer, separation, identification, and analysis. Extraction is a way to selectively remove and preconcentrate compounds of interest from a complex matrix. In this work, HS-SPME is the extraction method being utilized. SPME is an extraction method where a fused silica fiber that has been coated with a sorbent polymer serves as the stationary phase used to extract compounds that have partitioned into the headspace. The selection of coating, pore size, and fiber length can be fine-tuned to tailor analysis to the compounds of interest. Common commercially available options are polydimethylsiloxane (PDMS), divinylbenzene (DVB), and Carboxen® (CAR). Separation can be further optimized with the time and temperature setting of the device. The SPME fiber is exposed to the headspace of the vial for a specified amount of time. It sorbs compounds through selectivity designed from all of the given parameters, then is removed from the vial and brought to the inlet to allow thermal desorption to occur. In the inlet, the sample is transferred onto the column for separation. This can be done either with split or splitless injection. Split injection is a method for compounds at high concentration or where a low-end limit of detection is not needed for the study; splitless injection is applied for trace level compounds, or a low level of detection is necessary. The liner of the inlet can be chosen to manipulate

geometric design, volume, packing, and deactivation. One of the major advantages of this microextraction technique is the use of significantly less solvent than exhaustive extraction methods. SPME uses ratios of less than one hundred microliters or milligrams of solvent to volumes of sample greater than one milliliter. Additionally, it makes it possible to measure compounds in a complex matrix rather than a hyper-concentrated solution. To understand grape germplasm, compounds must be analyzed in conjunction, to understand how they interact with one another and at what concentration they exist at in untreated berries. Once the sample is on the column, it can be separated by boiling point and polarity. Having a longer column increases resolution but also increases the duration of analysis and, of course, cost.

A mass spectrometer is utilized to identify compounds as they elute from the column. For a mass spectrometer to detect a compound, it must be ionized. Many methods have been developed for ionization, including laser, electrospray, chemical, ambient, and electron. In electron ionization – the method utilized in this work, compounds leaving the GC enter 70 eV of energy from an electron beam, creating radical cations. Radicals are highly unstable species, so they begin to fragment quickly. Every compound has a unique fragmentation pattern that allows it to be identified with precision.

MATERIALS AND METHODS

STANDARDS AND SAMPLES

Standards of methyl anthranilate (≥ 99%), linalool (≥ 97%), nerol (≥ 97%), myrcene, α-terpineol, 1,4-cineole, β-damascenone, vanillin, eugenol, β-ionone (≥ 95%), hexanal, and *trans*-2-hexenal were acquired from Sigma-Aldrich (St. Louis, MO, USA). Eucalyptol was procured from Fluka Analytical (Charlotte, NC, USA). Geraniol (≥ 99%), α-terpineol (≥ 97%), guaiacol (≥ 99%), and α-ionone (≥ 90%) were purchased from Acros Organics (Geel, Belgium), and hexanol was received from MP biomedicals, LLC (Irvine, CA, USA). Forty-eight grape samples of *Vitis acerifolia*, *Vitis aestivalis*, *Vitis amurensis*, *Vitis labrusca*, *Vitis riparia*, *Vitis rupestris* , and *Vitis vulpine* were retrieved from the USDA germplasm during 2019. These grape clippings were all harvested on September 20, 2019 from different vines in a handful of rows. Thirty samples of *Vitis acerifolia*, *Vitis cinerea*, *Vitis labrusca*, *Vitis palmeta*, *Vitis riparia*, *Vitis rupestris*, and *Vitis vulpine* came from the USDA germplasm in 2020. The grapes from 2020 differed in row, vine, and harvest date.

PROCEDURE

Grape juice samples were analyzed for the specified odorants using headspace solid phase microextraction (HS-SPME) on instrumentation produced by LEAP CombiPALAutosampler (Carrboro, NC, USA) working in conjunction with a Shimadzu GCMS-TQ8040 (Kyoto, Japan). The method was adapted from that published by Burzynski-Chang et. al. in 2018 [9]. 50 grams of whole berries were frozen, destemmed, gently crushed, then blended first at medium speed (30 seconds), then high speed (30 seconds) for a total of one minute in a Waring stainless steel 250 mL blender (McConnellsburg, PA, USA) that had been pre-cooled. 5 grams of grape

homogenate for each sample was inserted into 20 mL amber SPME vials purchased from Sigma-Aldrich (St. Louis, MO, USA) in duplicate, diluted with phosphate buffer (5 mL, 0.1 M pH 7.0) to loosen the slurry, along with 3 grams of NaCl to encourage compounds into their volatile form and 50 µL of internal standard, which is added to better understand variation between samples. The internal standard was comprised of d_3-methyl anthranilate, d_{12}-hexanal, d_3-guaiacol, d_3-vanillin, and d_3-linalool. Concentrations of these compounds within the internal standard after addition to samples were 50 ppb, 620 ppb, 20 ppb, 100 ppb, and 20 ppb, respectfully. One deuterated standard was used for each class of compound (phenylpropanoids, C6 aldehydes/C6 alcohols, volatile phenols, phenolic aldehydes, and terpenes) to eliminate costs that would be procured doing so for individual native compounds. Samples were immediately agitated until salt was no longer visible within the vial. Samples were all stored frozen when not being analyzed.

Each of the grape macerate samples was processed with a triple phase SPME fiber of polydimethylsiloxane/carboxen/divinylbenzene (PDMS/Carboxyl/DVB; 80 µm thickness) that is 10 mm in length (Agilent Technologies, Basel, Switzerland). 15 minutes were allotted for extraction at 60 ˚C. The SPME fiber was allowed 3 minutes at 230 ˚C for desorption in splitless mode within the injector and another 3 minutes for purging. Purge flow was set to 50 mL/min.

Helium was the carrier gas, at a flow rate of 0.76 mL per minute, on the Factor Four VF-WAXms (30 m X 0.25 mm X 0.25 µm) column from Varian (Palo Alto, CA). For each sample, the temperature began at 40 ˚C, held for 5 minutes, then began ramping 5 ˚C/min until 195 ˚C was reached. Ramping continued at 20 ˚C/min until 240 ˚C was reached, and finally held for the remaining 5 minutes of processing. Mass Spectral analysis was conducted with electron ionization at 70 eV. Monitoring observed mass to charge ratios between 25 and 250. Quantifier and Qualifier ions are

Table 1: Quantifier and qualifier ions, given in mass to charge ratios, used to identify the compounds of interest.

Compound	Quantifier Ion (*m/z*)	Qualifier Ion (*m/z*)
Methyl Anthranilate	151	119
Ethyl Anthranilate	165	119
d3-Methyl Anthranilate	124	154
Hexanal	56	72
trans-2-Hexenal	69	83
Hexanol	56	69
d12-Hexanal	64	48
Eugenol	164	149
d3-Guaiacol	127	109
Benzaldehyde	106	77
Vanillin	152	151
d3-Vanillin	154	81
Linalool	71	121
d3-Linalool	124	74
Geraniol	123	69
Nerol	121	93
Myrcene	93	69
Eucalyptol	154	108
α-Terpineol	136	59
1,4-Cineol	71	111
β-Ionone	177	135

listed in Table 1 for each compound. The quantifier ion is used in quantification of the peak on the chromatogram and is oftentimes the most abundant fragment. The qualifier ion is one used to confirm identification of the specified analyte. Data was processed to determine the area under the curve of the peak created as each of the compounds were emitted. It was assumed that the response factor of each compound would be equivalent to the deuterated internal standard of that compound class. This peak area was then correlated to concentration with the use of a four-point calibration of standard solutions with known concentrations, beginning at or below the sensory threshold. These values can be seen in Table 2. By spiking four levels of the compounds of interest into vials prepared identically to sample solutions, a linear range of concentration was created to establish correlation with peak area on the Shimadzu GCMS Postrun Analysis Software (Kyoto, Japan). Each concentration was tested in triplicate to ensure repeatability.

Table 2: Concentrations of compounds in standards used to create a calibration curve correlating peak area and concentration.

Compound	Sensory Threshold (µg/L)	Calibration Curve (values given in ppb)			
		Point 1	Point 2	Point 3	Point 4
MA	45 [10]	15	45	135	405
EA	45 [10]	15	45	135	405
Hexanal	0.075 [11]	10	30	90	360
trans-2-Hexenal	0.097 [11]	10	30	90	360
Hexanol	0.4 [11]	10	30	90	360
Eugenol	100 [12]	30	90	270	810
Benzaldehyde	350 [11]	115	345	1035	3105
Vanillin	7 [13]	10	30	90	360
Linalool	20-50 [10]	15	45	135	405
Geraniol	30 [11]	15	45	135	405
Nerol	400 [11]	130	390	1170	3510
Myrcene	0.012 [14]	10	30	90	360
Eucalyptol	1.1 [15]	10	30	90	360
α-Terpineol	400 [11]	130	390	1170	3510
1,4-Cineole	0.63 [16]	10	30	90	360
β-Ionone	1.3 [17]	10	30	90	360

RESULTS AND DISCUSSION

With the described procedure, it was possible to confidently identify each of the aroma compounds sought. Selected ion chromatograms are shown in Figures 2-17 below alongside their mass spectra, highlighting quantifier and qualifier peaks. Ideal peaks should have a Gaussian distribution of time versus intensity, or response. Deviations from this curve can allow eluting compounds to bleed into one another, make them difficult to analyze reliably, and suggest conditions that are occurring within instrumentation that should be remedied. Some peaks showed tailing, an extension of the eluting compound that is asymmetric with the front of the peak. Peak tailing initially seemed to be taking place exclusively on polar compounds, such as benzaldehyde (Figure 3), which presented a defined shoulder in some samples. This shoulder had an ideal mass spectrum matching that of the benzaldehyde peak. Since the compound is a polar aldehyde with a carbonyl group, it was believed that the inlet liner had active sites within it causing the delay in elution and creation of the peak shoulder [35]. However, additional compounds were discovered to be tailing upon further analysis of the data. Some examples include myrcene (Figure 13) and eucalyptol (Figure 14). As the tailing occurred more sporadically than we initially thought, it was determined that the issue was more likely to be thermal decomposition of analytes in the inlet.

The relative standard deviation (%RSD) value was calculated for each set of sample replicates to determine variability from the mean of peak area ratios of the compounds of interest to the labeled internal standard. Some variability in concentration is expected while using the SPME technique and with analytes produced by biological processes, which can vary with environmental conditions; however, values had a much more expansive range than anticipated due to the human error

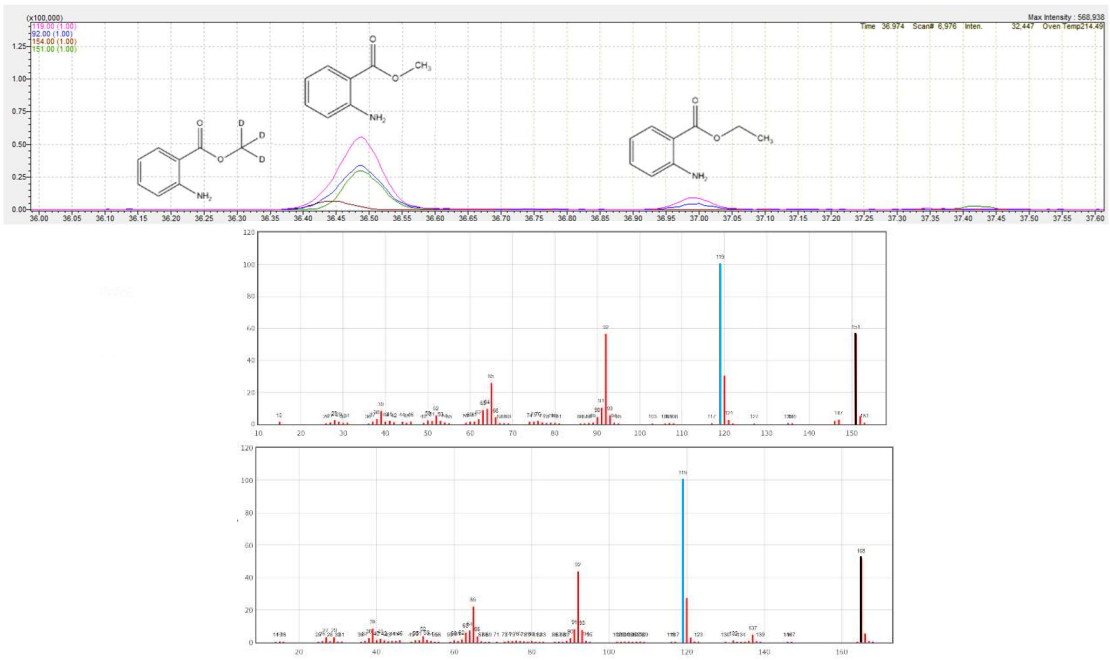


Figure 2: (a) Example chromatogram of MA (left), EA (right), and d_3-MA (brown curve); (b) representative EI mass spectrum of MA [18]; (c) representative EI mass spectrum of EA [19].

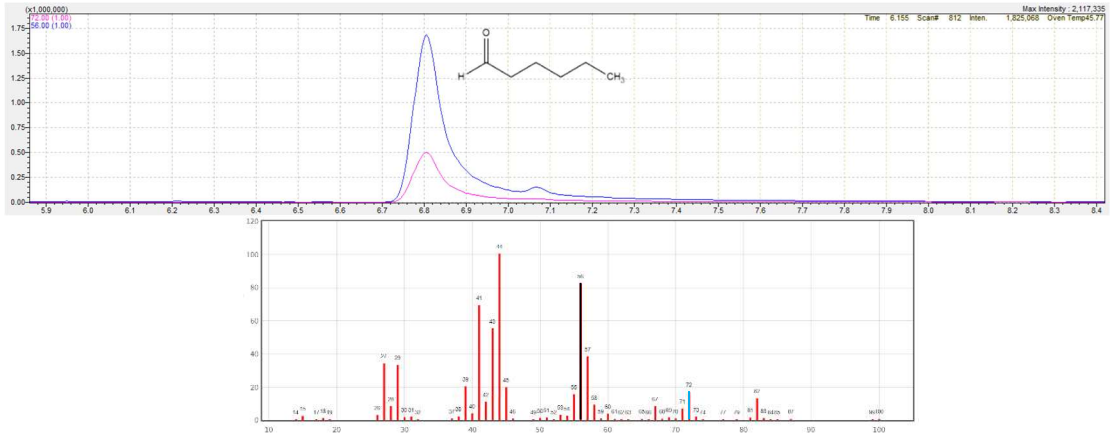


Figure 3: (a) Example chromatogram of Hexanal; (b) representative EI mass spectrum of Hexanal [20].

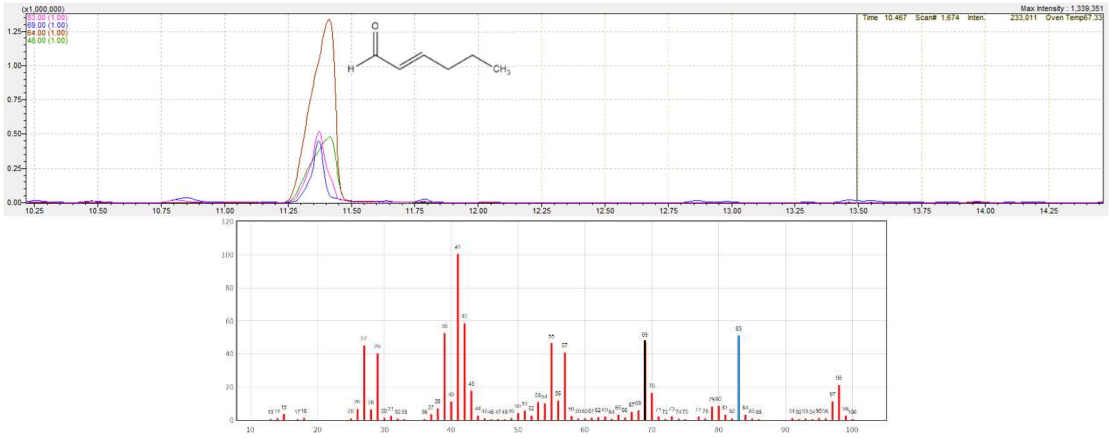


Figure 4: (a) Example chromatogram of *trans*-2-Hexenal; (b) representative EI mass spectrum of *trans*-2-Hexenal [21].

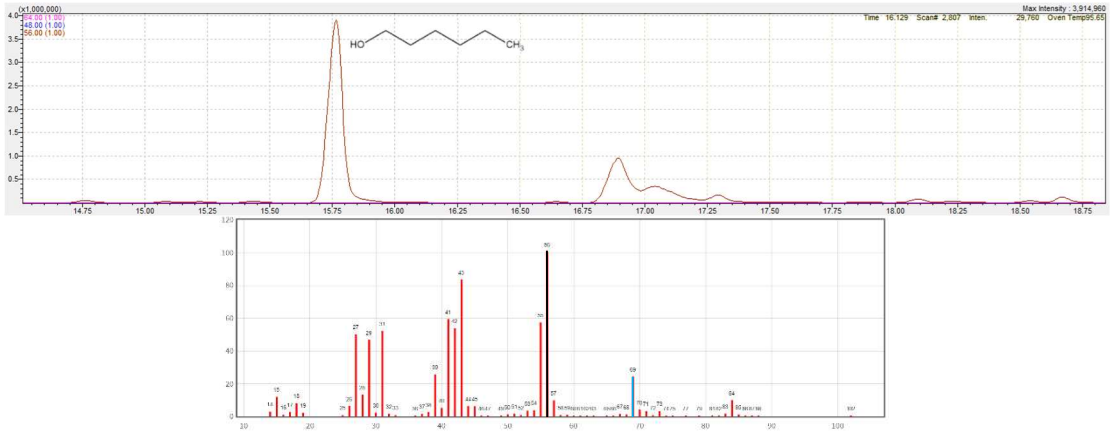


Figure 5: (a) Example chromatogram of Hexanol; (b) representative EI mass spectrum of Hexanol [22].

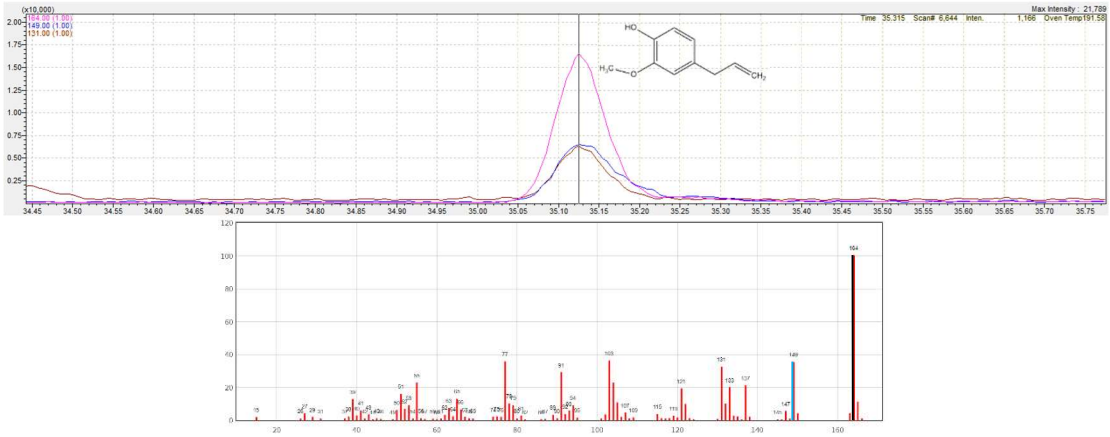


Figure 6: (a) Example chromatogram of Eugenol; (b) representative EI mass spectrum of Eugenol [23].

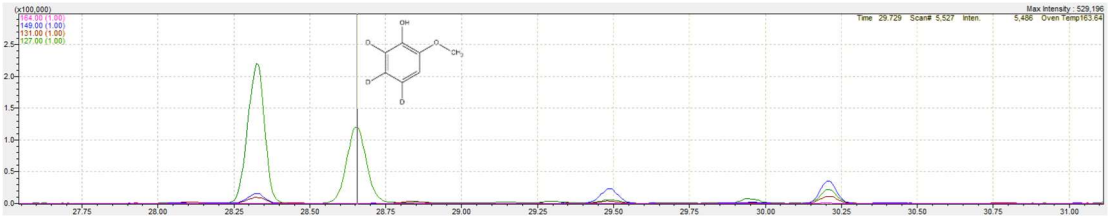


Figure 7: Example chromatogram of d_3-Guaiacol;

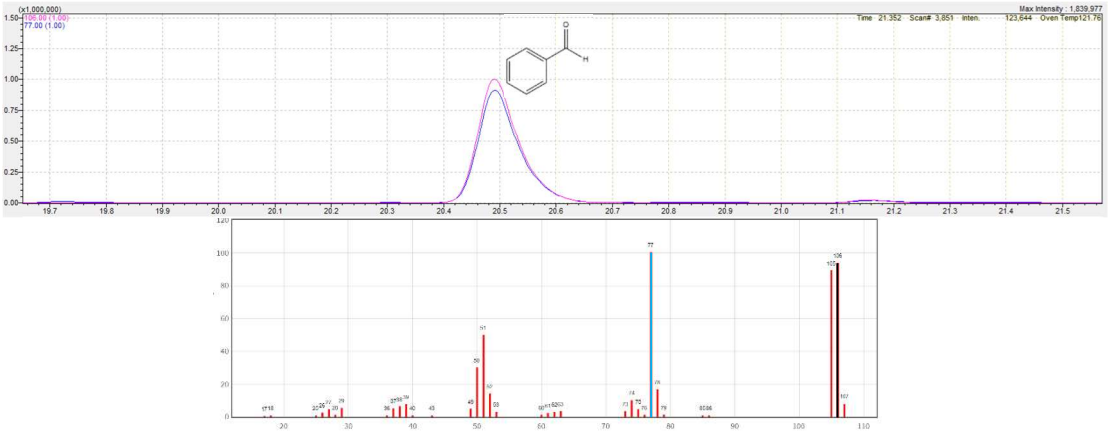


Figure 8: (a) Example chromatogram of Benzaldehyde; (b) representative EI mass spectrum of Benzaldehyde [24].

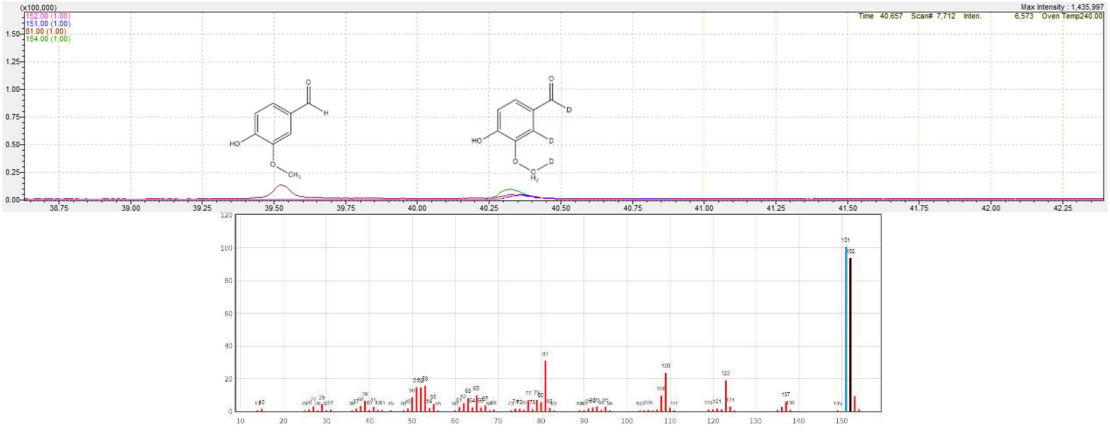


Figure 9: (a) Example chromatogram of Vanillin (left) and d_3-Vanillin (right); (b) representative EI mass spectrum of Vanillin [25].

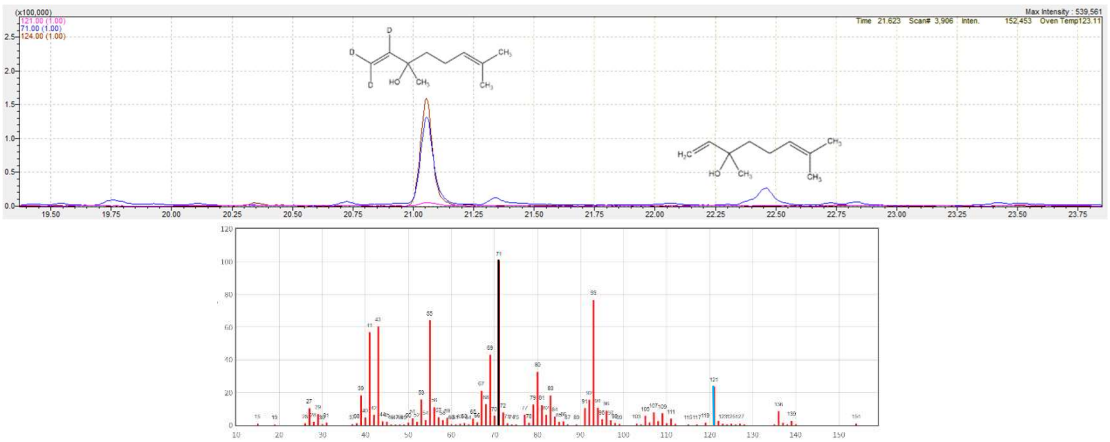


Figure 10: (a) Example chromatogram of d_3-Linalool (left) and Linalool (right); (b) representative EI mass spectrum of Linalool [26].

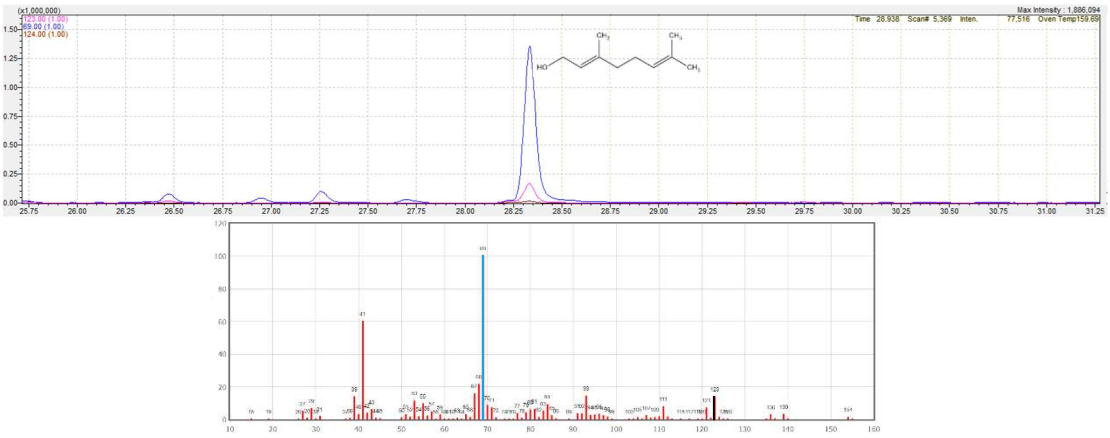


Figure 11: (a) Example chromatogram of Geraniol; (b) representative EI mass spectrum of Geraniol [27].

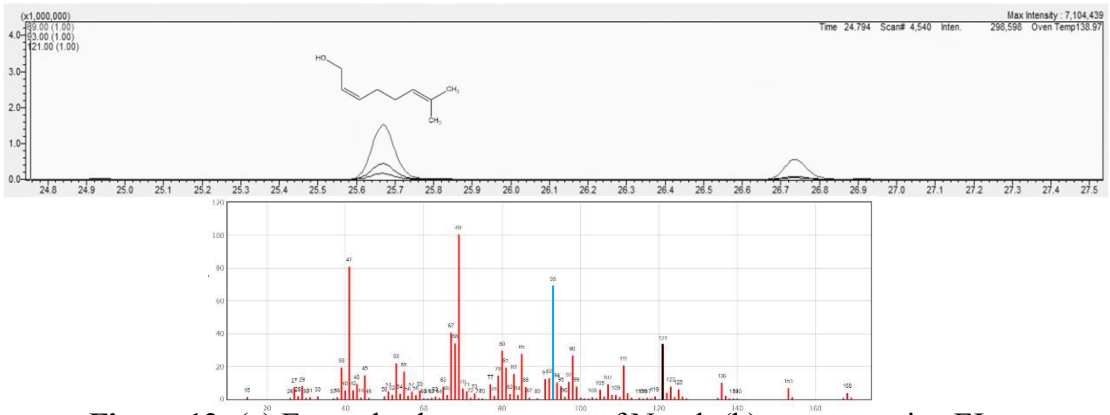


Figure 12: (a) Example chromatogram of Nerol; (b) representative EI mass spectrum of Nerol [28].

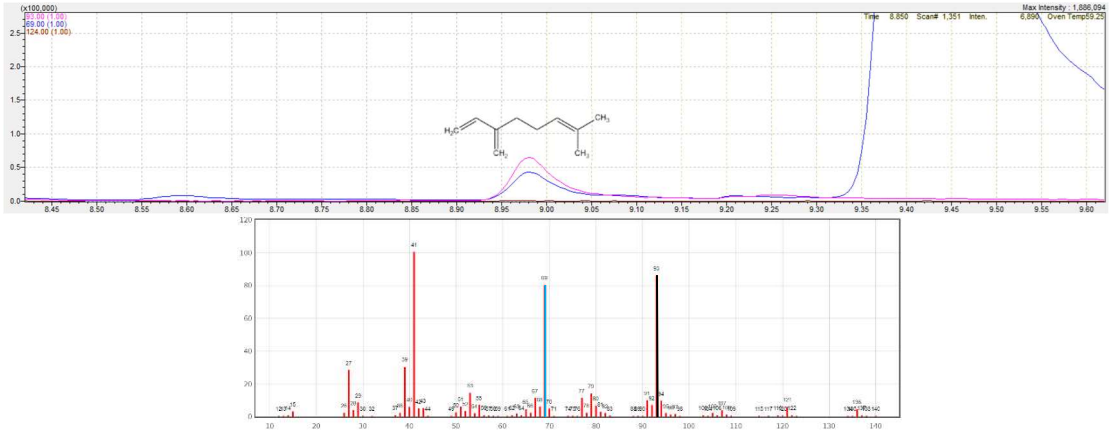


Figure 13: (a) Example chromatogram of Myrcene; (b) representative EI mass spectrum of Myrcene [29].

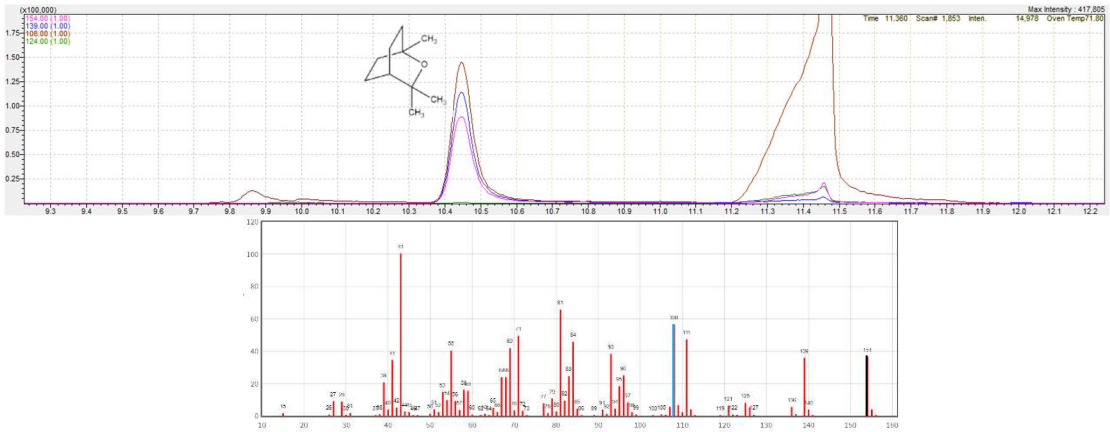


Figure 14: (a) Example chromatogram of Eucalyptol; (b) representative EI mass spectrum of Eucalyptol [30].

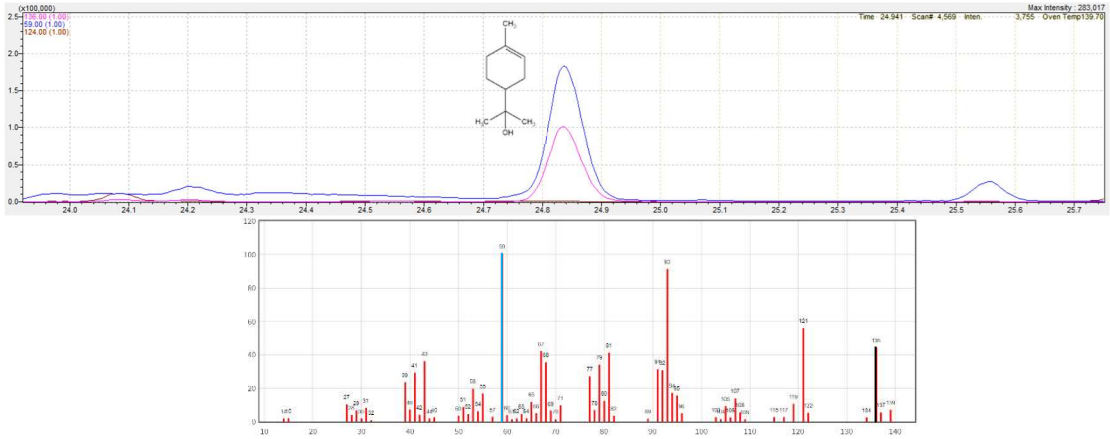


Figure 15: (a) Example chromatogram of α-Terpineol; (b) representative EI mass spectrum of α-Terpineol [31].

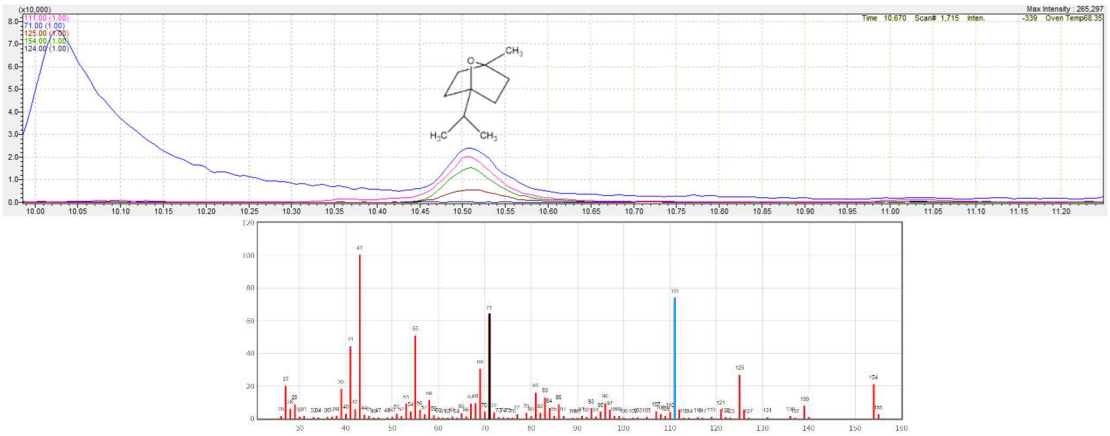


Figure 16: (a) Example chromatogram of 1,4-Cineole; (b) representative EI mass spectrum of 1,4-Cineole [32].

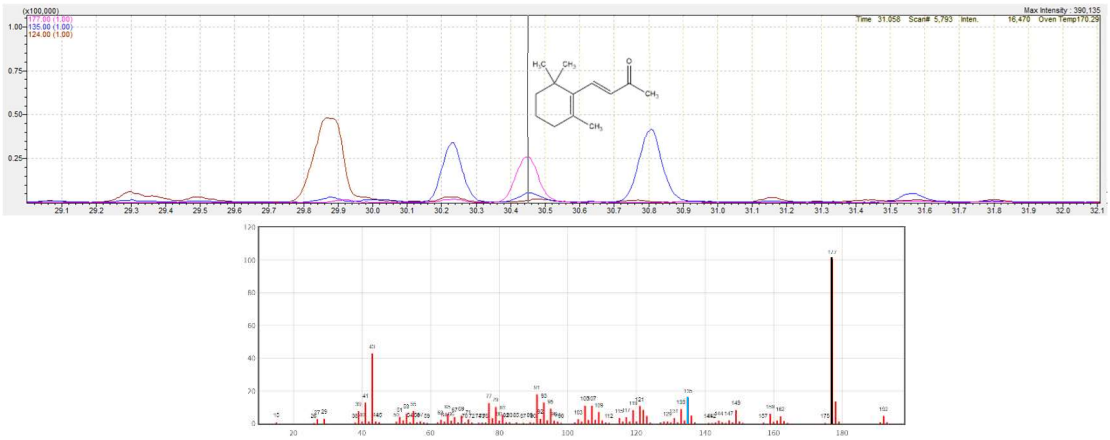


Figure 17: (a) Example chromatogram of β-Ionone; (b) representative EI mass spectrum of β-Ionone.

involved with sample preparation compounded with the variability of grapes sampled from different rows, vines, and species. Values for sample duplicates within a single compound included data points of both a few percent and exceeding 100%. While replicates should generally have similar variability, the sample size is only two for each macerate analyzed. That is far too small to get an accurate understanding of the variability taking place. For comparison, the %RSD value was also calculated for each of the aroma compounds (all samples, all replicates) to serve as a measurement of variability within the population being studied. These measurements still vary and include outliers; however, they more consistently fall within a smaller range. Most fall within 30-50% relative standard deviation, which is acceptable for trace-level measurements with the internal standards utilized.

Raw peak area data (Appendix 1) were first normalized to the internal standards added to the samples to evenly account for response factor. These values were then normalized by each analyte so that the highest occurrence is equal to one (Appendix 2). To produce the heat map provided in Figure 18, a calibration curve was created for individual analytes to convert the recorded areas into concentrations in units of parts per billion (ppb). The matrix allows you to examine the specimens studied by analyte or species. “No Data” indicates that the sample data could not be analyzed; this was always because of the breakage of the SPME fiber during a programed run of samples. “Below LOD” indicates that the compound was not detected with the instrument’s level of sensitivity.

Vitis vinifera is known for its neutral qualities and ideal growing abilities. Isoprenoids generally occur at levels that are far too low to affect sensory perception because they are below compound sensory thresholds. In juice, linalool has been measured at 70 µg/L bound and 32 µg/L free, while nerol, geraniol, and α-terpineol materialize at 20/11 µg/L, 42/22 µg/L, and 114/8 µg/L (bound/free), respectfully.

	MA (mg/L d3-MA equivalents)	EA (µg/L d3-MA equivalents)	Hexanal (µg/L d3-Linalool equivalents)	*trans*-2-Hexenal (µg/L d3-Linalool equivalents)	Hexanol (µg/L d3-Linalool equivalents)	Eugenol (µg/L d3-Guaiacol equivalents)	Vanillin (µg/L d3-Vanillin equivalents)	Linalool (µg/L d3-Linalool equivalents)	Geraniol (µg/L d3-Guaiacol equivalents)	Nerol (µg/L d3-Guaiacol equivalents)	Myrcene (µg/L d3-Guaiacol equivalents)	Eucalyptol (µg/L d3-Guaiacol equivalents)	α-Terpineol (µg/L d3-Guaiacol equivalents)	1,4-Cineole (µg/L d3-Guaiacol equivalents)	β-Ionone (µg/L d3-Guaiacol equivalents)
Acerifolia	2.18	6.83	9.66	24.33	20.78	0.01	Below LOD	1.25	0.14	0.73	0.03	10.32	0.12	48.24	0.34
Acerifolia	16.84	Below LOD	8.33	63.08	45.31	0.02	0.22	1.20	0.33	0.96	0.03	1.40	0.24	7.12	0.62
Acerifolia	2.16	Below LOD	9.77	139.18	101.71	Below LOD	Below LOD	0.85	0.03	Below LOD	0.01	2.32	0.06	12.05	0.59
Aestavalis	3.45	Below LOD	14.22	486.06	197.85	0.05	Below LOD	1.57	0.24	1.27	0.10	0.17	0.19	Below LOD	0.88
Amurensis	0.05	Below LOD	8.58	244.78	137.88	Below LOD	Below LOD	Below LOD	0.30	Below LOD	0.02	Below LOD	0.12	Below LOD	0.64
Amurensis	2.64	Below LOD	17.43	374.76	160.61	0.00	0.23	1.59	0.20	Below LOD	0.02	Below LOD	Below LOD	Below LOD	0.86
Labrusca	36.44	140.92	1.58	39.36	10.29	0.01	0.24	4.19	0.34	1.10	0.03	14.29	1.42	64.69	0.31
Labrusca	13.69	22.05	3.09	55.67	29.99	Below LOD	0.05	Below LOD	0.35	0.36	0.12	Below LOD	0.11	Below LOD	0.36
Labrusca	16.01	Below LOD	0.28	1.49	6.87	0.04	Below LOD	1.35	1.19	0.42	0.04	2.30	0.40	10.06	0.12
Labrusca	17.87	Below LOD	3.94	69.29	11.42	0.01	0.09	0.92	0.73	0.83	0.07	0.37	0.40	1.59	0.34
Labrusca	7.03	Below LOD	1.54	22.04	4.84	0.01	0.13	Below LOD	0.22	0.05	0.05	Below LOD	0.05	Below LOD	0.43
Labrusca	5.73	8.41	3.21	52.93	78.41	0.01	Below LOD	2.05	1.15	1.05	0.80	0.51	0.73	Below LOD	0.34
Labrusca	12.03	29.16	4.45	11.81	73.73	0.00	0.14	1.83	0.55	0.21	0.01	7.80	0.37	36.12	0.29
Riparia	Below LOD	Below LOD	1.79	52.71	56.20	Below LOD	Below LOD	2.83	0.03	Below LOD	0.02	7.95	0.37	30.92	0.72
Riparia	3.37	10.49	2.72	41.14	133.70	0.01	0.33	Below LOD	0.05	Below LOD	0.01	0.14	0.07	Below LOD	0.75
Riparia	0.45	Below LOD	8.58	71.65	75.93	Below LOD	0.33	Below LOD	0.03	0.23	0.01	0.38	0.05	0.99	0.59
Riparia	3.95	15.66	2.08	48.38	20.88	Below LOD	0.24	Below LOD	1.97	7.16	0.15	0.92	0.14	4.52	1.11
Riparia	0.82	2.70	6.21	97.14	31.38	Below LOD	0.23	1.68	0.06	0.50	0.11	0.23	0.19	1.03	0.82
Riparia	1.35	5.40	7.05	24.75	27.57	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	0.01	19.57	0.05	86.34	1.20
Riparia	2.08	6.66	11.48	50.39	38.17	0.01	Below LOD	Below LOD	0.07	0.51	0.01	0.10	0.07	Below LOD	0.65
Riparia	0.60	1.91	3.69	15.25	21.54	Below LOD	0.04	Below LOD	Below LOD	Below LOD	0.02	Below LOD	0.03	Below LOD	0.73
Riparia	3.79	13.57	4.77	14.30	17.90	Below LOD	Below LOD	Below LOD	0.05	Below LOD	0.02	4.36	0.07	21.10	0.69
Riparia	2.03	7.69	4.44	11.85	15.02	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	0.01	0.23	0.10	Below LOD	0.95
Riparia	3.22	10.95	1.28	26.18	7.85	Below LOD	0.04	Below LOD	0.15	0.48	0.02	Below LOD	0.19	Below LOD	0.25
Riparia	0.12	Below LOD	4.06	11.92	41.18	0.00	Below LOD	Below LOD	0.01	Below LOD	0.01	1.32	0.06	5.97	1.10
Riparia	1.62	5.79	3.65	80.88	11.52	0.01	0.04	2.03	0.56	2.58	0.21	0.49	0.54	1.99	0.73
Riparia	0.42	0.67	4.27	12.72	24.74	0.00	0.07	Below LOD	Below LOD	Below LOD	0.01	1.65	0.05	7.60	0.95
Riparia	1.33	5.29	3.51	25.93	13.16	Below LOD	Below LOD	0.15	Below LOD	Below LOD	0.01	1.54	0.09	7.78	0.27
Riparia	0.58	1.38	6.43	21.03	61.06	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	0.01	0.15	0.04	Below LOD	0.40
Riparia	7.35	22.69	5.82	16.43	16.69	Below LOD	0.37	Below LOD	0.08	Below LOD	0.01	Below LOD	0.07	Below LOD	1.01
Riparia	1.57	2.67	3.13	74.50	225.84	Below LOD	0.27	Below LOD	Below LOD	Below LOD	0.04	0.09	0.08	Below LOD	1.26
Riparia	0.23	Below LOD	10.14	41.80	14.87	0.01	Below LOD	0.48	Below LOD	Below LOD	0.03	5.66	0.09	26.18	0.77
Riparia	0.71	2.21	9.42	51.15	106.40	0.01	0.09	Below LOD	Below LOD	Below LOD	0.01	Below LOD	0.05	Below LOD	0.55
Riparia	Below LOD	Below LOD	5.49	31.89	97.94	Below LOD	0.05	Below LOD	Below LOD	Below LOD	0.02	0.40	0.25	Below LOD	0.81
Riparia	0.84	Below LOD	12.52	25.43	25.50	Below LOD	Below LOD	0.46	Below LOD	Below LOD	0.02	11.00	0.14	53.47	0.81
Rupestris	2.22	7.43	4.84	65.67	96.56	Below LOD	0.33	0.65	Below LOD	Below LOD	0.02	Below LOD	0.11	Below LOD	0.51
Rupestris	0.22	Below LOD	5.48	67.24	72.59	0.04	0.14	Below LOD	Below LOD	Below LOD	0.00	0.45	0.04	3.15	0.66
Rupestris	0.19	Below LOD	7.25	40.99	26.00	Below LOD	0.15	Below LOD	Below LOD	Below LOD	0.01	0.25	0.05	1.62	0.52
Vulpina	0.12	Below LOD	6.09	50.62	189.89	Below LOD	0.21	Below LOD	Below LOD	Below LOD	0.04	1.57	1.64	7.46	0.74
Vulpina	Below LOD	Below LOD	6.82	167.47	188.15	Below LOD	0.21	1.85	0.28	0.69	0.16	0.70	4.54	Below LOD	0.82

Figure 18: Heat map of grape samples analyzed versus analyte concentration. Nine samples have been removed from the table because of SPME fiber breakage.

Typical concentration of MA is in the range of 0.06 to 0.6 µg/L. Additionally, C6 compounds such as hexanal, *trans*-2-hexenal, and hexanol show up at 240 µg/kg, 800 µg/kg, and 2.2 mg/L in must. Finally, eugenol and vanillin are introduced at varying levels depending on the toasting of the oak the must or wine should come in contact with. After a light toasting, eugenol will reach approximately 1.87 µg/g, and vanillin can be up to 27.17 µg/g. These values become 1.27 µg/g for eugenol and 49.81 µg/g for vanillin after contact with medium-level toasted wood. Finally, there is 1.65 µg/g eugenol and 25.45 µg/g vanillin after a heavy or high toast [6, Table 12.2].

Vitis acerifolia has a relatively neutral profile, having low presence of nearly all odorants relative to their concentration in other varieties. *V. acerifolia* is desired because of this neutral quality, lacking typical "off-aromas" of common North American grape varieties like Concord, which are believed to make poor wines. Neutral flavor and aroma make *V. acerifolia* a great candidate for crossbreeding with varieties with stronger flavors to impart its ability to thrive in North American winters, resistance to drought, and general wellness. The most prominent aroma compound is *trans*-2-hexenal, followed by hexanol and 1,4-cineole; however, all are inconsistent from vine to vine in testing. Samples with high concentration of MA, vanillin, and eucalyptol may indicate that these compounds experience more variation with environmental conditions in the vineyard. These samples had the most regular level of hexanal in each cluster. Hexanal is slightly elevated for the entire population evaluated, so these berries will exude stronger green flavor associated with immaturity.

Only two clippings of *Vitis aestavalis* were acquired from the germplasm, and the evaluation of one was interrupted by breakage of the SPME fiber. Results cannot be considered highly reliable because one independent measurement does not permit statistical analysis; the data may be suggestive but is obviously incomplete.

Additionally, ripe fruit from this variety is known to be more variable [34]. The cluster contained the highest point for two of the aroma compounds: *trans*-2-hexenal and eugenol. While not the maximums from the study, the macerate has very high concentrations of hexanal and hexanol, and β-ionone. The hexanol point is substantially higher than that typically found in *V. vinifera*. This would suggest a sweet berry with almond, apple, plum, spice, or clove notes.

The analysis of only two samples of *Vitis amurensis* also may not present definitive evidence of a flavor profile. In any case, relatively few identifiable flavor compounds could be detected in *Amurensis*. The grapes have less complexity than some of the others but have high levels of the compounds that are present. This species contains the maximum concentration of hexanal as well as notable quantities of *trans*-2-hexenal, hexanol, vanillin, and β-ionone. This grape is known for acidity with a high sugar content, which follows expectations of these shown trends.

Eight of the forty-eight samples from 2019 were *Vitis Labrusca*. Nerol, hexanol, hexanal, and α-terpineol were consistently low. EA and myrcene show themselves to be typically quite low in this species – except that the cultivar showed an unusual concentration maximum for these compounds. The first of these two particular specimens runs high on most compounds of interest, so it can be categorized as an outlier. Geraniol and linalool are common among each of the *Labrusca* samples and contribute to its characteristic floral and rosy features. There is consistent presence of MA with this compound serving as the highest or second largest quantity in each clipping. Methyl anthranilate is said to be responsible for grapey notes referred to as "foxy," and this is expected because *Vitis Labrusca* is known as the "fox grape."

Over half of our grape samples represented *Vitis riparia.* This species is already heavily utilized in the industry where its hardy root stock and disease resistance is much appreciated. The berries could be just as useful with their fruity and

slightly floral aroma. The strongest presence is β-ionone, known for smelling similarly to violet blossoms and suppling herbaceousness. This is followed by hexanal and vanillin. The degree of MA, EA, eugenol, myrcene, and α-terpineol is steadily low throughout the entire sampling. The twenty-first sample of *Riparia* is an outlier, exuding ballooned levels of hexanol in comparison to its neighbors. This can also be said of the eighteenth sample, which has the highest level of vanillin.

Neither geraniol nor nerol could be detected within *Vitis rupestris*. Concentration of MA, EA, *trans*-2-hexenal, myrcene, eucalyptol, and 1,4-cineole is quite low as well. The notable compounds investigated in this species are hexenol, vanillin, and β-ionone. Vanillin is uncommon in grapes prior to some intentional treatment but consistent in this variety. While the berries alone would not reach equivalent levels without oak contact, *V. rupestris* could have the potential to impart some oak aromas without barreling if other matrix effects do not mask the vanillin characteristics.

The last species of grape included in this study is *Vitis vulpina.* EA and eugenol have gone undetected in this variety; MA, geraniol, nerol, and 1,4-cineole are detected at low quantities. Hexanol, β-ionone, and α-terpineol are prominent aromas. The maximum concentration of α-terpineol throughout the entire study occurred within the clippings of *Vitis vulpina*. Should the concentration of hexanol not reach too high, the alcohol should maintain a characteristic of sweetness. The grape berries should exude floral flavor while highlighting lilac, violet, and raspberry.

CONCLUSIONS

In this work, a novel study of seven grape cultivars revealed the aroma compounds that are the foundation of the smell and flavor we have come to know from these berries. Each variety highlighted characteristics that set it apart from its counterparts and would make them useful for propagation studies within grape breeding. Sharing parentage with *Vitis vinifera* would result in second generation vines with high plant heartiness, quality, and unique profiles. *Vitis acerifolia* imparts a neutral flavor while accentuating green notes. The consistent nature of those aromas would make this variety an excellent choice in working to create the profile of a New Zealand Sauvignon blanc. *Vitis labrusca* proves its nickname with elevated concentrations of MA. This gives the grapes a "foxy" aroma known well from concord grapes typically used in Welch's™. *Vitis riparia* is highly floral thanks to β-ionone. β-ionone is also present in *Vitis vulpina* alongside α-terpineol. This plants berries will be sweet with hints of lilac should the hexanol remain at a level that prevents a solvent aroma from taking hold over the others. Finally, *Vitis aestavalis* and *Vitis amurensis* were studied; unfortunately, this investigation does not contain concrete results due to low sampling number. Should further study provide parallel data, these varieties would produce the most interesting flavor. *V. amurensis* indicates high acidity paired with sweetness to counteract the intensity. *V. aestavalis* contains peak levels of *trans*-2-hexenal and eugenol, resulting in aromas of almonds, plums, cloves, and apples.

This area of research leaves an expanse for continuing with future work. Thirty samples were collected from the USDA germplasm during 2020; these grapes have been prepared for analysis using the same procedure as described above for those retrieved in 2019. The data produced from 2020 has not been analyzed. Doing so would add further data points to the cultivars collected during both years – *Vitis*

acerifolia, *Vitis aestavalis*, *Vitis labrusca*, *Vitis riparia*, *Vitis rupestris*, and *Vitis vulpina*. Quantifying additional samples of these grapes would offer a greater understanding of variation that takes place year to year, as well as what characteristics can be considered consistent within the species. I would also expand the aroma compounds being explored in the specimens. In this work, sixteen odorants were evaluated. Though we had hoped to assess twenty-seven, time became a limitation that prevented this from occurring prior to the conclusion of the study. Including 2-aminoacetophenone, *cis*-rose-oxide, β-damascenone, guaiacol, α-ionone, ethyl octanoate, ethyl decanoate, syringol, rotundone, and hotrienol would be a useful next step for the study. With ample time, the investigation could be broadened to characterize additional species of grapes with the goal of creating a library of odorant profiles for grape breeders to reference at no cost. The free access of knowledge would broaden the ability of growers to produce unique varieties of grapes with specific flavor profiles, which would then allow an explosion of innovation.

REFERENCES

1. Casscles, J. S. E. S. Rogers and the Origins of American Grape Breeding. *Arnoldia* **2019**, *77* (2). https://arboretum.harvard.edu/stories/e-s-rogers-and-the-origins-of-american-grape-breeding/ (accessed 2022-06-18).

2. Clark, L. American Bugs Almost Wiped Out France's Wine Industry. *Smithsonian Magazine,* March 2015. https://www.smithsonianmag.com/smart-news/american-bugs-almost-wiped-out-frances-wine-industry-180954619/ (accessed 2022-06-17)

3. Cain, D. Grapevine 'IFG Seven.' US 0055476 P1, 2013.

4. Kiel, A. Top Beverage Trends for 2022 Include Bold Flavors, a Return to Simplicity. *Worldtea News (New York, NY)*, December 1, 2021. https://www.worldteanews.com/issues-trends/top-beverage-trends-2022-include-bold-flavors-return-simplicity (accessed 2022-06-15).

5. Denig, V. Top wine trends for 2022: styles, sustainability and supply. *Decanter*. https://www.decanter.com/features/top-wine-trends-for-2022-styles-sustainability-and-supply-471680/ (accessed 2022-06-15).

6. Waterhouse, A. L.; Sacks, G. L.; Jeffery, D. W. *Understanding Wine Chemistry*, 1st ed.; John Wiley & Sons: Chichester, West Sussex, 2016.

7. Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 6th ed.; CRC Press, 2010.

8. Urban Horticulture Institute at Cornell University. *European (Viitis vinifera) Wine Grape Varieties*. http://www.hort.cornell.edu/reisch/grapegenetics/bulletin/wine/winetext5.html (accessed 2022-07-20).

9. Burzynski-Chang, E. A.; Ryona, I.; Reish, B. I.; Gonda, I.; Foolad, M. R.; Giovannoni, J. J.; Sacks, G. L. HS-SPME-GC-MS Analyses of Volatiles in Plant Populations–Quantitating Compound x Individual Matrix Effects. *Molecules* **2018**, *23* (10), 2436. DOI: 10.3390/molecules23102436

10. Perry, D. M.; Hayes, J. H. Effects of Matrix Composition on Detection Threshold Estimates for Methyl Anthranilate and 2-Aminoacetophenone. *Foods* **2016**, *5* (2), 35. DOI: 10.3390/foods5020035

11. Sánchez-Palomo, E.; Díaz-Maroto, M. C.; Pérez-Coello, M. S. Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC-MS. *Talanta* **2005**, *66* (5), 1152-1157. DOI: 10.1016/j.talanta.2005.01.015

12. Choudhury, R.; Paudel, P.; Sharma, A. K.; Webb, S.; Ware, M. Evaluating the Merit of a Syringol Derived Fluorophore as a Charge Transfer Probe for Detection of Serum Albumins. *J. Photochem. Photobiol., A* **2022**, *422*. DOI: 10.1016/j.jphotochem.2021.113563

13. Meilgaard, M. C. Individual differences in sensory threshold for aroma chemicals added to beer. *Food Qual. Prefer.* **1993**, *4* (3), 153-167. DOI: 10.1016/0950-3293(93)90158-3

14. Dziadas, M.; Jeleń, H. H. Analysis of terpenes in white wines using SPE-SPME-GC/MS approach. *Anal. Chim. Acta* **2010**, *677* (1), 43-49. DOI: 10.1016/j.aca.2010.06.035

15. Capone, D. L. *Eucalyptol (1,8-cineole) in wine.* Australian Wine Research Institute. https://www.awri.com.au/wp-content/uploads/2013/08/capone-W07-AWITC15.pdf (accessed 2022-02-08).

16. Antalick, G.; Tempère, S.; Šuklje, K.; Blackman, J. W.; Deloire, A.; de Revel, G.; Schmidtke, L. M. Investigation and Sensory Characterization of 1,4-Cineole: A Potential Aromatic Marker of Australian Cabernet Sauvignon Wine. *J. Agric. Food Chem.* **2015**, *63* (41), 9103-9111. DOI: 10.1021/acs.jafc.58b03847

17. Miller, G. H. Appendix A. In *Whisky Science*; Springer: Cham, Switzerland, 2019.

18. NIST Mass Spectrometry Data Center, *Methyl anthranilate Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C134203&Units=SI&Mask=200#Mass-Spec (accessed 2022-06-14).

19. NIST Mass Spectrometry Data Center, *Benzoic acid, 2-amino-, ethyl ester Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C87252&Mask=200#Mass-Spec (accessed 2022-06-14).

20. NIST Mass Spectrometry Data Center, *Hexanal Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C66251&Mask=200#Mass-Spec (accessed 2022-06-14).

21. NIST Mass Spectrometry Data Center, *2-Hexenal, (E)- Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C6728263&Mask=200#Mass-Spec (accessed 2022-06-14).

22. NIST Mass Spectrometry Data Center, *1-Hexanol Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C111273&Mask=200#Mass-Spec (accessed 2022-06-14).

23. NIST Mass Spectrometry Data Center, *Eugenol Mass Spectrum*. https://webbook.nist.gov/cgi/inchi?ID=C97530&Mask=200#Mass-Spec (accessed 2022-06-14).

24. NIST Mass Spectrometry Data Center, *Benzaldehyde Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C100527&Mask=200#Mass-Spec (accessed 2022-06-14).

25. NIST Mass Spectrometry Data Center, *Vanillin Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C121335&Mask=200#Mass-Spec (accessed 2022-06-14).

26. NIST Mass Spectrometry Data Center, *Linalool Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C78706&Mask=200#Mass-Spec (accessed 2022-06-14).

27. NIST Mass Spectrometry Data Center, *Geraniol Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C106241&Mask=200#Mass-Spec (accessed 2022-06-14).

28. NIST Mass Spectrometry Data Center, *Nerol, methyl ether Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=U352641&Mask=200#Mass-Spec (accessed 2022-06-14).

29. NIST Mass Spectrometry Data Center, *.beta.-myrcene Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C123353&Mask=200#Mass-Spec (accessed 2022-06-14).

30. NIST Mass Spectrometry Data Center, *Eucalyptol Mass Spectrum*. https://webbook.nist.gov/cgi/inchi?ID=C470826&Mask=200#Mass-Spec (accessed 2022-06-14).

31. NIST Mass Spectrometry Data Center, *.alpha.-terpineol Mass Spectrum*. https://webbook.nist.gov/cgi/inchi?ID=C98555&Mask=200#Mass-Spec (accessed 2022-06-14).

32. NIST Mass Spectrometry Data Center, *7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)- Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C470677&Mask=200#Mass-Spec (accessed 2022-06-14).

33. NIST Mass Spectrometry Data Center, *trans-.beta.-ionone Mass Spectrum*. https://webbook.nist.gov/cgi/cbook.cgi?ID=C79776&Mask=200#Mass-Spec (accessed 2022-06-14).

34. Missouri Botanical Gardens. *Vitis aestivalis*. https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?taxonid=287538 (accessed 2022-07-09).

35. Crawford Scientific. *Troubleshooting GC peak shapes*. https://www.crawfordscientific.com/chromatography-blog/post/gc-troubleshooting-peak-shapes (accessed 2022-08-16).

36. Jackson, R. S. Vineyard Practice. In *Wine Science*, 4th ed. Academic Press: Cambridge, MA, 2014.

37. Christie, C. A Kern County grape that tastes like cotton candy. *The Bakersfield Californian Bakersfield, CA)*, August 9, 2013, updated September 13, 2016. https://www.bakersfield.com/news/a-kern-county-grape-that-tastes-like-cotton-

candy/article_82a44d1c-3565-5904-94db-7c39a3271505.html (accessed 2022-09-06).

APPENDIX 1

Raw area data from Shimadzu GCMS Postrun Analysis Software. Nine samples have been removed from the table because of SPME fiber breakage.

	MA	EA	d3-MA	Hexanal	*trans*-2-Hexenal	Hexanol	d12-Hexanal	Eugenol	d3-Guaiacol	Benzaldehyde	Vanillin	d3-Vanillin	Linalool	Geraniol	Nerol	Myrcene	Eucalyptol	α-Terpineol	1,4-Cineole	β-Ionone	d3-Linalool
Acerifolia	277,466	36,281	26,041	12,630,700	1,132,279	2,318,858	29,448	5,548	247,899	942,545	Below LOD	32,289	149,178	30,644	25,782	38,122	344,046	20,522	453,014	52,377	678,323
Acerifolia	7,875,469	Below LOD	81,393	9,869,053	3,357,897	5,501,638	15,973	26,678	327,451	1,328,164	15,640	49,248	150,825	129,467	39,631	81,155	48,236	60,196	64,680	95,430	781,389
Acerifolia	280,551	Below LOD	26,737	11,020,732	5,539,492	9,792,794	29,433	Below LOD	331,110	1,365,372	Below LOD	25,638	87,467	5,271	Below LOD	20,244	67,321	8,808	98,676	77,936	576,222
Aestavalis	195,552	Below LOD	18,513	20,749,610	25,037,978	24,602,711	27,134	59,784	317,836	4,285,146	Below LOD	25,140	204,546	55,080	50,463	177,941	6,216	37,641	Below LOD	148,641	735,235
Amurensis	12,018	Below LOD	53,106	16,307,209	16,437,653	22,349,244	39,188	Below LOD	589,305	3,999,894	Below LOD	88,845	Below LOD	89,765	Below LOD	47,842	Below LOD	30,672	Below LOD	142,080	959,888
Amurensis	187,724	Below LOD	14,657	25,555,358	19,419,586	20,089,049	27,008	3,668	332,164	970,116	10,338	40,929	211,209	45,558	Below LOD	28,880	Below LOD	46,174	Below LOD	147,350	740,699
Labrusca	10,246,543	1,718,012	57,867	3,268,391	2,879,731	1,829,017	14,888	14,899	398,143	3,184,981	8,314	58,725	786,747	111,204	61,853	66,493	760,842	394,715	969,954	74,189	1,054,793
Labrusca	4,317,447	642,800	80,916	8,203,690	5,172,890	6,726,634	18,413	Below LOD	375,641	1,522,139	22,186	216,479	Below LOD	153,181	27,743	378,748	Below LOD	40,284	Below LOD	116,623	1,378,740
Labrusca	22,206,353	Below LOD	285,414	969,618	186,036	2,064,008	15,468	79,223	552,476	881,414	Below LOD	150,004	428,657	661,959	41,219	187,322	207,275	186,938	255,159	47,419	1,779,856
Labrusca	12,250,774	Below LOD	141,110	10,085,838	6,262,788	2,492,104	11,155	14,499	366,002	1,623,388	8,807	87,330	213,464	294,652	59,111	227,247	24,252	136,272	29,201	99,954	1,291,996
Labrusca	3,566,586	Below LOD	108,659	3,945,836	2,017,096	1,060,859	17,316	10,801	408,091	1,127,570	9,173	68,954	Below LOD	97,033	3,209	151,314	Below LOD	18,767	Below LOD	131,420	1,318,137
Labrusca	1,731,288	117,338	62,109	7,111,509	4,130,096	15,067,232	31,366	25,384	521,055	4,723,513	Below LOD	19,055	427,135	422,373	67,110	2,190,881	29,679	225,055	Below LOD	88,710	1,184,910
Labrusca	490,663	51,074	8,397	9,232,519	865,441	13,036,654	34,397	5,252	460,816	1,795,026	11,819	77,513	343,030	181,241	12,341	33,216	413,003	101,947	538,972	69,255	1,047,046
Riparia	Below LOD	Below LOD	Below LOD	2,664,165	2,768,207	7,123,399	38,081	Below LOD	360,847	1,159,276	Below LOD	82,430	380,139	7,008	Below LOD	32,615	301,697	73,945	330,821	125,076	750,634
Riparia	400,210	53,785	22,992	3,895,433	2,104,665	16,285,998	13,843	10,277	286,825	1,565,941	9,931	26,948	Below LOD	12,156	Below LOD	24,018	6,257	12,281	Below LOD	122,768	738,019
Riparia	46,354	Below LOD	24,055	11,682,082	3,476,340	8,891,788	22,518	Below LOD	252,011	708,856	20,723	35,260	Below LOD	6,716	8,894	22,494	12,929	9,093	10,905	97,127	710,805
Riparia	564,398	96,149	29,437	5,385,182	4,174,450	4,405,615	29,543	15,842	308,541	1,022,183	7,151	28,165	Below LOD	763,184	488,569	419,234	57,947	44,241	80,561	339,665	1,247,331
Riparia	127,354	18,469	32,421	11,235,464	6,236,484	4,843,049	24,651	Below LOD	409,106	579,851	11,853	49,432	279,006	16,919	26,624	236,420	10,799	45,770	13,478	175,011	928,744
Riparia	186,669	32,155	32,354	14,528,304	1,801,865	4,843,526	30,528	Below LOD	276,132	785,465	Below LOD	39,527	Below LOD	Below LOD	Below LOD	27,815	1,029,973	12,511	1,280,282	286,937	1,040,523
Riparia	209,899	29,392	18,976	13,946,236	2,186,518	3,970,182	28,352	5,453	269,641	2,175,003	Below LOD	21,031	Below LOD	12,828	16,724	19,064	3,003	10,827	Below LOD	91,439	619,970
Riparia	163,921	22,458	45,539	7,704,382	1,125,197	3,842,894	31,857	Below LOD	378,047	861,749	11,353	148,071	Below LOD	Below LOD	Below LOD	39,356	Below LOD	8,138	Below LOD	177,854	1,058,762
Riparia	488,010	75,903	29,741	10,070,866	1,078,605	3,231,049	34,122	Below LOD	315,520	1,014,127	Below LOD	51,045	Below LOD	17,710	Below LOD	38,306	235,149	18,432	328,896	169,357	1,065,825
Riparia	444,046	72,076	44,953	9,274,100	874,081	2,673,850	23,081	Below LOD	267,402	801,026	Below LOD	97,516	Below LOD	Below LOD	Below LOD	24,404	12,334	27,345	Below LOD	231,645	1,054,433
Riparia	2,058,293	280,122	151,267	3,611,021	2,613,457	1,888,511	22,405	Below LOD	496,010	874,220	7,656	130,571	Below LOD	70,240	37,927	53,634	Below LOD	69,967	Below LOD	80,353	1,410,160
Riparia	18,098	Below LOD	32,049	10,122,889	1,046,422	8,745,265	34,530	8,765	437,064	650,990	Below LOD	71,013	Below LOD	5,774	Below LOD	26,873	85,231	18,935	109,261	334,571	1,294,858
Riparia	434,085	61,343	80,220	10,142,707	7,964,233	2,732,507	22,399	7,909	357,291	606,919	6,040	83,013	518,648	253,414	202,352	716,810	35,731	203,440	38,746	241,501	1,423,086
Riparia	218,405	14,946	107,184	8,193,689	873,937	4,060,636	33,206	3,149	463,889	883,477	17,004	133,034	Below LOD	Below LOD	Below LOD	28,992	83,123	13,757	108,637	224,841	1,006,737
Riparia	195,881	33,508	30,377	6,895,704	1,801,719	2,207,029	31,612	Below LOD	280,669	499,316	Below LOD	71,183	26,598	Below LOD	Below LOD	23,480	77,225	23,277	110,213	61,171	993,385
Riparia	49,157	5,008	17,442	7,318,354	844,436	5,927,585	33,659	Below LOD	288,978	645,376	Below LOD	22,589	Below LOD	Below LOD	Below LOD	19,135	4,393	6,258	Below LOD	52,599	575,691
Riparia	824,465	106,109	25,320	5,426,071	526,979	1,318,709	18,370	Below LOD	251,027	1,085,163	6,645	17,831	Below LOD	12,472	Below LOD	8,597	Below LOD	8,481	Below LOD	106,254	464,815
Riparia	281,455	20,544	36,826	4,437,568	3,728,552	27,281,990	5,630	Below LOD	466,111	776,573	11,949	40,981	Below LOD	Below LOD	Below LOD	71,550	3,157	15,091	Below LOD	207,305	715,352
Riparia	23,480	Below LOD	20,932	13,726,323	1,999,500	1,717,047	44,704	15,246	358,817	737,851	Below LOD	30,333	59,212	Below LOD	Below LOD	46,608	195,716	16,639	255,139	120,403	683,752
Riparia	108,005	14,349	31,127	14,852,799	2,849,047	14,305,996	50,383	Below LOD	458,590	1,820,480	6,410	69,392	Below LOD	Below LOD	Below LOD	25,174	Below LOD	9,708	Below LOD	101,531	796,185
Riparia	Below LOD	Below LOD	Below LOD	9,211,735	1,889,320	14,004,655	30,735	Below LOD	357,864	1,223,066	6,194	108,184	Below LOD	Below LOD	Below LOD	38,642	17,340	55,896	Below LOD	158,526	846,811
Riparia	63,905	Below LOD	15,667	14,264,144	1,023,650	2,477,997	25,792	Below LOD	220,968	750,406	Below LOD	13,973	47,212	Below LOD	Below LOD	32,594	320,243	22,163	438,460	107,160	575,399
Rupestris	236,409	33,995	21,934	5,068,858	2,424,543	8,625,399	21,027	Below LOD	186,311	757,525	5,026	14,734	61,161	Below LOD	Below LOD	21,596	Below LOD	14,815	Below LOD	61,369	528,746
Rupestris	17,707	Below LOD	21,989	15,886,274	6,932,080	17,878,644	37,213	59,143	425,288	8,891,165	7,372	43,136	Below LOD	Below LOD	Below LOD	14,960	33,522	15,424	64,051	221,170	1,461,187
Rupestris	24,556	Below LOD	27,272	16,792,597	3,354,252	5,135,796	58,142	48,952	458,924	1,114,011	4,695	29,902	Below LOD	Below LOD	Below LOD	16,123	14,802	16,864	26,927	139,415	1,169,568
Vulpina	26,957	Below LOD	45,596	9,891,164	2,905,074	26,306,700	19,610	7,364	285,158	1,162,490	21,691	94,788	Below LOD	Below LOD	Below LOD	68,432	65,078	354,478	87,260	139,477	820,364
Vulpina	Below LOD	Below LOD	Below LOD	13,015,691	11,288,603	30,613,830	23,078	7,339	379,329	1,896,375	10,490	46,521	318,664	85,495	36,588	353,180	33,960	1,152,876	Below LOD	181,901	963,522

APPENDIX 2

Heat map of grape samples analyzed versus aroma compounds. Data provided is area as given in Shimadzu GCMS Postrun Analysis Software. Nine samples have been removed from the table because of SPME fiber breakage.

	MA	EA	Hexanal	*trans*-2-Hexenal	Hexanol	Eugenol	Vanillin	Linalool	Geraniol	Nerol	Myrcene	Eucalyptol	α-Terpineol	1,4-Cineole	β-Ionone
Acerifolia	0.06	0.05	0.47	0.04	0.02	0.11	Below LOD	0.30	0.07	0.10	0.03	0.53	0.03	0.56	0.27
Acerifolia	0.46	Below LOD	0.72	0.28	0.09	0.34	0.59	0.29	0.17	0.13	0.04	0.07	0.05	0.08	0.49
Acerifolia	0.06	Below LOD	0.40	0.20	0.07	Below LOD	Below LOD	0.20	0.02	Below LOD	0.02	0.12	0.01	0.14	0.46
Aestavalis	0.09	Below LOD	0.82	1.00	0.19	1.00	Below LOD	0.38	0.12	0.18	0.13	0.01	0.04	Below LOD	0.70
Amurensis	0.00	Below LOD	0.44	0.44	0.12	Below LOD	Below LOD	Below LOD	0.15	Below LOD	0.03	Below LOD	0.03	Below LOD	0.51
Amurensis	0.07	Below LOD	1.00	0.76	0.15	0.06	0.63	0.38	0.10	Below LOD	0.02	Below LOD	0.05	Below LOD	0.69
Labrusca	1.00	1.00	0.24	0.21	0.03	0.19	0.66	1.00	0.17	0.15	0.03	0.73	0.31	0.75	0.24
Labrusca	0.38	0.16	0.47	0.29	0.07	Below LOD	0.13	Below LOD	0.18	0.05	0.15	Below LOD	0.02	Below LOD	0.29
Labrusca	0.44	Below LOD	0.07	0.01	0.03	0.72	Below LOD	0.32	0.60	0.06	0.06	0.12	0.09	0.12	0.09
Labrusca	0.49	Below LOD	0.96	0.59	0.05	0.20	0.25	0.22	0.37	0.12	0.09	0.02	0.09	0.02	0.27
Labrusca	0.19	Below LOD	0.24	0.12	0.01	0.13	0.35	Below LOD	0.11	0.01	0.06	Below LOD	0.01	Below LOD	0.34
Labrusca	0.16	0.06	0.24	0.14	0.10	0.24	Below LOD	0.49	0.58	0.15	1.00	0.03	0.16	Below LOD	0.27
Labrusca	0.33	0.21	0.28	0.03	0.08	0.06	0.38	0.44	0.28	0.03	0.02	0.40	0.08	0.42	0.23
Riparia	Below LOD	Below LOD	0.07	0.08	0.04	Below LOD	Below LOD	0.68	0.02	Below LOD	0.02	0.41	0.08	0.36	0.57
Riparia	0.09	0.07	0.31	0.16	0.25	0.18	0.89	Below LOD	0.03	Below LOD	0.02	0.01	0.01	Below LOD	0.60
Riparia	0.01	Below LOD	0.54	0.16	0.08	Below LOD	0.88	Below LOD	0.02	0.03	0.02	0.02	0.01	0.01	0.47
Riparia	0.11	0.11	0.17	0.16	0.03	Below LOD	0.63	Below LOD	0.99	1.01	0.18	0.05	0.03	0.05	0.88
Riparia	0.02	0.02	0.47	0.27	0.04	Below LOD	0.61	0.40	0.03	0.07	0.14	0.01	0.04	0.01	0.65
Riparia	0.04	0.04	0.50	0.06	0.03	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	0.01	1.00	0.01	1.00	0.95
Riparia	0.06	0.05	0.52	0.08	0.03	0.10	Below LOD	Below LOD	0.03	0.07	0.02	0.00	0.02	Below LOD	0.51
Riparia	0.02	0.01	0.25	0.03	0.03	Below LOD	0.11	Below LOD	Below LOD	Below LOD	0.02	Below LOD	0.01	Below LOD	0.58
Riparia	0.10	0.10	0.31	0.03	0.02	Below LOD	Below LOD	Below LOD	0.03	Below LOD	0.02	0.22	0.01	0.24	0.55
Riparia	0.06	0.05	0.42	0.04	0.02	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	0.01	0.01	0.02	Below LOD	0.76
Riparia	0.09	0.08	0.17	0.12	0.02	Below LOD	0.10	Below LOD	0.08	0.07	0.02	Below LOD	0.04	Below LOD	0.20
Riparia	Below LOD	Below LOD	0.31	0.03	0.05	0.09	Below LOD	Below LOD	0.01	Below LOD	0.01	0.07	0.01	0.07	0.88
Riparia	0.04	0.04	0.47	0.37	0.02	0.10	0.12	0.48	0.28	0.36	0.26	0.03	0.12	0.02	0.58
Riparia	0.01	0.00	0.25	0.03	0.02	0.04	0.19	Below LOD	Below LOD	Below LOD	0.02	0.08	0.01	0.09	0.75
Riparia	0.04	0.04	0.23	0.06	0.01	Below LOD	Below LOD	0.04	Below LOD	Below LOD	0.01	0.08	0.02	0.09	0.21
Riparia	0.02	0.01	0.24	0.03	0.04	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	0.02	0.01	0.01	Below LOD	0.31
Riparia	0.20	0.16	0.31	0.03	0.02	Below LOD	0.99	Below LOD	0.04	Below LOD	0.01	Below LOD	0.01	Below LOD	0.80
Riparia	0.04	0.02	0.83	0.70	1.00	Below LOD	0.73	Below LOD	Below LOD	Below LOD	0.05	0.00	0.02	Below LOD	1.00
Riparia	0.01	Below LOD	0.32	0.05	0.01	0.21	Below LOD	0.12	Below LOD	Below LOD	0.04	0.29	0.02	0.30	0.61
Riparia	0.02	0.02	0.31	0.06	0.06	Below LOD	0.23	Below LOD	Below LOD	Below LOD	0.02	Below LOD	0.01	Below LOD	0.44
Riparia	Below LOD	Below LOD	0.32	0.06	0.09	Below LOD	0.14	Below LOD	Below LOD	Below LOD	0.02	0.02	0.06	Below LOD	0.65
Riparia	0.02	Below LOD	0.58	0.04	0.02	Below LOD	Below LOD	0.11	Below LOD	Below LOD	0.03	0.56	0.03	0.62	0.64
Rupestris	0.06	Below LOD	0.26	0.13	0.09	Below LOD	0.88	0.15	Below LOD	Below LOD	0.02	Below LOD	0.02	Below LOD	0.40
Rupestris	0.01	Below LOD	0.45	0.19	0.10	0.69	0.38	Below LOD	Below LOD	Below LOD	0.01	0.02	0.01	0.04	0.52
Rupestris	0.01	Below LOD	0.31	0.06	0.02	0.53	0.39	Below LOD	Below LOD	Below LOD	0.01	0.01	0.01	0.02	0.41
Vulpina	0.00	Below LOD	0.53	0.16	0.28	0.13	0.57	Below LOD	Below LOD	Below LOD	0.04	0.08	0.36	0.09	0.59
Vulpina	Below LOD	Below LOD	0.60	0.52	0.27	0.10	0.56	Below LOD	Below LOD	Below LOD	0.19	0.04	1.00	Below LOD	0.65