



Article

Analysis of the Aroma Volatile Profile of Muscadine Grape Germplasm by Headspace Solid-Phase Microextraction Coupled with Gas Chromatography-Mass Spectrometry

Gaurab Bhattarai ¹, Orestis Giannopoulos ², Ramsey Nathanael Corn ², Camille E. E. McAvoy ², Angelos Deltsidis ², Margaret L. Worthington ³ and Patrick J. Conner ^{2,*}

¹ Institute of Plant Breeding, Genetics & Genomics, University of Georgia, Athens, GA 30602, USA; gbhattarai@uga.edu

² Department of Horticulture, University of Georgia-Tifton Campus, Tifton, GA 31793, USA; orestis.g@uga.edu (O.G.); ramsey.corn@uga.edu (R.N.C.); ceemcavoy@uga.edu (C.E.E.M.); adeltsidis@uga.edu (A.D.)

³ Department of Horticulture, University of Arkansas, Fayetteville, AR 72701, USA; mlworthi@uark.edu

* Correspondence: pconner@uga.edu

Abstract: Muscadine grapes (*Vitis rotundifolia*) are native to the southeastern U.S., where they are valued for their unique flavor and fruity aroma. Despite having a diverse aroma profile, muscadine germplasm is virtually unexplored in terms of its aroma volatile content and composition, which is crucial in determining the value of its products. The aim of this research was to characterize 24 muscadine genotypes with distinct uses and origin for their aroma-related volatile profiles using the headspace solid-phase microextraction method coupled with gas-chromatography mass spectrometry. In total, 63 volatile compounds were detected, and genotypes significantly differed for 43 of the volatile compounds. We also profiled the aroma volatile content and composition of the commercially cultivated muscadine cultivar Carlos at various stages of berry ripeness. Characteristic differences were observed in the composition of the volatile compounds as ripening progressed. This is the first study to have evaluated the aroma volatile composition of a wide variety of muscadine germplasms, including juice and fresh fruit cultivars, as well as the related species *Vitis popenoei* and its complex hybrids between *V. rotundifolia* and *Vitis vinifera*. The results obtained from this study will help identify muscadine genotypes and better design crosses to produce fresh fruit and wine selections with the desired aroma profiles. This knowledge will lead to the development of new muscadine cultivars and significantly contribute to the expansion of muscadine use in the future.

Keywords: *Vitis rotundifolia*; *Muscadinia*; flavor; quality; GC-MS; HS-SPME



Citation: Bhattarai, G.; Giannopoulos, O.; Corn, R.N.; McAvoy, C.E.E.; Deltsidis, A.; Worthington, M.L.; Conner, P.J. Analysis of the Aroma Volatile Profile of Muscadine Grape Germplasm by Headspace Solid-Phase Microextraction Coupled with Gas Chromatography-Mass Spectrometry. *Horticulturae* **2023**, *9*, 1054. <https://doi.org/10.3390/horticulturae9091054>

Academic Editors: Antonio Amores-Arocha and Ana Jiménez-Cantizano

Received: 25 August 2023
Revised: 15 September 2023
Accepted: 18 September 2023
Published: 20 September 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Muscadine grapes (*V. rotundifolia*) are a native North American fruit found commonly throughout the southeastern U.S. with excellent adaptation to hot, humid summers and warm winters. It belongs to the *Muscadinia* subgenera in the genus of *Vitis* [1–4]. The subgenus *Muscadinia* has only two species: *V. rotundifolia* Michx. and *V. popenoei* J.H. Fennel [5]. Muscadines are a regional fruit with commercial production concentrated in the southern piedmont of North Carolina, the eastern coastal plain of North Carolina and South Carolina, and the piedmont and coastal plain of Georgia [6]. Production also extends to other states, especially Florida and Arkansas, but in lesser amounts. This fruit crop possesses tremendous potential for sustainable fruit systems in this region as it is much more productive than bunch grapes when grown in low-input systems [7]. Muscadines are primarily produced for fresh-market sales and processed products such as wine and juice. Muscadines have a high antioxidant capacity similar to that of blueberries and blackberries, leading to them often being described as a “superfruit” [8,9].

Muscadines are prized throughout their native range for their unique flavor. The basic tastes (sweetness, sourness, and bitterness) impacting fruit flavor are perceived by the taste receptors on the tongue, while volatile compounds are responsible for typical aromas (smells) and aromatic flavors (perceived while in the mouth). The combination of taste and olfaction provide the sensation of flavor [10,11]. Muscadines have very pronounced aromas that are often described as “fruity”, “foxy”, or “candy-like”. The aroma of fresh muscadines is very desirable to those who know and love the fruit, but it can be overwhelming to those who are only familiar with *V. vinifera* grapes. Desired flavors vary depending on whether the fruit is to be used for fresh market, juice, or wine. Muscadine grape germplasm is highly diverse for appealing fruit aromas, likely in part to facilitate the location and consumption of dehiscent berries by an array of frugivore seed dispersers [12]; however, muscadine germplasm is virtually unexplored for its aroma-related volatile composition and content.

Baek et al. [13] performed a gas chromatography-mass spectrometry (GC-MS) study of ‘Carlos’ muscadine grape juice and identified 33 volatile compounds related to aroma. Among them, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furanol) was the most abundant, which, along with o-aminoacetophenone, was thought to give the candy and foxy-like aroma notes to muscadine juice. At low concentrations, furaneol has a pineapple- or strawberry-like aroma. It has also been found in other North American *Vitis* species, including *Vitis labrusca*. However, at higher concentrations, the same compound gives off an undesirable caramel-like aroma, which is often avoided in wine made from bunch grapes (*V. vinifera*). This study also found several other predominant compounds and their aromas such as 2,3-butanedione (buttery/cream cheese), ethyl butanoate (bubble gum/fruity), ethyl 2-methylbutanoate (green apple/fruity), and 2-phenylethanol (rosy). In a separate study of ripe ‘Coward’ muscadine grapes by Lee et al. [14], volatile esters associated with fruity, floral, and pleasant odors were detected. Wine samples of ‘Welder’ and ‘Noble’ muscadine grapes produced relatively high concentrations of alcohols and esters of fatty acids [15]. Recently, Deng et al. [16] investigated the aroma-related volatile profile of five commercially grown muscadine cultivars. This study was able to identify 44 compounds, including esters, aldehydes, alcohols, fatty acids, terpenes, ketones, and furan via the solid-phase microextraction (SPME) GC-MS procedure. They found that geraniol and cinnamyl alcohol were the key volatile components that distinguished the Alachua cultivar from the rest. This study also showed that (Z)-3-hexenal, and (E)-2-hexenol were more prominent in the Fry and Granny Val cultivars, respectively. Although these studies indicate the presence of a wide variety of aroma-related volatile compounds in muscadine, focusing on only a few pure *V. rotundifolia* muscadine cultivars was not sufficient to investigate and exploit this genetic resource.

Fruit ripeness is the key attribute in muscadines that determines the appropriate time of harvesting fruits for both fresh market and juice production. This is especially important for fruits like muscadines with a relatively short shelf-life (about four weeks) and harvesting window (late summer to early autumn). The composition and abundance of aroma volatiles are linked with the fruit maturity and level of ripeness. Studies have shown differences in volatile composition in the different ripening stages of bunch grapes [17], raspberries [18], bananas [19], lulos [20], figs [21], and muskmelons [22]. However, no research has been conducted to understand the effect of berry ripeness on the aroma volatile composition of muscadines.

In this research, we investigated the aroma-related volatile profile of a wide variety of muscadine germplasms, including closely related species and hybrids that have been an important gene pool in muscadine breeding. Additionally, we identified volatile compounds and their abundance pattern at various ripening stages in a commercial muscadine wine cultivar, Carlos. The systematic characterization of the volatile compound profile of key germplasms will aid muscadine breeding by giving breeders the ability to select and combine appropriate parents for the desired flavor profile of the product. This eventually helps fulfill consumers’ demand for specific muscadine flavors in the market, and it will ultimately boost muscadine utilization. Similarly, understanding the pattern of volatile

abundance at various stages of fruit ripeness could provide information for the muscadine industry in understanding how berry ripening stage will influence the flavor profile of muscadine products.

2. Materials and Methods

2.1. Plant Materials and Sampling

The muscadine vines for germplasm characterization were grown at the University of Georgia (UGA) breeding program's experimental vineyards located in Tifton, GA, USA (lat. 31°28'39.81" N, long. 83°31'39.61" W). A list of these genotypes, their utility, and derivation is presented in Table 1. Vines were planted 3.04 m apart within the row and trained to two cordons on a 1.5 m high wire. Vines were irrigated and received commercial level care, which includes fertilization and fungicide applications as recommended by Poling et al. [23]. For the experiment to study the aroma volatiles at different stages of ripening, the fruit samples collected from the Carlos cultivar were planted at a commercial vineyard (Paulk Vineyards) in Wray, GA, USA.

Table 1. Muscadine germplasm used in aroma-volatile characterization.

Genotype	Female Parent	Male Parent	Berry Color ^z	Reference	Notes
AM-195	AM-19	AM-1	<u>Black</u>	M.L.W. breeding records	Fresh-market selection.
AM-77	Carlos	NC. 67A015-26	<u>Black</u>	M.L.W. breeding records	Red wine and juice breeding selection.
Carlos	Howard	NC. 11-173	<u>Bronze</u>	[24]	Leading white wine muscadine cultivar.
Cowart	Higgins	Ga. 28	<u>Black</u>	[24]	Fresh-market cultivar with a pronounced aroma.
Fennel's 3-way hybrid	Fennel's 2-way hybrid	<i>V. popenoei</i>	Black	[25]	Fennel's 2-way hybrid is 'Scuppernong' × <i>V. rotundifolia</i> var. <i>munsoniana</i> .
Fry	Ga. 19-13	Ga. 19-11	<u>Bronze</u>	[24]	Leading bronze fresh-market cultivar.
Ga. 13-3-36	Ga. 6-9-91	Ga. 6-1-217	Bronze	P.J.C. breeding records	Ga. 18-5 is a grandparent, Ga. 13-3-36 10.9% <i>V. vinifera</i> and 89.1% <i>V. rotundifolia</i> .
Ga. 1-6-14	Scarlet	Tara	<u>Bronze</u>	P.J.C. breeding records	Fresh-market muscadine selection with a "honey" flavor.
Ga. 18-5	Ga. 14-32	Ga. 12-2-1	Black	P.J.C. breeding records	Interspecific hybrid that is 43.75% <i>V. vinifera</i> and 56.25% <i>V. rotundifolia</i> .
Golden Isles	Fry	Ga. 19-6	<u>Bronze</u>	[24]	White wine muscadine cultivar released for more neutral flavored wine.
Hall	Fry	Tara	<u>Bronze</u>	[24]	Fresh-market muscadine cultivar.
Lane	Supreme	Tara	<u>Black</u>	[24]	Fresh-market muscadine cultivar.
Magnolia	Unnamed seedling	Topsail × Tarheel	<u>Bronze</u>	[24]	White wine muscadine cultivar.
Magoon	Thomas	Burgaw	<u>Black</u>	[24]	Black fresh-market muscadine cultivar.
Noble	Thomas	Tarheel	<u>Black</u>	[24]	Leading red wine muscadine cultivar.
Oh My!	JB99-1-4-15	JB03-20-1-21	Bronze	[24,26]	Stenospermocarpic seedless quasi-BC2 hybrid with an 86.9% <i>V. rotundifolia</i> background.
Paulk	Supreme	Tara	<u>Black</u>	[24]	Fresh-market muscadine cultivar.
Pineapple	Fry	Senoia	<u>Bronze</u>	[24]	Fresh-market muscadine cultivar with a "pineapple" flavor.
Ruby Crisp	Supreme	Tara	Red	[24]	Fresh-market muscadine cultivar with a red color and mild flavor.
Scuppernong	Unknown	Unknown	<u>Bronze</u>	[24]	Bronze colored native selection.
Southern Home	Summit	Fla. P9-15	Black	[25]	Interspecific hybrid of <i>V. rotundifolia</i> , <i>V. popenoei</i> , and <i>V. vinifera</i> .
Supreme	Black Fry	Dixieland	<u>Black</u>	[24]	Leading black fresh-market muscadine cultivar.
Tarheel	Luola	V68 R14 B2	<u>Black</u>	[24]	Older red wine muscadine cultivar.
<i>V. popenoei</i> DVIT 2970	Unknown	Unknown	Purple		Native selection of <i>V. popenoei</i> .

^z Underlined genotypes were used in the study of difference in the aroma volatile composition between black and bronze muscadines.

Muscadine berries ripen asynchronously, and berries are harvested individually rather than in clusters. Therefore, most muscadine cultivars require several pickings to remove all

the ripe fruit. For germplasm characterization, berries judged to be at optimal commercial ripeness (fully colored and with some softness), as well as free from defects, were harvested. Fruits were harvested at different times depending upon the harvest period of the genotype, but all fruits were harvested in the month of August. Four berry samples were collected separately from the two vines of each genotype on two different days (two samples each day and a sample from each vine). Depending on berry size, a sample of at least five berries were combined for each replicate (more berries were required per replicate for small-berry-sized genotypes). For the ripening stage study, fruit samples of the Carlos cultivar were collected on the same day from five separate vines with berries at various stages of ripening. Fruits were collected ranging from green, firm, and unripe to dark bronze, soft, and overripe. Fruits were harvested, brought into the lab, sorted as described below, and processed for GC-MS, as well as a fruit quality study, within 24 h. In addition to the analysis of aroma volatiles, several fruit-quality- and flavor-related traits were measured for the muscadine samples. A sample of 10 berries were separated from the same samples harvested for GC-MS study as a biological replicate; in addition, color, firmness, total soluble solids (TSS), and titratable acidity (TA) were measured.

2.2. Sample Preparation for Quality and Volatile Analysis

Fruits were washed with distilled water and dried with paper towels. Berries were then cut open into halves and the seeds were removed. For aroma volatile analysis, the halves of at least five berries were then collected for each sample in a 50 mL centrifuge tube and homogenized for 20 s using Power Gen 500 (Thermo Fisher Scientific, Waltham, MA, USA). Five grams of the homogenized samples were immediately pipetted into the 20 mL amber glass vials containing 5 g of a saturated salt (NaCl) solution and vortexed for homogenization. The addition of salt to the homogenized sample lowers the partitioning coefficient (K) for some volatiles and thus increases their concentration in the headspace. A total of 10 μ L of 1000 ppm 1-Heptanol (Sigma-Aldrich Co., Saint Louis, MO, USA) was added as an internal standard (IS) in the vials for the relative quantification of volatiles in a homogenized sample. The vials were then stored at -20°C until analysis.

For the ripening stage study, 'Carlos' berries were first density sorted by floating berries in sodium chloride brine solutions of 8%, 9%, 10%, and 11% [27] to determine the four grades of the berries (Stages 1–4) (Figure 1). Berries of increasing ripeness sunk in progressively denser brine. Sorted berries were then immediately rinsed with distilled water and processed as outlined above.

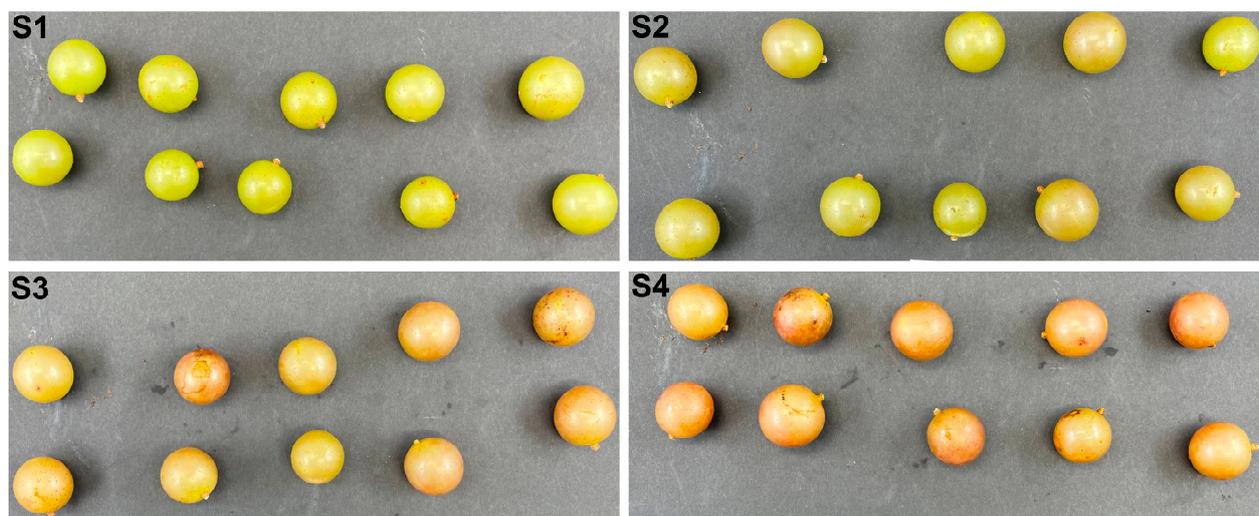


Figure 1. Four ripening stages in 'Carlos' muscadine berries (S1: unripe, S2: slightly ripe, S3: fully ripe, and S4: overripe).

Skin color was measured using a Chroma Meter CR-400 (Konica Minolta Sensing Americas Inc., Wayne, NJ, USA). Five different color components (L*: Lightness, a*: Red/Green value, b*: Blue/Yellow value, C*: Chroma, and h: Hue) were measured. Fruit firmness was measured using a FirmTech 2 Automatic Fruit Firmness tester (BioWorks, Inc., Wamego, KS, USA). Total soluble solids (TSS) and titratable acidity (TA) are commonly used indicators of fruit maturity, ripening, and flavor. For both TA and TSS measurement, the same protocol of homogenization was followed as used for the volatile analysis; in addition, the homogenized muscadine samples in 20 mL tubes were centrifuged at 4000 rpm for 30 min at 4 °C. The solid portion was separated by filtering with the cheesecloth and the supernatant flow, which was collected and stored immediately at −20 °C until analysis. TSS was measured using a digital refractometer (PAL-1; Minato-ku City, Tokyo, Japan). Six grams of a filtered sample was used for TA analysis. A 0.1 N NaOH solution was used as the titrant, and the percent TA was measured using a Mettler Toledo DL15 titrator (Greifensee, Switzerland).

2.3. Sample Incubation and GC-MS Conditions

The homogenized fruit samples were incubated for 30 min at 40 °C with continuous agitation at a speed of 250 rpm. After equilibration, the volatile compounds were collected using a 1 cm SPME-fiber-assembly Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA, USA), and this was achieved by exposing the fiber to the headspace for 30 min under the same temperature. The fibers were activated before sampling according to the instructions of the manufacturer. After the incubation, the SPME fiber was inserted directly into the injection port for desorption (4 min at 250 °C) in a splitless mode. An ultra-inert liner of straight geometry and a 0.75 mm inner diameter (Agilent Technologies Inc., Santa Clara, CA, USA) was used. Aroma volatiles were analyzed using an Agilent 7890A gas chromatography system that was connected with a 5977B mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). The sample preparation was fully automated and carried out by a Gerstel MultiPurpose Sampler (MPS) (GERSTEL GmbH & Co. KG, Mülheim, Germany), which was coupled with GC-MS. Helium gas with a 99.9% purity was used as a carrier gas. A back-inlet purge flow rate was maintained at 3 mL min^{−1}, and a constant gas flow rate of 1.2 mL min^{−1} was utilized through the column. Volatiles were separated using an Agilent HP-5MS (30 m × 250 μm × 0.25 μm) (Agilent Technologies Inc., Santa Clara, CA, USA) column. The GC-MS methodology for this experiment was optimized based on the literature of previous research on muscadine volatile analysis, and it was modified according to the equipment and the needs of the specific cultivars used in this project. The oven temperature was programmed at an initial temperature of 35 °C for 7 min. The oven temperature was increased to 120 °C at 8 °C min^{−1}, held for 5 min, ramped to 150 °C at 4 °C min^{−1}, and then held for 2 min. The post run temperature was set at 280 °C for 5 min before returning to 35 °C. The thermal Aux 2 MSD transfer line, ion source, and quadrupole mass detector temperature values were set to 250 °C, 230 °C, and 150 °C, respectively. The solvent delay time was set to 2 min in order to avoid detection of the unnecessary carbon dioxide peak in the chromatogram. The fragmentation data from a mass spectrometer were collected in scan mode from *m/z* 25 to 300. The mass spectra in the electron impact ionization (ME-EI) mode were recorded at an ionization energy of 70 eV. MS data were analyzed in Agilent MSD software ChemStation F.01.03 (Santa Clara, CA, USA), and volatile compounds were identified by comparing the mass spectral data with the NIST 2.0 reference library (National Institute of Standards and Technology, Gaithersburg, MA, USA). The peak area of volatiles was normalized against the peak area of the internal standard. Furthermore, their relative concentration in a sample (ng/g of fresh fruit) were calculated with respect to the known concentration of the internal standard.

2.4. Statistical Analysis

Statistical analysis was performed in R statistical software, version 4.2.1 [28]. One-way ANOVA (analysis of variance) and Tukey's HSD (honestly significant difference) test

was performed with four biological replicates for germplasm characterization study and five biological replicates for the maturity study. Principal component analysis (PCA) was carried out, and genotype and variable biplots were generated to visualize the difference between muscadine genotypes, as well as to identify the correlated volatile compounds or classes for such variation. Heatmaps were generated for both germplasm and ripeness studies using the 'pheatmap' package in R [29]. Euclidian distance between samples or volatile compounds were calculated and a complete method of clustering was applied to generate heatmaps. Pure *V. rotundifolia* genotypes were divided into black or bronze categories, and a two-sample t-test was performed to identify the volatile compounds that significantly differ between two color classes. To correct the type I error due to multiple testing, a Bonferroni correction was applied to the *p*-values obtained.

3. Results and Discussion

3.1. Role of Berry Maturity on Volatile Composition

Ripening 'Carlos' berries were density sorted into four grades from the least mature (stage 1) to most mature (stage 4). Stage 1 berries were very firm, partially green, and clearly unripe. Stage 4 berries were dark bronze in color, very soft, obviously overripe, and would likely be rejected for fresh fruit sales. Stages 2 and 3 were more similar to each other, with stage 3 berries being darker in color (Figure 1, Table S1). Both stages would likely be considered acceptable for fresh fruit sales, but experienced pickers would recognize stage 3 by its darker color and increased softness; as such, this stage would be targeted for harvest. The berry color darkened, the firmness and titratable acidity decreased, and the sugar content increased with increasing maturity classification (Table S1).

The 'Carlos' berries produced 18 different volatile compounds, 15 of which varied significantly among the four fruit maturity stages (Table S2). A heatmap of the volatile compounds in ripening fruit indicated two distinct clusters: compounds that increase in maturing fruit and compounds that decrease with maturity (Figure 2). A cluster of four volatiles, i.e., Eucalyptol, 2-hexenal, (E)-, Hexanal, and 2,4-hexadienal, (E, E)-, decreased with maturity, especially from stage two to stage three. These volatiles impart fresh mint-like, fresh green, freshly cut grass, unripe, and citrus odors (Table S2). These volatiles were among the most dominant aroma volatiles at ripening stages 1 and 2. Three out of four of the volatiles were from the aldehyde class, and one (Eucalyptol) was a monoterpene. The second cluster of volatiles, which increased with berry maturity, primarily belonged to the ester class. Among the 14 volatile compounds, 10 were from the ester, 1 from the sesquiterpene, 2 from the alcohol, and 1 from the aldehyde chemical class. These volatiles produced floral, fruity, warm, peppery, sweet, rose, and mango-like aromas. The increased abundance of these volatile compounds in ripening stages 3 and 4 marked the onset and progression, respectively, of the berry maturation in the muscadine.

A previous study conducted on ripe berries from five muscadine cultivars has shown similar categories of volatile compounds (ethyl hexanoate, ethyl octanoate, benzene acetaldehyde, 1-octanol, and phenylethyl alcohol) being abundant in 'Carlos' [16]. Similarly, among the 21 positively identified volatile compounds in 'Carlos' juice samples, ethyl acetate, butanoic acid, ethyl ester, hexanal, hexanoic acid, ethyl ester, benzeneacetaldehyde (phenyl acetaldehyde), and 2-phenylethyl alcohol were detected with concentrations ranging from 1.3 to 51 folds higher compared to the detectable aroma threshold (in ppb) [13]. Baek et al. [13] found furaneol to be amongst the most important aromatic compounds in ripe 'Carlos' juice samples by following a liquid-liquid continuous method of extraction (LLCE). However, it was not detected in our study likely due to the difference in extraction methods between the two studies. Future studies can implement extraction methods that allow for the better detection of this unstable and polar aromatic volatile compound [30,31]. A proteome analysis of ripening 'Carlos' berries indicated 55 proteins with a change of ± 1.5 -fold, and these were recognized to be associated with flavor and aroma components [32]. The enzymes associated with terpenes, benzenoids, fatty acid

degradation, and phenylpropanoid pathways were all detected during the ripening of the 'Carlos' berries.

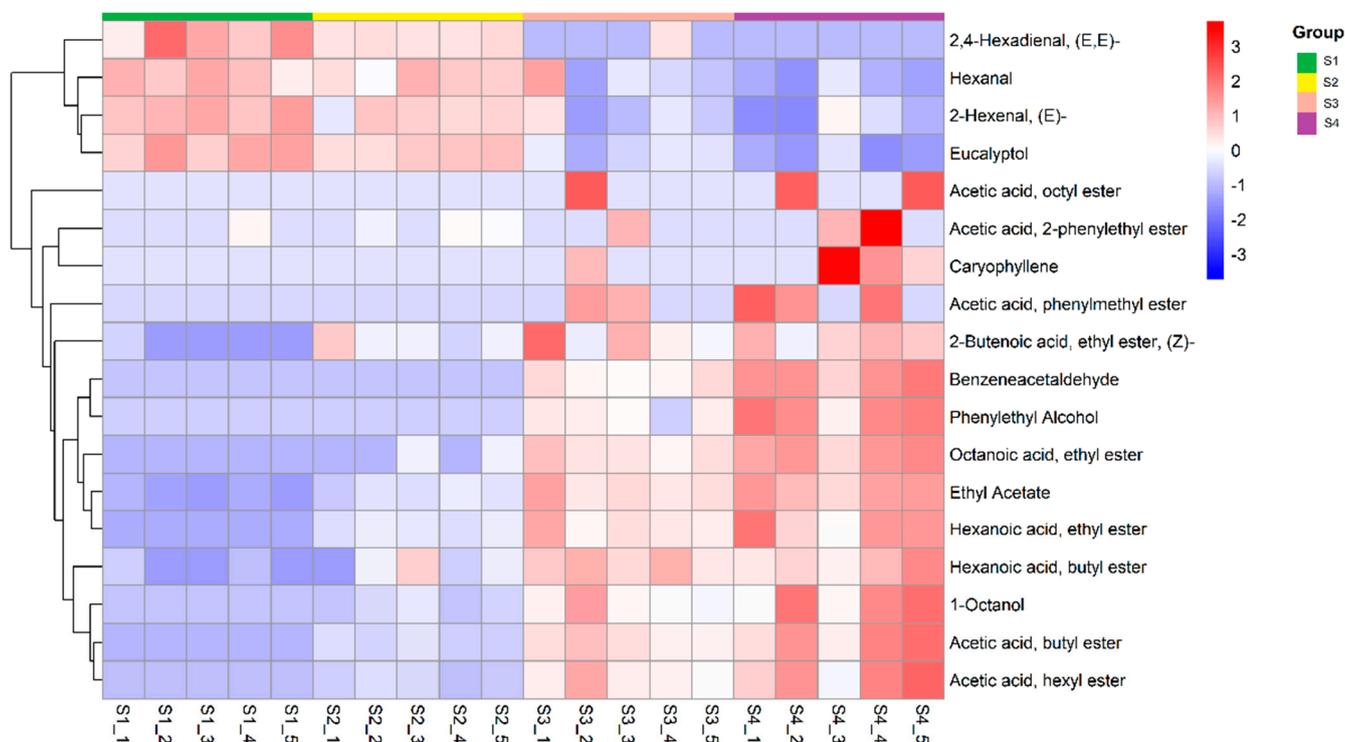


Figure 2. Heatmap showing the cluster of volatile compounds at various ripening stages of the 'Carlos' muscadine cultivar. (S1–S4: the four different stages of increasing berry ripeness).

The volatile profile we obtained in this study can also be compared with the profile obtained in bunch grapes (*V. vinifera*). Gu et al. [17] monitored four red wine grape varieties (*V. vinifera* cvs. Cabernet Sauvignon, Cabernet Gernischtet, Cabernet Franc, and Merlot) near harvest time for their aroma volatile composition and found a very similar pattern of volatile composition during berry ripening. As the ripening progressed, the content of favorable bound aroma compounds such as free alcohols, esters, and terpenes increased, and the content of C-6 aldehydes such as 2-hexenal, (E)-, and hexanal decreased in most cultivars. Similarly, Yang [33] found most esters tended to accumulate during and after maturation, while C-6 volatiles increased until early maturation and then decreased.

3.2. Diversity of Aroma Volatiles in Muscadine Germplasm

In total, 63 aroma-related volatile compounds were detected in 24 different muscadine genotypes. These compounds can be compared to the 45 [34], 60 [35], and 94 [36] volatiles detected in other various groups of the *Vitis* germplasm. The abundance of the 43 volatile compounds were significantly different in at least one cultivar (Table S3). Based on the functional group, these volatile compounds represented seven different chemical classes: aldehyde, alcohol, ester, furan, monoterpene, aromatic hydrocarbon, and sesquiterpene. In the principal component (PC) analysis performed, each of the 43 volatile compounds were considered as a separate variable. Furthermore, the first (PC1) and second (PC2) components explained 23.9% and 12.4% of total variation in the aroma profile due to the effect of the genotypes (Figure 3). The cumulative contribution of the first ten principal components explained 84.4% of the total variation (Figure 4, Table S4), indicating that there is large variation among the genotypes for their content and composition of aroma volatiles, thus resulting in the scattered contribution of several PCs. The clustering of genotypes and volatile compounds clearly shows the variation between genotypes for aroma volatiles (Figure 5). The PCA loading plot indicated that the separation of genotypes in the first

principal component was strongly correlated with the volatile compounds that belong to the aldehyde, furan, ester, and alcohol classes (Figure 6, Table S5). The ester and alcohol class volatile compounds had a positive correlation, while the aldehyde and furan class compounds were negatively correlated with the first principal component. The second PC was positively affected by the monoterpene content of the genotypes. *V. popenoei* DVIT 2970 had both the lowest PC1 and PC2 score, and it was slightly separated from the other genotypes. *V. popenoei* is one of two species of the *Muscadinia* subgenera. It is a native of southern Mexico [37] with a very thick skin, and this accession lacks the typical fruity aroma of *V. rotundifolia*. *V. popenoei*, which has not been used in muscadine breeding to develop new cultivars due to the exception of its appearance in the pedigree of the complex interspecific hybrid cultivar Southern Home [25]. The volatile composition showed that DVIT 2970 had relatively large amounts of C-6 aldehydes [hexanal (47.1%); 2,4-hexadienal, (E, E)- (3.9%); 2-hexenal, (E)- (43.6%)], furan (Furan, 2-pentyl- (0.56%)), as well as low ester and alcohol class volatiles. Another genotype that has a lower PC1 and PC2 score is Fennel's 3-way hybrid, which is the early genotype developed from a complex cross between *V. rotundifolia* var. *rotundifolia*, *V. rotundifolia* var. *munsoniana*, and *V. popenoei*. *V. popenoei* is the immediate parent of Fennel's 3-way hybrid, although DVIT 2970 is not the accession of *V. popenoei* that was used as the parent [38]. Like *V. popenoei*, Fennel's 3-way hybrid had a relatively large content of hexanal (19.4%) and 2-hexenal, (E)- (79%) volatiles. Interestingly, the pure muscadine cultivar Cowart appeared distinct and well separated from the rest of the genotypes in the first PC. 'Cowart' is a fresh market muscadine cultivar with a strong fruity aroma that was released in 1968. It has a relatively high amount of ester and alcohol, as well as low aldehydes class volatiles compared to other genotypes (Figures 3 and 5). 'Lane' was separated on the second PC, and this separation was correlated with its high level of monoterpene-class volatile compounds. The primary monoterpene compounds with a positive correlation with PC2 were 3-carene, D-limonene, beta-pinene, Citronellol, Geranic acid, and Citral. Cultivars Lane, Paulk, and Ruby Crisp share the same pedigree ('Supreme' × 'Tara'). Additionally, breeding selection Ga. 1-6-14 has one parent in common ('Scarlet' × 'Tara'), and breeding selection AM-195 was derived from a cross between selections AM-19 (Supreme × Tara) and AM-1 (open pollination of Tara). These genotypes were clustered close to each other in a PCA plot that indicated that their genetic similarity may also affect their volatile composition. Ga. 18-5 is a complex hybrid that is 43.75% *Euvoitis* (Figure S1), and it has a prominent aroma that differs from most muscadines. Ga. 18-5 has significantly higher concentrations of esters (Table S3), such as ethyl acetate (fruity); butanoic acid and ethyl ester (fruity, juicy, and pineapple); 2-butenic acid, ethyl ester, and (Z)- (fruity, mango-like); 2-hexenoic acid and ethyl ester (pleasant, rum-like, fruity, green, and sweet with a juicy undertone); and heptanoic acid and ethyl ester (fruity and grape-like). The genotypes 'Oh My!', 'Tarheel', 'Golden Isles', 'Noble', 'Carlos', and 'Magnolia' were concentrated mostly in the third quadrant of the PCA plot along with Fennel's 3-way hybrid and DVIT 2970. These genotypes have a proportionately higher concentration of aldehyde, as well as low alcohol, ester, and terpene-class aroma volatiles. 'Tarheel' and 'Noble' are closely related to each other as 'Noble' is a direct descendent of 'Tarheel'. 'Noble', 'Carlos', and 'Magnolia' muscadines are popular wine cultivars and comprise a large proportion of the muscadine wine industry; in addition, 'Golden Isles' was released as a wine cultivar but was never planted on a wide scale [39]. 'Oh My!' is a seedless cultivar developed from a complex hybridization of muscadine with *V. vinifera* [26]. All these cultivars were high in the aldehyde class and low in the ester- and alcohol-class volatile compound concentrations (Table S3).

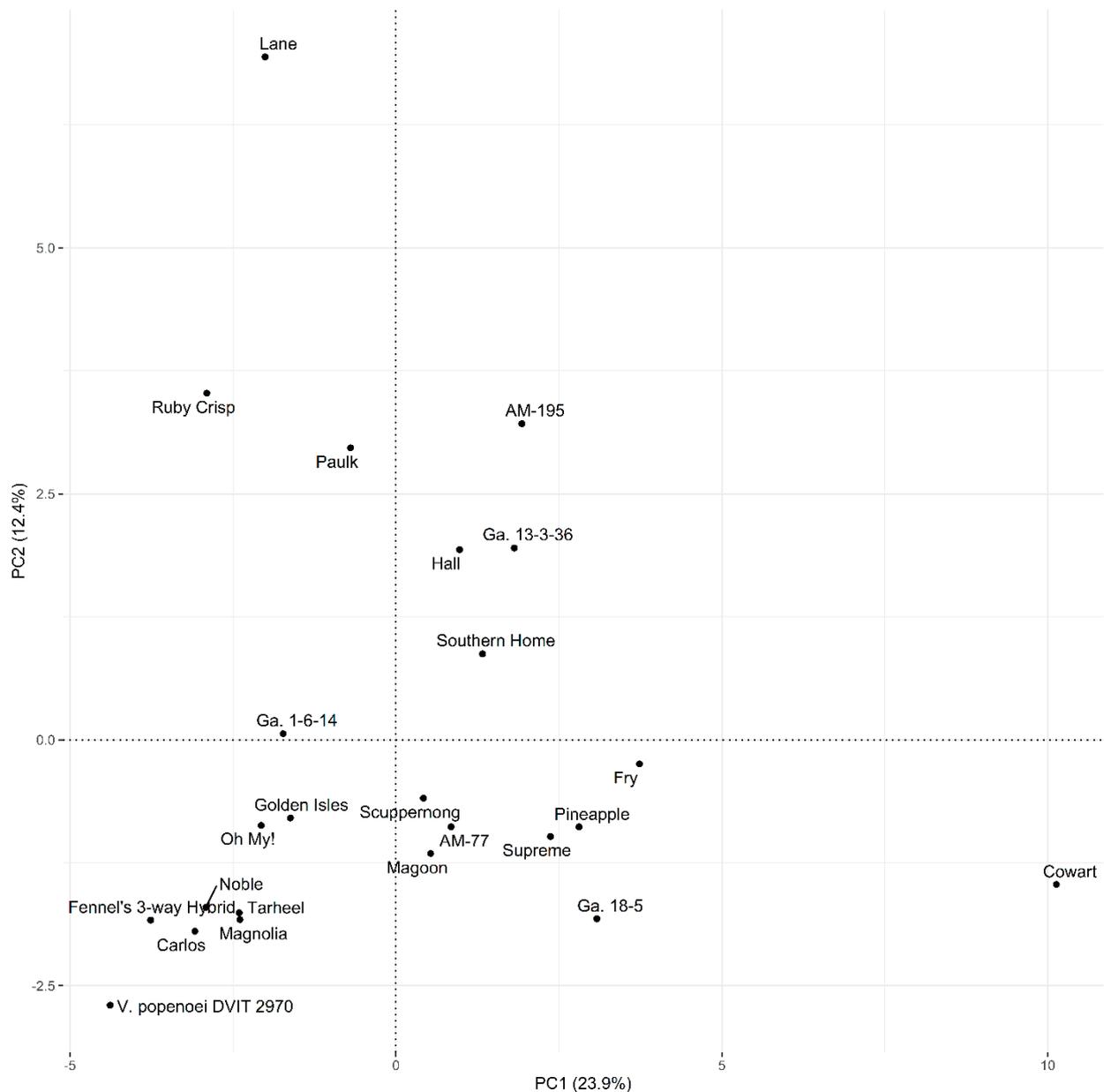


Figure 3. Principal component analysis (PCA) score plot of the aroma volatiles of the twenty-four muscadine genotypes.

To further reduce the dimensionality of the volatile composition data and to better understand how genotypes differ for various chemical classes of aroma volatiles, the concentration of individual volatiles belonging to the same chemical class were grouped together and used to perform the ANOVA and principal component analyses. The muscadine genotypes were significantly different for each chemical class of volatile compounds (Table S6). The PCA results showed a similar genotype distribution pattern to that obtained from the PCA using individual volatile compounds (Figure S2). The first and second PC explained 34.2% and 17.6% of the total variation, respectively (Figure S3). This result is very similar to the PCA results reported by Deng et al. [16], with their PC1 and PC2 explaining 36.7% and 18.9% of the total variation (although they had only five commercial muscadine cultivars included in their analysis). The PCA loading plot showed aldehyde, furan, ester, alcohol, aromatic hydrocarbon, and sesquiterpene as the important classes through which to separate the genotypes in PC1 and the monoterpene class for separation in PC2 (Figure S4).

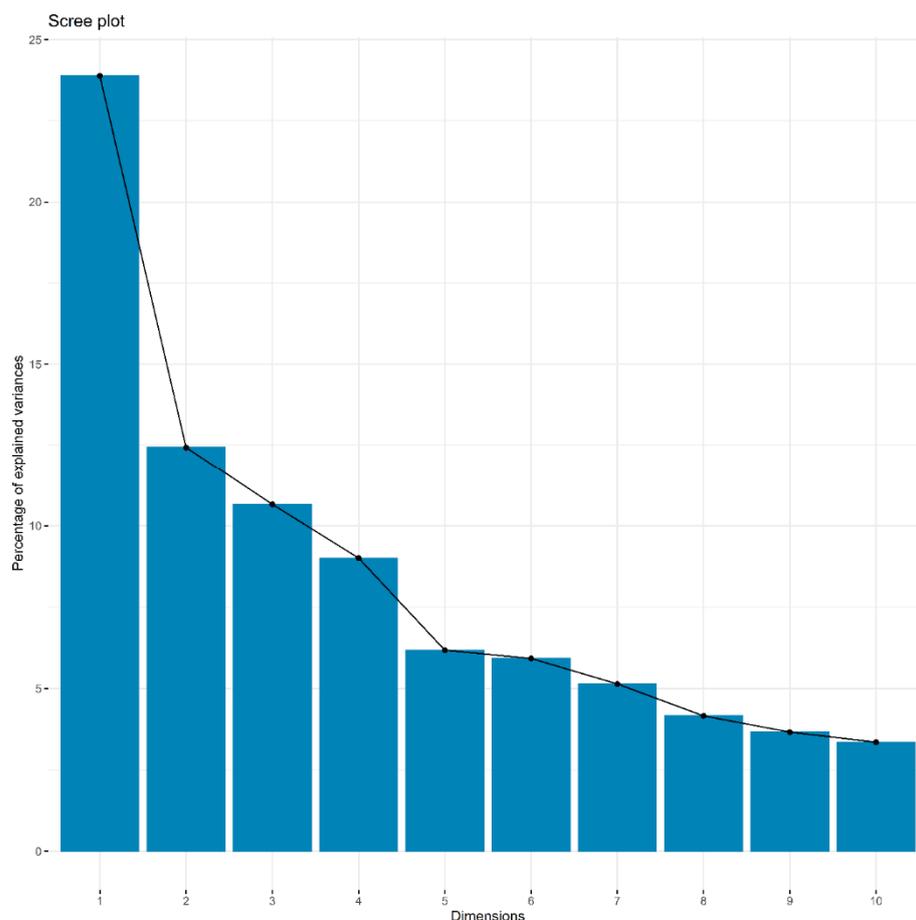


Figure 4. Scree plot showing the percentage of variation in the composition of volatile compounds between genotypes, which was explained by the first ten principal components obtained from PCA.

Aldehydes were the most abundant volatile class in this germplasm, representing about 45.8% of the total volatile abundance (Table S3). Aldehydes have been found to be the predominant volatiles produced in ripe grape berries in studies that investigated a wide variety of grape species [34–36]. Among the aldehydes, the C-6 volatiles hexanal and 2-hexenal, (E)- were the predominant volatiles in this muscadine germplasm, averaging about 45.2% of the volatiles across the samples (Table S3). These compounds produced green aromas and were found across all of the muscadine samples. In an evaluation of berry aroma volatiles in a range of Chinese wild grape species, Rahman et al. [34] identified 45 aroma volatiles, and the C-6 volatiles were the predominant volatiles in most of the grape species studied. Hexenal and 2-hexenal, (E)- are also abundant in *V. vinifera* table grape breeding lines [40].

Esters were the second most abundant volatile class and the most diverse class within the muscadine germplasm studied here, whereby 31 different esters were identified that represented, on average, 44.5% of the total volatile abundance (Table S3). Among the predominant esters, there were the following: ethyl acetate (sweet, grape, and cherry); 2-butenic acid, ethyl ester, and (Z)- (pungent and sour caramellike); and acetic acid and butyl ester (apple, banana, and glue). These represented about 30.1% of the total volatiles. Comparably, Rahman et al. [34] and Liu et al. [36] found esters to be most abundant in *V. labrusca*, where they represented 15.8% to 24.3% of all volatiles; meanwhile, in other grape species, esters were detected in relatively small amounts or not detected at all. The actual abundance of esters measured in *V. labrusca* ranged from 121.4 ng/g [36] to 592 ng/g [35]. The abundance of esters in muscadine germplasm measured here ranged from 13.3 ng/g to 6162.4 ng/g (Table S3). The relatively high abundance and diversity of the esters in

muscadine germplasm with their characteristic fruity and sweet aromas are likely why muscadines are well-known for their fruity aroma [13].

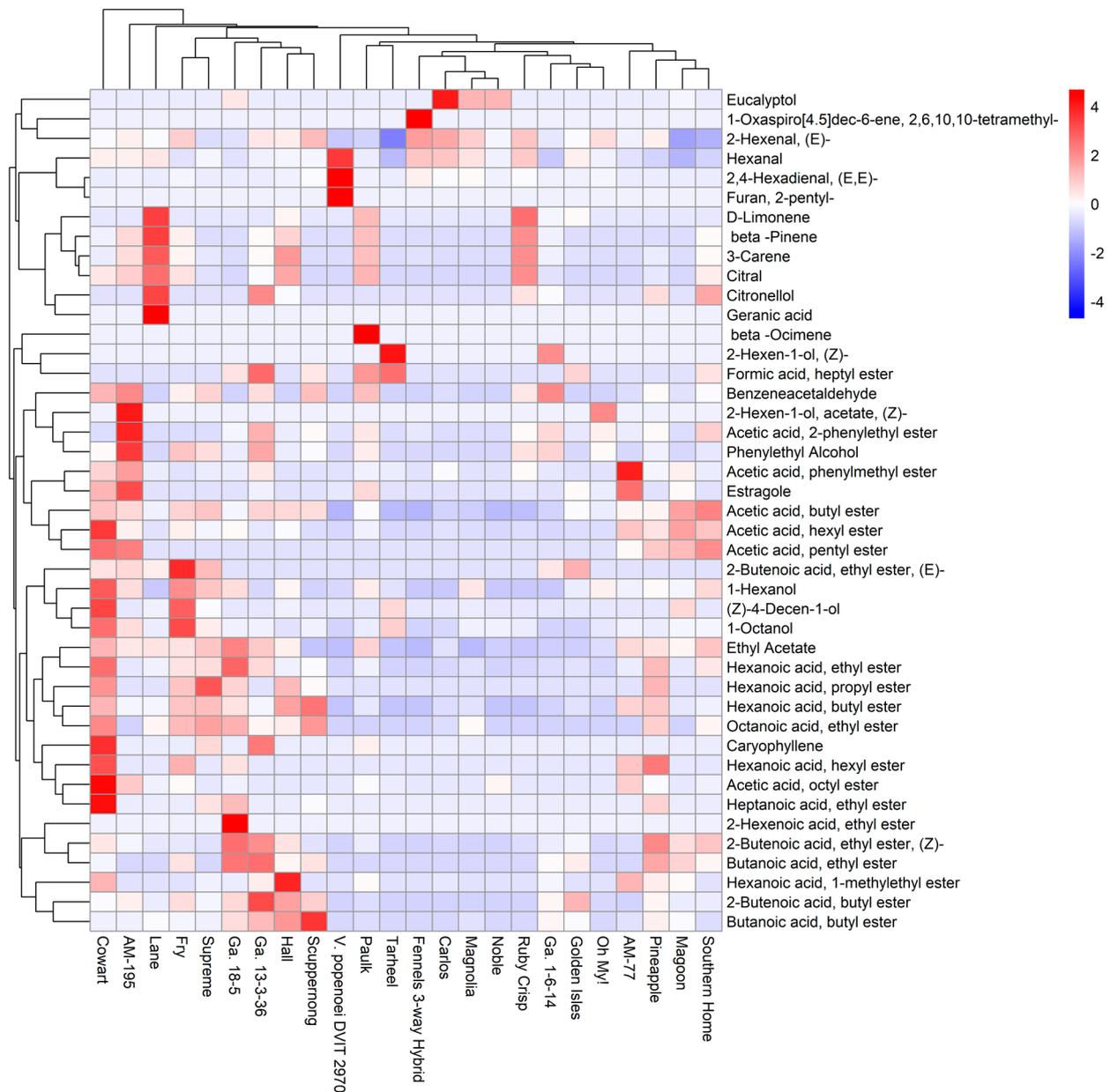


Figure 5. Heatmap generated by the hierarchical clustering of aroma volatile compounds in the 24 muscadine genotypes. Genotypes are presented in columns and aroma volatiles are in rows. The color in each cell represents the relative concentration of the volatile compounds.

Monoterpenes were produced in relatively low abundance in these muscadine accessions, and the predominant monoterpene was C-carene (nutmeg) (Table S3). Amongst the other grape species, monoterpenes are predominantly found in *V. vinifera* accessions. Monoterpenes in ripe *V. vinifera* berries represent 17.4% of all volatiles [36] in terms of the absolute amounts produced. Moreover, 171.5 ng/g is very similar to the average amounts produced in muscadine germplasm (172.4 ng/g), where monoterpenes are only 3.2% of the volatiles produced. This difference in relative abundance highlights the large amount of aldehyde and ester volatiles produced in muscadine in comparison to *V. vinifera*.

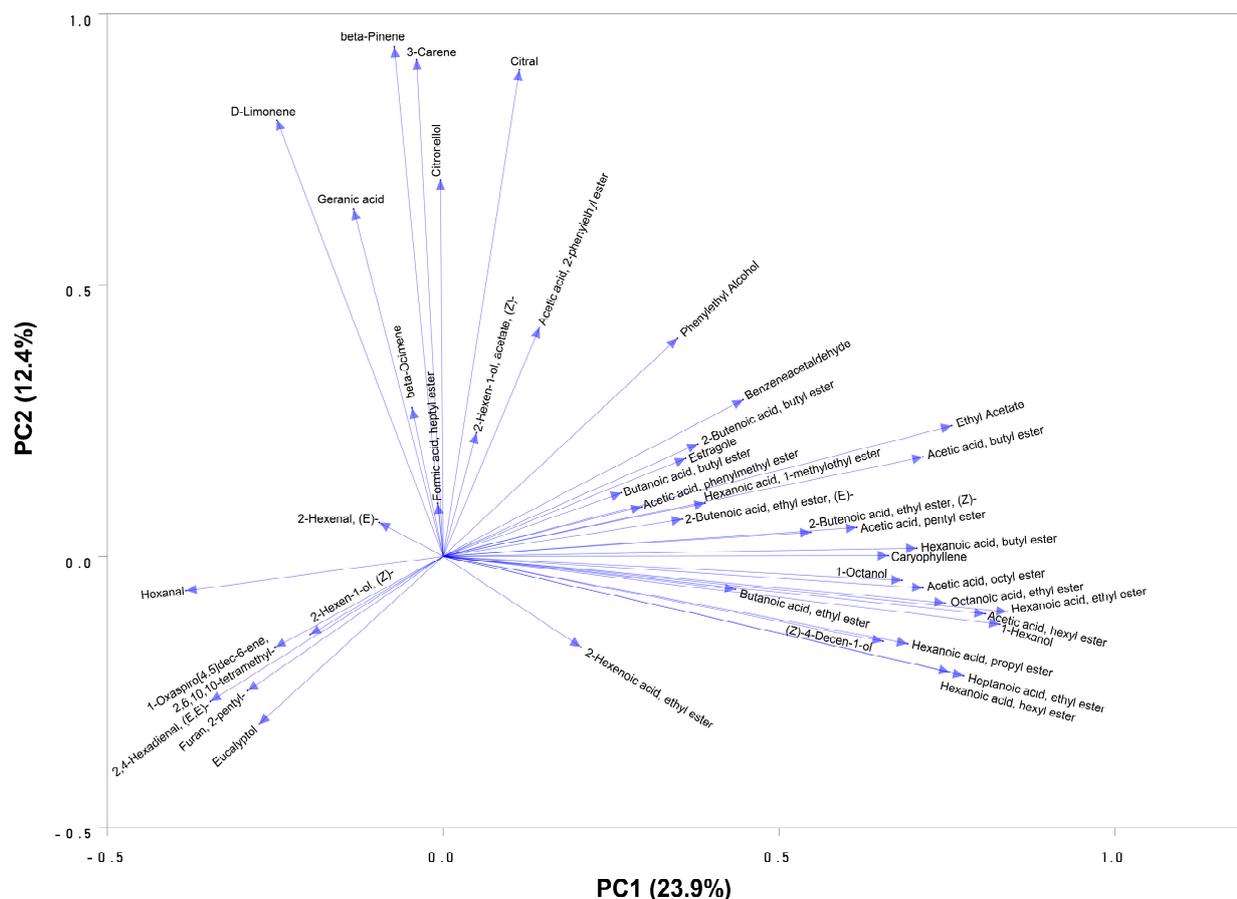


Figure 6. PCA loading biplot showing the contribution of each volatile compounds to the first (PC1) and second (PC2) principal components. Length of arrow represents the amount of contribution made by the volatile compound.

3.3. Differences in the Aroma Volatile Composition between Black and Bronze Muscadines

Berry color in muscadines is genetically controlled with bronze-colored berries resulting from a recessive glutathione S-transferase4 (VrGST4) mutation that leads to a lack of anthocyanin pigmentation in the native selection ‘Scuppernong’ [41,42]. Most muscadine cultivars can be grouped as black or bronze due to their berry color, though there are some cultivars that have red or pink berries. Fresh fruit vineyards typically plant both black and bronze varieties in approximately equal proportions as consumers vary in their preference for color type. We grouped 17 of the pure *V. rotundifolia* muscadine cultivars used in this study into black and bronze categories (Table 1), as well as performed two sample t-tests for all the volatile compounds detected. As expected, the color measurement showed that black and bronze muscadines are significantly different in their lightness scale ($L^*(C)$) (Table S7). We identified six volatile compounds that differed between the black and bronze cultivars (Bonferroni adjusted p -value < 0.05) (Figure 7). Black muscadine cultivars had a significantly higher concentration of ethyl acetate (fruity aroma) and estragole (odor description: sweet, phenolic, anise, harsh, spice, green, herbal, and minty), while two aldehyde-class (2-hexenal, (E)-; 2,4-hexadienal, (E, E)-), and two ester-class (butanoic acid, ethyl ester; 2-butenic acid, butyl ester) volatiles were significantly more abundant in bronze muscadines. Deng et al. [16] performed a partial least-squares discriminate analysis (PLS-DA) that considered black and bronze muscadine color classes as categorical response variables, as well as found a better separation among the five muscadine grape cultivars for their fruit volatile composition. They identified several volatile compounds as potential metabolic markers to distinguish between black and bronze muscadine samples. Ethyl acetate and 2-hexenal, (E)- were identified in both studies, suggesting the existence of in-

herent differences between black and bronze muscadines for aromatic volatile composition, as well as providing a basis for the further exploration of the genetic and biosynthetic pathways involved in the volatile composition of black and bronze muscadine cultivars.

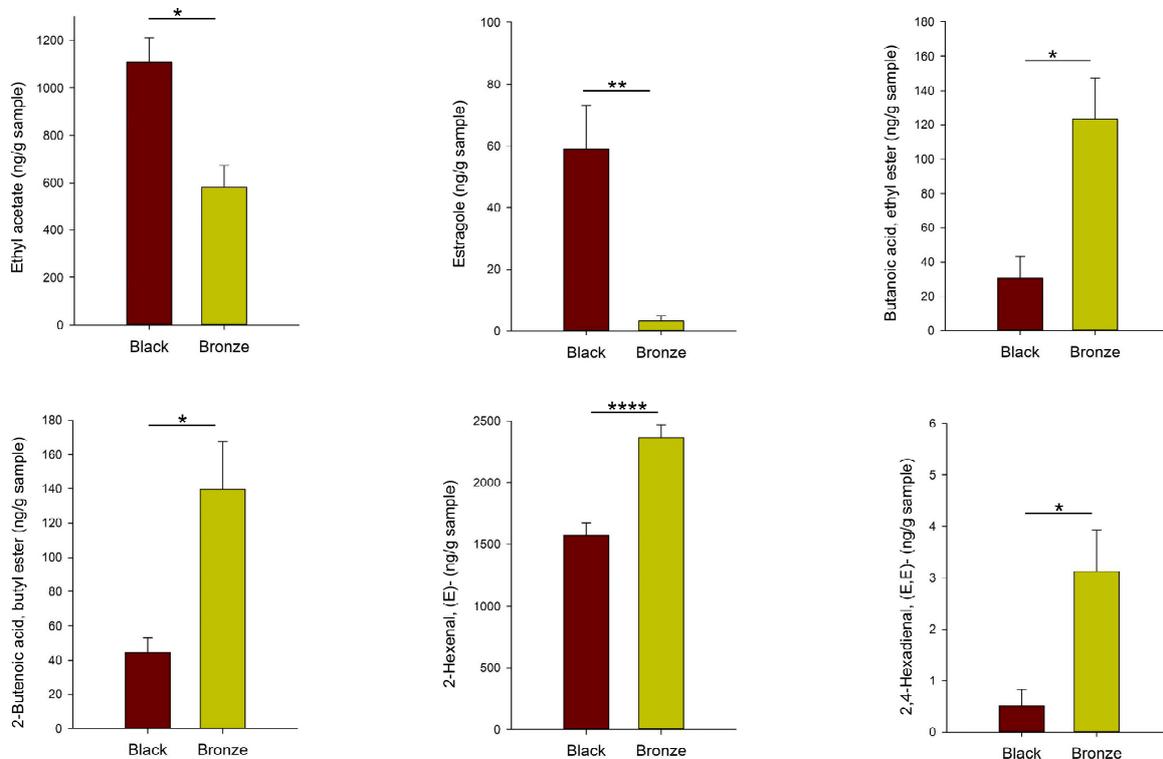


Figure 7. The significantly different (adjusted p -value < 0.05) aroma volatiles between the black ($n = 9$) and bronze ($n = 8$) muscadines obtained from the two sample t -tests. * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$.

4. Conclusions

Significant variation was detected in the muscadine germplasm for the composition of aroma-related volatiles. Aldehyde, ester, alcohol, and primary monoterpene aroma volatiles were crucial in distinguishing the muscadine genotypes. Aldehydes and furan-class volatiles were more abundant in early muscadine selections, wild genotypes, and juice cultivars, while fresh-market cultivars were mainly characterized by their relatively large quantity of esters, alcohols, and primary monoterpenes. A ripening study revealed a distinctive pattern of aroma volatile composition in the muscadine berries of the cultivar Carlos during fruit ripening. Similar to other studies on fruits and vegetables, we successfully implemented the SPME method of aroma volatile extraction in this study. However, this method might not be ideal for capturing important volatiles, like furaneol, in muscadine grapes. In the future, researchers can explore and incorporate different extraction methods to ensure the successful extraction and detection of furaneol. The results from this study provide an important basis for the utilization of muscadine germplasm in muscadine breeding programs for the selection of parents and the development of cultivars with desired flavor profiles. Similarly, consumer preference studies can be carried out and combined with GC/MS analysis to identify the specific volatiles that contribute to the desired or unwanted flavor in muscadines. Additionally, the pattern of aroma-volatile composition in ripening berries offers valuable information for the development and implementation of metabolic markers to ensure the ripening quality of muscadines.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae9091054/s1>, Supplementary information are provided in two separate files: supplementary file.xlsx and supplementary figures.docx. Figure S1: Pedigree of

the muscadine selection Ga. 18-5 showing its development via complex hybridization steps. Figure S2: PCA plot of the twenty-four muscadine genotypes obtained using the total amount of volatile compounds in each chemical class as variables. Figure S3: Scree plot showing percentage of variation in the composition of volatile compound class between the genotypes explained by the first seven principal components obtained from PCA. Figure S4: PCA loading biplot showing the contribution of each volatile compounds class to the first (PC1) and second (PC2) principal components. Length of arrow represents the amount of contribution made by the volatile compound class. Table S1: Color, firmness, titratable acidity (TA), and total soluble solid (TSS) contents in the ‘Carlos’ berries at various stages of maturity. Table S2: Volatile concentration (ng/g of sample) at various stages of berry ripening in the ‘Carlos’ muscadine cultivar. Table S3: Volatile composition (ng/g of sample) of the muscadine genotypes. Table S4: PC scores of the muscadine genotypes with each volatile compound as a separate variable. Table S5: PCA loadings showing the contribution of each volatile compound to the different principal components. Table S6: Abundance of volatile compounds (ng/g of sample) of the various chemical classes in muscadine genotypes. Table S7: Color, firmness, titratable acidity (TA), and total soluble solid (TSS) contents of muscadine genotypes.

Author Contributions: Conceptualization, P.J.C.; Methodology, P.J.C., A.D., G.B. and O.G.; Software, G.B., O.G. and R.N.C.; Validation, G.B. and A.D.; Formal Analysis, G.B.; Investigation, G.B., O.G., R.N.C. and C.E.E.M.; Resources, P.J.C. and A.D.; Data Curation, G.B.; Writing—Original Draft Preparation, G.B., P.J.C. and O.G.; Writing—Review and Editing, G.B., P.J.C., O.G., A.D. and M.L.W.; Visualization, G.B.; Supervision, P.J.C. and A.D.; Project Administration, P.J.C.; Funding Acquisition, P.J.C., M.L.W., A.D. and G.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Southern Region Small Fruit Consortium (Project ID: 2022 R-10) and by the funding received from the John Ingle Innovation in Plant Breeding award by Gaurab Bhattarai from the University of Georgia, Research Foundation, Inc.

Data Availability Statement: The data presented in this study are available in supplementary file.xlsx and supplementary figures.docx.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Hedrick, U.P.; Booth, N.O.; Taylor, O.M.; Wellington, R.; Dorsey, M.J. *The Grapes of New York*; 15th Annual Report; State of New York, Department of Agriculture: New York, NY, USA, 1908; Volume 3, Part II.
- Bailey, L.H. *The Standard Cyclopedia of Horticulture*; Macmillan: New York, NY, USA, 1908.
- Moore, M.O. Classification and systematics of eastern North American *Vitis L. (Vitaceae)* north of Mexico. *SIDA Contrib. Bot.* **1991**, *14*, 339–367.
- Radford, A.E.; Ahles, H.E.; Bell, C.R. *Manual of the Vascular Flora of the Carolinas*; University of North Carolina Press: Chapel Hill, NC, USA, 2010.
- Wen, J. Vitaceae. In *The Families and Genera of Vascular Plants*; Kubitz, K., Ed.; Springer: Berlin/Heidelberg, Germany, 2007; pp. 467–479.
- Hoffmann, M.; Conner, P.; Brannen, P.; Burrack, H.; Mitchem, W.; Cline, B.; Perkins-Veazie, P.; Poling, B. Muscadine Grape Production Guide for the Southeast. North Carolina State Ext. AG-94. 29 January 2021. Available online: <https://smallfruits.org/files/2020/07/muscadine-grape-production-guide-southeast.pdf> (accessed on 5 October 2022).
- Hickey, C.C.; Smith, E.D.; Cao, S.; Conner, P. Muscadine (*Vitis rotundifolia* Michx., syn. *Muscandinia rotundifolia* (Michx.) small): The resilient, native grape of the southeastern US. *Agriculture* **2019**, *9*, 131. [[CrossRef](#)]
- Wang, S.Y.; Lin, H.S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140–146. [[CrossRef](#)] [[PubMed](#)]
- Pastrana-Bonilla, E.; Akoh, C.C.; Sellappan, S.; Krewer, G. Phenolic content and antioxidant capacity of muscadine grapes. *J. Agric. Food Chem.* **2003**, *51*, 5497–5503. [[CrossRef](#)] [[PubMed](#)]
- Bartoshuk, L.M.; Klee, H.J. Better fruits and vegetables through sensory analysis. *Curr. Biol.* **2013**, *23*, R374–R378. [[CrossRef](#)]
- Colantonio, V.; Ferrão, L.F.V.; Tieman, D.M.; Bliznyuk, N.; Sims, C.; Klee, H.J.; Munoz, P.; Resende, M.F., Jr. Metabolomic selection for enhanced fruit flavor. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2115865119. [[CrossRef](#)]
- Rodríguez, A.; Alquézar, B.; Peña, L. Fruit aromas in mature fleshy fruits as signals of readiness for predation and seed dispersal. *New Phytol.* **2013**, *197*, 36–48. [[CrossRef](#)]
- 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*, 249–252. [[CrossRef](#)]

14. Lee, B.; Lin, P.C.; Cha, H.S.; Luo, J.; Chen, F. Characterization of volatile compounds in Cowart muscadine grape (*Vitis rotundifolia*) during ripening stages using GC-MS combined with principal component analysis. *Food Sci. Biotechnol.* **2016**, *25*, 1319–1326. [[CrossRef](#)]
15. Lamikanra, O. Aroma constituents of muscadine wines1. *J. Food Qual.* **1987**, *10*, 57–66. [[CrossRef](#)]
16. Deng, H.; He, R.; Long, M.; Li, Y.; Zheng, Y.; Lin, L.; Dong, L.; Zhang, X.; Liao, M.; Lv, X.; et al. Comparison of the Fruit Volatile Profiles of Five Muscadine Grape Cultivars (*Vitis rotundifolia* Michx.) using HS-SPME-GC/MS Combined with Multivariate Statistical Analysis. *Front. Plant Sci.* **2021**, *12*, 2302. [[CrossRef](#)] [[PubMed](#)]
17. Gu, X.; Zhang, X.; Wang, K.; Lv, X.; Li, R.; Ma, W. GC-MS Untargeted Analysis of Volatile Compounds in Four Red Grape Varieties (*Vitis vinifera* L. cv) at Different Maturity Stages near Harvest. *Foods* **2022**, *11*, 2804. [[CrossRef](#)] [[PubMed](#)]
18. Yang, Y.N.; Zheng, F.P.; Yu, A.N.; Sun, B.G. Changes of the free and bound volatile compounds in *Rubus corchorifolius* L. f. fruit during ripening. *Food Chem.* **2019**, *287*, 232–240. [[CrossRef](#)] [[PubMed](#)]
19. Zhu, X.; Li, Q.; Li, J.; Luo, J.; Chen, W.; Li, X. Comparative study of volatile compounds in the fruit of two banana cultivars at different ripening stages. *Molecules* **2018**, *23*, 2456. [[CrossRef](#)] [[PubMed](#)]
20. Corpas Iguarán, E.; Taborda Ocampo, G.; Tapasco Alzate, O. Identification of volatile compound markers during the ripening and senescence of lulo (*Solanum quitoense* Lam.). *J. Food Sci. Technol.* **2018**, *55*, 437–442. [[CrossRef](#)] [[PubMed](#)]
21. Zidi, K.; Kati, D.E.; Bachir-Bey, M.; Genva, M.; Fauconnier, M.L. Comparative study of fig volatile compounds using headspace solid-phase microextraction-gas chromatography/mass spectrometry: Effects of cultivars and ripening stages. *Front. Plant Sci.* **2021**, *12*, 1312. [[CrossRef](#)]
22. Senesi, E.; Di Cesare, L.F.; Prinzevalli, C.; Scalzo, R.L. Influence of ripening stage on volatiles composition, physicochemical indexes and sensory evaluation in two varieties of muskmelon (*Cucumis melo* L. var *reticulatus* Naud). *J. Sci. Food Agric.* **2005**, *85*, 1241–1251. [[CrossRef](#)]
23. Poling, E.B.; Mainland, C.M.; Bland, W.T.; Cline, B.; Sorensen, K.A. *Muscadine Grape Production Guide for North Carolina; AG-94; NC State Extension: Raleigh, NC, USA, 2003; Available online: <https://content.ces.ncsu.edu/muscadine-grape-production-guide> (accessed on 2 March 2023).*
24. Conner, P.J.; Worthington, M.L. Muscadine Grape Breeding. In *Plant Breeding Reviews*; Goldman, I., Ed.; John Wiley & Sons: Hoboken, NJ, USA, 2022. [[CrossRef](#)]
25. Mortensen, J.A.; Harris, J.W.; Hopkins, D.L.; Andersen, P.C. ‘Southern Home’: An interspecific hybrid grape with ornamental value. *HortScience* **1994**, *29*, 1371–1372. [[CrossRef](#)]
26. Bloodworth, P.J. Grapevine Plant Named ‘JB06-43-6-22’. US Plant Patent 31,010, 11 February 2019.
27. Lanier, M.R.; Morris, J.R. Evaluation of Density Separation for Defining Fruit Maturities and Maturation Rates of Once-over Harvested Muscadine Grapes. *J. Am. Soc. Hortic. Sci.* **1979**, *104*, 249–252. [[CrossRef](#)]
28. R Core Team, R. A Language and Environment for Statistical Computing. Available online: <https://www.r-project.org/> (accessed on 11 February 2022).
29. Kolde, R. Pheatmap: Pretty Heatmaps. R Package Version 1.0.12. 2019. Available online: <https://CRAN.R-project.org/package=pheatmap> (accessed on 11 February 2022).
30. Chen, Y.; Sidisky, L.M. Quantification of 4-hydroxy-2, 5-dimethyl-3-furanone in fruit samples using solid phase microextraction coupled with gas chromatography–mass spectrometry. *J. Chromatogr. A* **2011**, *1218*, 6817–6822. [[CrossRef](#)]
31. Dutra, M.D.C.P.; de Souza, J.F.; Viana, A.C.; de Oliveira, D.; Pereira, G.E.; dos Santos Lima, M. Rapid determination of the aromatic compounds methyl-anthranilate, 2'-aminoacetophenone and furanone by GC-MS: Method validation and characterization of grape derivatives. *Food Res. Int.* **2018**, *107*, 613–618. [[CrossRef](#)] [[PubMed](#)]
32. Kambiranda, D.; Basha, S.M.; Singh, R.K.; He, H.; Calvin, K.; Mercer, R. In depth proteome analysis of ripening muscadine grape berry cv. Carlos reveals proteins associated with flavor and aroma compounds. *J. Proteome Res.* **2016**, *15*, 2910–2923. [[CrossRef](#)] [[PubMed](#)]
33. Yang, C.; Wang, Y.; Wu, B.; Fang, S.; Li, S. Volatile evolution of three table grapes with different flavour during and after maturation. *Food Chem.* **2011**, *128*, 823–830. [[CrossRef](#)]
34. Rahman, F.U.; Nawaz, M.A.; Liu, R.; Sun, L.; Jiang, J.; Fan, X.; Liu, C.; Zhang, Y. Evaluation of volatile aroma compounds from Chinese wild grape berries by headspace-SPME with GC-MS. *Food Sci. Technol.* **2022**, *42*, e54320. [[CrossRef](#)]
35. Yang, C.; Wang, Y.; Liang, Z.; Fan, P.; Wu, B.; Yang, L.; Wang, Y.; Li, S. Volatiles of grape berries evaluated at the germplasm level by headspace-SPME with GC-MS. *Food Chem.* **2009**, *114*, 1106–1114. [[CrossRef](#)]
36. Liu, X.; Fan, P.; Jiang, J.; Gao, Y.; Liu, C.; Li, S.; Liang, Z. Evolution of volatile compounds composition during grape berry development at the germplasm level. *Sci. Hort.* **2022**, *293*, 110669. [[CrossRef](#)]
37. Fennell, J.L. Two new North American species of *Vitis*. *J. Wash. Acad. Sci.* **1940**, *30*, 15–19. Available online: <https://www.jstor.org/stable/24530082> (accessed on 11 February 2022).
38. Cao, S.; Stringer, S.; Gunawan, G.; McGregor, C.; Conner, P.J. Genetic diversity and pedigree analysis of muscadine grape using SSR markers. *J. Am. Soc. Hortic. Sci.* **2020**, *145*, 143–151. [[CrossRef](#)]
39. Basiouny, F.M.; Himelrick, D.G. *Muscadine Grapes*; ASHS Press: Alexandria, VA, USA, 2001.
40. Maoz, I.; Kaplunov, T.; Raban, E.; Dynkin, I.; Degani, O.; Lewinsohn, E.; Lichter, A. Insights into the chemosensory basis of flavor in table grapes. *J. Sci. Food Agric.* **2020**, *100*, 1405–1417. [[CrossRef](#)]

41. Ismail, A.; Gajjar, P.; Park, M.; Mahboob, A.; Tsoleva, V.; Subramanian, J.; Darwish, A.G.; El-Sharkawy, I. A recessive mutation in muscadine grapes causes berry color-loss without influencing anthocyanin pathway. *Commun. Biol.* **2022**, *5*, 1012. [[CrossRef](#)]
42. Varanasi, A.; Worthington, M.; Nelson, L.; Brown, A.; Chizk, T.M.; Threlfall, R.; Howard, L.; Conner, P.; Figueroa-Balderas, R.; Massonnet, M.; et al. Glutathione S-transferase: A candidate gene for berry color in muscadine grapes (*Vitis rotundifolia*). *G3 Genes | Genomes | Genet.* **2022**, *12*, jkac060. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.