Progress Report 2022 R-10

Title: Evaluation of aroma volatiles in muscadine cultivars and breeding selections

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Objectives:

1. **Determine the effect of vineyard location on muscadine aroma volatiles.**
2. **Determine the effect of fruit ripeness on muscadine aroma volatiles.**
3. **Examine a wide array of muscadine breeding germplasm for variation in aroma volatiles.**

Justification and Description:

Desired muscadine berry quality parameters vary by intended use, fresh-market or juice. Factors influencing quality can broadly be divided into those influencing taste and aroma including sugar and acid content and their ratios and aroma volatiles, and those influencing texture which includes skin thickness and tenderness, flesh firmness, adherence of flesh to the skin, and seed size and number. Cultivated and wild grape species display an enormous diversity of aroma producing volatiles which are capable of producing an array of flavors (Lin et al, 2019). The predominant compounds contributing to the aroma profile of grape berries are mono- and sesquiterpenes, methoxypyrazines, furan derivatives, lipoxygenase pathway products, and phenyl-propanoid pathway products. Attempts to use non-*vinifera* species as germplasm in breeding interspecific wine grapes, or as wine grapes themselves, has been complicated by the presence of negatively perceived flavors (Lin et al., 2019).

Muscadine grapes are known for having pronounced aromas variously described as “fruity”, “foxy”, or “candy-like”. These aromas can vary substantially from *Vitis vinifera*, perhaps due to muscadine evolving for dissemination by mammals rather than birds. Muscadine aroma is generally seen as desirable, especially for those familiar with muscadines and consuming it as a fresh fruit. However, these aromas can be overwhelming to those not used to them, and they sometimes impart undesirable attributes to processed products like wine. Variation for aroma has long been recognized in muscadine germplasm, with one juice cultivar, ‘Golden Isles’, released specifically for its less pronounced muscadine aroma (Lane and Bates, 1987). Unfortunately, a tendency for ‘Golden Isles’ to overcrop, and the disease susceptibility of the cultivar, prevented it from being widely grown. However, this release does indicate that useful variability for aroma volatiles exists in muscadine breeding germplasm.

Despite their importance, and both positive and negative attributes, muscadine aroma volatiles have not been extensively studied. An examination of 'Welder' and 'Noble' muscadine wine (Lamikanra, 1987) found that higher alcohols and esters of fatty acids were the largest group of flavor components, with some differences in concentrations found between the two cultivars. More recent analysis found 42 aroma-active volatiles in 'Noble' muscadine juice (Gurbuz et al., 2018). Sensory panelists found aroma attributes of whole muscadine berries to be relatively low in intensity, but did detect differences in grape/overall, grape/muscadine and fruity aroma attributes (Felts et al., 2018). Total sugars in berries was positively correlated with several aroma attributes, suggesting berry ripeness strongly affects aroma.

Gas chromatography/mass spectrometry identified 33 aroma-active in 'Carlos' muscadine grape juice. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furaneol) was the most intense aroma compound (Baek et al., 1997). Furaneol and o-aminoacetophenone were thought to give the candy and foxy-like aromas notes to muscadine juice. These compounds are present in both free and glycosidically bound forms (Baek et al., 1999). Furaneol has a pineapple- or strawberry-like aroma at low concentrations, but exhibits a caramel-like aroma at higher concentrations. Furaneol has also been found in *V. labrusca*, but is not found *V. vinifera* and is undesirable in some wines at high concentrations. Other predominant compounds and their aromas were 2,3-butanedione (buttery/cream cheese), ethyl butanoate (bubble gum/fruity), ethyl 2-methylbutanoate (green apple/fruity), 2-phenylethanol (rosy). Lee et al. (2016) found that ripe 'Cowart' muscadine grapes produced volatile esters which are associated with fruity, floral and pleasant odors. The most abundant VOC detected was ethyl acetate, which was identified by Bake et al. (1997) as producing an ester aroma.

Analysis of aroma compounds is complicated by sample preparation leading to loss of some volatile compounds, environmental and temporal effects, and genotype variation. However, given the importance of aroma in differentiating muscadine from most other grape products, further exploration of this characteristic is warranted. Studies should focus on differences between cultivars with pronounced and mild muscadine aromas and aromas that are desirable in fresh and processed products. This study is aimed at sampling an array of muscadine germplasm important in both fresh and processed muscadine products. Most of these genotypes are known by breeders to have pronounced differences in aroma, but have not been studied analytically. In addition, we seek to establish how much variation is introduced by berry ripeness and vineyard location to better understand how broadly our results can be interpreted.

Experimental Plan:

Plant material. The majority of the plant material will be obtained from the University of Georgia (UGA) breeding program experimental vineyards. These vineyards receive commercial level care which includes fertilization, irrigation, and fungicide applications. However, for a few experiments as noted below, fruit may be obtained from Paulk Vineyards located in Wray, GA and from University of Arkansas Fruit Research Station, Clarksville, AR. Fruit will be harvested at optimal commercial ripeness (fully colored, some softness, and a soluble solids of at least 14) and free from defects. A sample of 10 berries will be combined for each replicate and three replicates will be examined for each genotype.

Sample analysis. Fruit will be washed with a commercial detergent and dried with paper towels. Berries will then be squeezed to extract juices for analysis. Juice samples will be stored at -80 °C until analysis. For headspace sampling and gas chromatography-mass spectrometry (GC-MS) analysis, samples will be incubated for 30 min at 40 °C. A 2-cm solid phase microextraction (SPME) fiber will be exposed to the headspace for 30 min at 40 °C. After exposure the SPME fiber will be inserted into the injector of a GC-MS for detection and quantification of aroma volatiles. Volatile compounds will be identified by comparison of their mass spectra with authentic standards and/or library entries.

Experiment 1. Effect of vineyard location on volatile profile.

Two cultivars, the popular juice cultivars 'Noble' and 'Carlos' will be examined from three locations: Tifton, GA, Wray, GA, and Clarksville AR. Samples from Tifton and Wray will be collected on the same day, while samples from Clarksville will be collected at a later date (muscadine ripening is several weeks later in AR) and immediately shipped to Tifton for analysis.

Experiment 2. Effect of berry ripeness on volatile profile.

'Carlos' berries of various maturity will be collected. Berries will then be density sorted by floating berries in sodium chloride brine solutions of 8%, 9%, 10% and 11% (Lanier and Morris, 1979) to give 4 grades of berries. Berries of increasing ripeness will sink in progressively denser brine. Once berries are sorted, they will be juiced and processed as outlined above to examine aroma profile variation due to ripeness.

Experiment 3. Evaluation of muscadine germplasm for aroma volatile variation.

Little systematic evaluation of muscadine germplasm has been carried out for aroma profile, but breeders know from experience that much variation exists in the germplasm. This knowledge will be used to select a range of cultivars for testing.

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Note: Sample are still being analyzed and verified. Results presented below are preliminary and may be revised as more data is obtained.

Experiment 1. Effect of vineyard location on volatile profile.

Samples were obtained of two cultivars, 'Carlos' and 'Noble' from three vineyards; Still Pond Vineyard near Arlington, GA; Paulk Vineyard near Wray, GA; and Ison's Vineyard near Brooks, GA (Fig. 1). Samples were taken from 5 vines of each of the two cultivars. Samples were then graded to a similar ripening stage using salt solution, ground and frozen. GC analysis of these samples have not yet been performed.

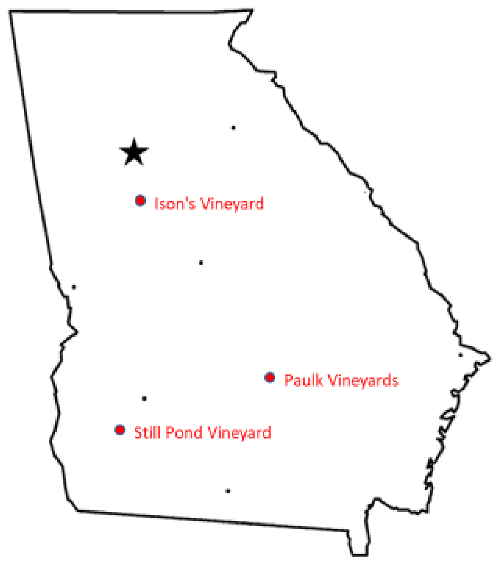


Fig. 1. Vineyard locations.

Experiment 2. Effect of berry ripeness on volatile profile.

Carlos berries of various maturities were harvested from Paulk Vineyards. Berry samples were collected from 5 replicate vines in the same vineyard row. Samples were immediately brought back to the lab and density sorted to provide four different maturity grades from unripe (S1) to overripe (S4) (Lanier and Morris, 1979). Samples were then ground and frozen. Several aroma volatiles were found to vary dramatically via ripeness (Fig. 2), with stages 1 and 2 generally varying from stages 3 and 4. These results emphasize the importance of sampling ripe berries for analysis.

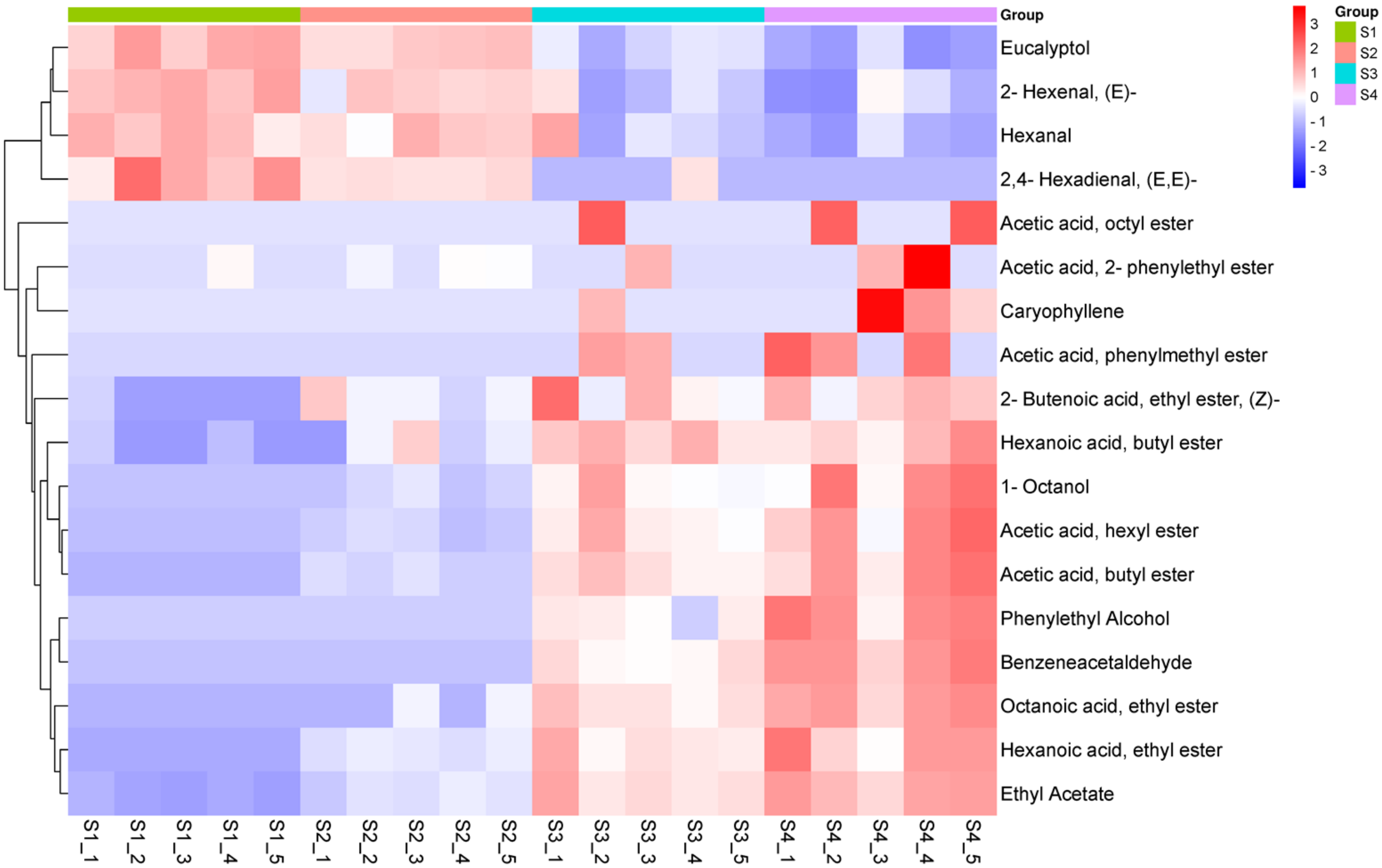


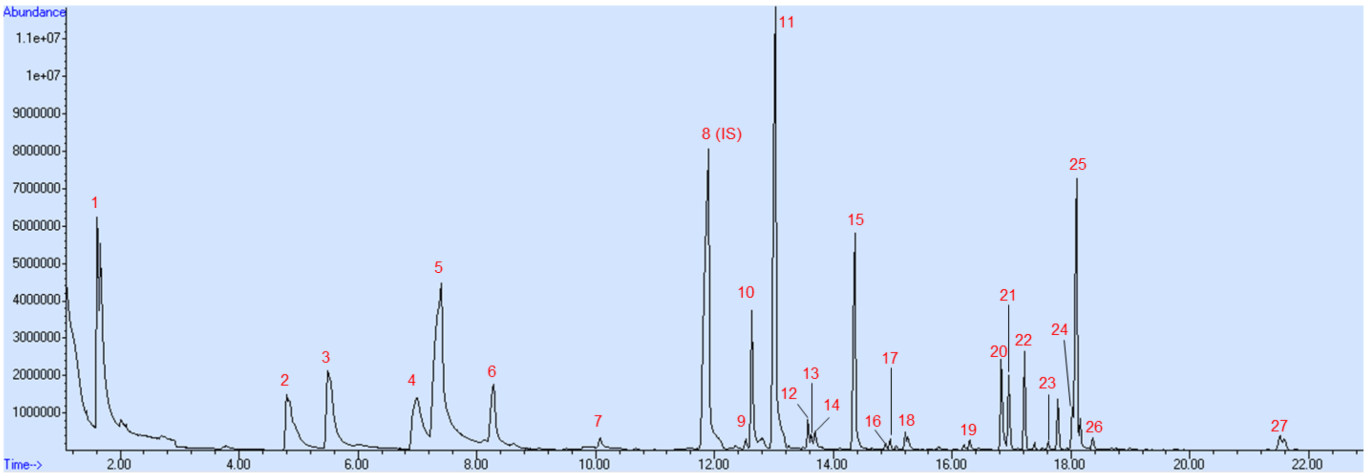
Fig. 2. Heatmap showing variation of 18 aroma volatiles in different ripening stages of 'Carlos' muscadine. Stage 1 is unripe and stage 4 is overripe.

Experiment 3. Evaluation of muscadine germplasm for aroma volatile variation. Samples were primarily collected from the UGA-Tifton Campus, but a few samples were also obtained from Paulk Vineyards and Ison's Vineyards. Cultivars and selections tested are listed in Table 3 below.

Table 3. Muscadine accessions used for aroma volatile analysis.

Genotype	**Value**
V. popenoei DVIT 2970	Related species
V. munsoniana 'Marsh'	Related species
Fennel's 3-way Hybrid	Related species hybrid
Southern Home	Wide hybrid with bunch grape ancestry
Oh My!	Seedless wide hybrid
Supreme	Popular fresh market cultivar
Fry	Popular fresh market cultivar
Cowart	Older fresh market cultivar, strong aroma
Magoon	Older cultivar, very pronounced aroma
Paulk	New fresh market cultivar
Hall	New fresh market cultivar
Lane	New fresh market cultivar, grape aroma
RubyCrisp	New fresh market cultivar, very mild aroma
AM-195	Arkansas selection, rosy aroma
AM-77	Arkansas selection, likely release
Ga. 1-6-14	Breeding selection, honey aroma
Pineapple	Older cultivar, pineapple aroma
Carlos	Popular bronze juice cultivar
Golden Isles	Juice cultivar, low aroma.
Tarheel	Older juice cultivar, high aroma
Magnolia	Older juice cultivar, high aroma
Scuppernong	Historical wine cultivar
Ga. 18-5	Wide hybrid with bunch grape ancestry
Ga. 13-3-36	Hybrid with Ga. 18-5

Preliminary aroma volatile analysis has been completed on each of these accessions. While there is some variation amongst the tested germplasm, we generally found about 26 major aroma volatiles (Fig. 3).



Peak	Aroma volatile compound
1	Ethyl Acetate
2	Hexanal
3	Acetic acid, butyl ester
4	2-Butenoic acid, ethyl ester, (E)-
5	2-Hexenal, (E)-
6	1-Hexanol
7	Acetic acid, pentyl ester
8	**1-Heptanol (Internal standard)**
9	Butanoic acid, butyl ester
10	Hexanoic acid, ethyl ester
11	Acetic acid, hexyl ester
12	Hexanoic acid, 1-methylethyl ester
13	Benzeneacetaldehyde
14	2-Butenoic acid, butyl ester
15	1-Octanol
16	Hexanoic acid, propyl ester
17	Heptanoic acid, ethyl ester
18	Phenylethyl Alcohol
19	Acetic acid, phenylmethyl ester
20	Hexanoic acid, butyl ester
21	Estragole
22	Acetic acid, octyl ester
23	n-Octanoic acid isopropyl ester
24	3-Carene
25	(Z)-4-Decen-1-ol
26	Citral
27	Hexanoic acid, hexyl ester

Fig. 3. Sample chromatogram and peak identification of muscadine aroma volatiles.

Principle component analysis grouped most cultivars relatively closely together, with a few major outliers (Fig. 4). V. popenoei (DVIT 2970) separated well away from other accessions. This Central American species has been little used in breeding and has reduced amounts of several volatiles normally found in muscadine. Ga. 18-5 is a wide hybrid muscadine with several *Euvitis* cultivars in its background and is fully fertile with muscadine. This selection varies from most muscadines in the content of several volatiles. The third outlier was, surprisingly, 'Cowart'. 'Cowart' is a well-known cultivar that was commonly used a self-fertile pollinizer in 'Fry' vineyards. It has been used extensively in breeding and appears in the pedigree of many cultivars including 'Supreme', 'Lane', 'Paulk', and 'RubyCrisp'; which were all included in this study. We are still in the process of verifying and analyzing these results.

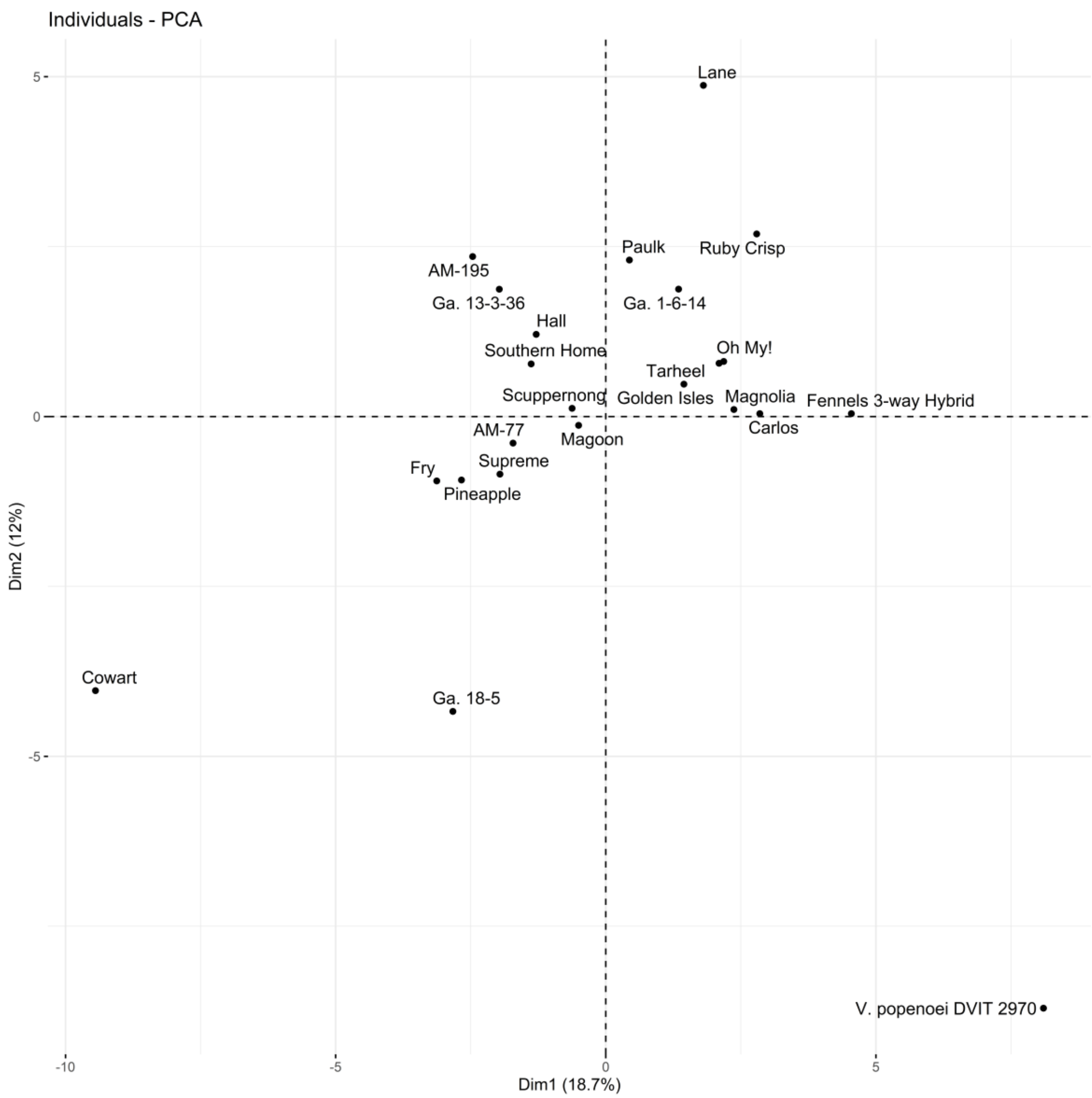


Fig. 4. Principle component analysis of aroma volatiles in muscadine germplasm.