Volatile metabolite analysis: Characterizing emissions from the grape cluster microenvironment in the field (in vivo) and in the laboratory from destructive sampling of berry skin and pulp using solid phase microextraction and simultaneous chemical and sensory analyses

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Background and Rationale: *Volatile metabolite analysis* (ii) is part of *Obj. 1b Characterization of changes in fruit composition during the ripening phase and how they influence grape chemistry / quality at harvest*. Cold climate cultivars such as Marquette, Frontenac, St. Croix, and La Crescent are new, with many unknowns regarding their fruit characteristics. This study sought to find a connection between known industry standards of sugar content (Brix), pH, and titratable acidity (TA) to volatile organic compounds (VOCs) present and released the fruit during ripening. VOCs emitted in-vivo, liberated from crushed berries, and resulting wines were analyzed using state of the art solid phase microextraction (SPME) and multidimensional gas chromatography – gas chromatography – olfactometry (mdGC-MS-O). Temporal evolutions of VOCs will potentially identify marker compounds that can be used to track fruit maturity and ripeness to support timing of harvest and winemaking decisions.

Treatments/Methods: Volatiles emitted from maturing grape clusters were sampled four times between veraison and harvest in 2013 growing season. New method for volatiles was developed. Gases released by grapes were collected using special sampling vessels made from modified 2 mL glass vials and SPME fibers. Vial modifications consisted of removing the bottom and flaring the edges. Thus a sampling vessel could easily adhere to grape surface under vacuum without injuring the grape surface (**Figure 1**). VOCs were collected on SPME fibers for 30 minutes at ambient temperatures. SPME fibers were wrapped in clean aluminum, placed in a cooler for subsequent analysis in the lab at Iowa State University. Brix measurements, pH, and titratable acidity (TA) were taken simultaneously with gas sampling. Samples were collected at two vineyard sites: (1) NE1020 Plot at Iowa State University Horticulture Station, (2) NE1020 Plot at the South Dakota State University NE Hansen Research Farm.

Berries were picked at the same time as volatiles, brought to the lab, crushed, placed in a clean vial. Volatiles emitted from crushed berries were collected using SPME and were analyzed using mdGC-MS-O. A method was developed for simultaneous chemical and sensory analysis of grape, juice and wine volatiles.

Figure 1. Sampling of volatile emissions from La Crescent grapes. A 2 mL glass vial with modified open and flared bottom was fitted with Teflon septa and screw cap (blue) was placed on a maturing grape. A slight vacuum was pulled using a 10 mL syringe and needle. A SPME fiber was inserted, and exposed for 30 minutes to collect volatiles in-situ (seen inside the vial as a black 10 mm long and ~0.3 mm diameter fiber). SPME fibers with collected volatiles were stored in cooler, transported to laboratory, and analyzed at Iowa State University using multidimensional gas-chromatography-mass spectrometry-olfactometry.

Results:

The summary of volatiles emitted in-vivo from maturing La-Crescent and St. Croix grapes in Brookings, SD and Ames, IA is shown in **Table 1**. Only seven compounds were detected in sufficient quantities enabling identification. Three volatiles (benzaldehyde; 1-hexanol, 2-ethyl-; acetaldehyde) were detected in in-vivo volatile emissions from both La Crescent and St. Croix grapes in Iowa. Three volatiles (decanal; nonanal; and 2-pentanone, 4-hydroxy-4-methyl-) were detected in in-vivo volatile emissions from both La Crescent and St. Croix grapes in South Dakota. 1-Hexanol, 2-ethyl- was the only compound common to La Crescent and both locations. The emergence of volatiles in South Dakota was delayed by about 10 days for St. Croix and 16 days for La Crescent. The emergence of volatiles emitted from St. Croix was approximately 1 week later compared with La Crescent in Iowa.

Table 1. Volatiles emitted in-vivo and identified by SPME-mdGC-MS-O in Brookings, SD (SDSU) & Ames, IA (ISU)

Volatile Compound	CAS	Cultivar	Julian Date(s)	Site
Benzaldehyde	100-52-7	La Crescent	225	ISU
1-Hexanol, 2-ethyl-	104-76-7	La Crescent	225	ISU
Acetaldehyde	75-07-0	La Crescent	225, 238, 243	ISU
Benzaldehyde	100-52-7	St. Croix	231	ISU
1-Hexanol, 2-ethyl-	104-76-7	St. Croix	231	ISU
Acetaldehyde	75-07-0	St. Croix	231, 243	ISU
1-Hexanol, 2-ethyl-	104-76-7	La Crescent	242	SDSU
Decanal	112-31-2	La Crescent	242	SDSU
2-Pentanone, 4-hydroxy-4-methyl-	123-42-2	La Crescent	242	SDSU
Nonanal	124-19-6	La Crescent	242	SDSU
1-Hexanol	111-27-3	St. Croix	242	SDSU
Decanal	112-31-2	St. Croix	242	SDSU
2-Pentanone, 4-hydroxy-4-methyl-	123-42-2	St. Croix	242	SDSU
Nonanal	124-19-6	St. Croix	242	SDSU

Summary of volatiles in La Crescent crushed berries is presented in **Figure 2**. Volatile organic compounds evolve with time. Total abundance of volatiles increases with stages of development and is correlated with brix. Composition of individual volatiles in berries is highly variable. Several predominant volatiles were identified (1-hexanol; cyclohexanol; 1,6-octadien-3-ol, 3,7-deminethyl (preliminary identification); cyclohexane, methyl-) while 32 other compounds tend to be detected at much lower and highly variable concentrations.

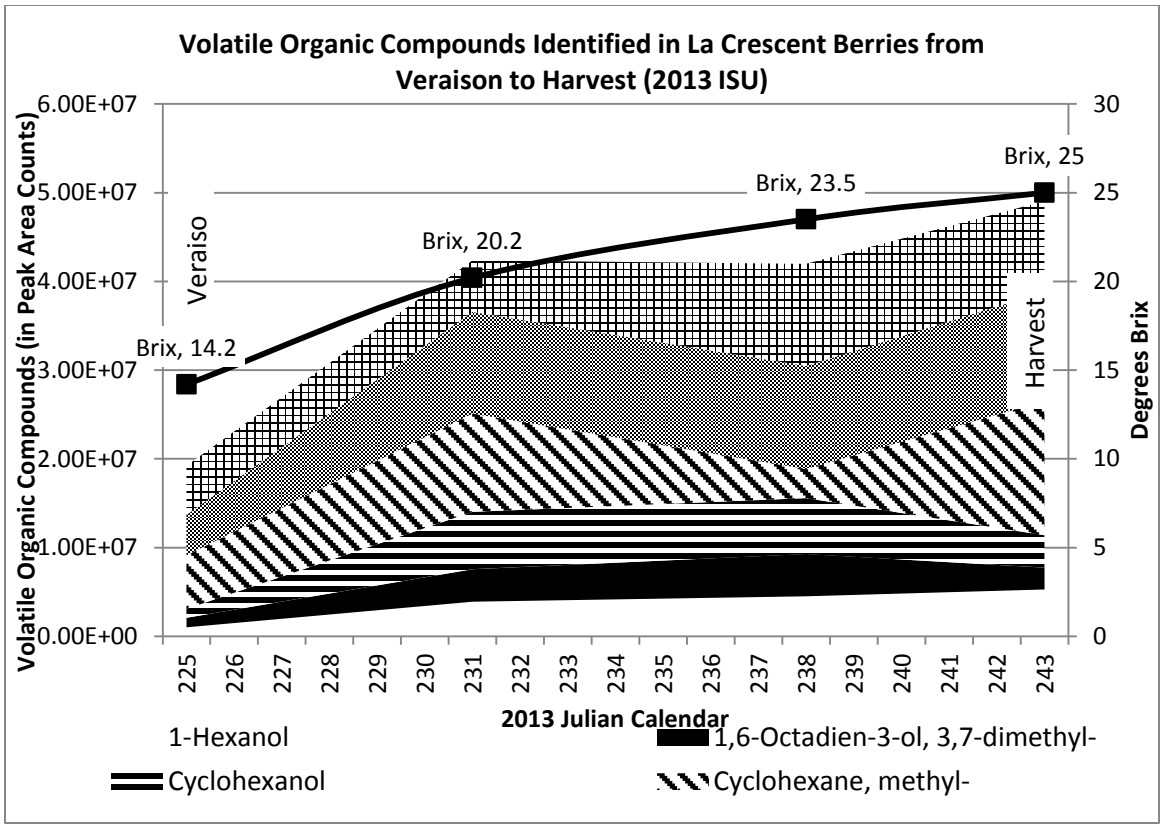

Figure 2. Evolution of volatile organic compounds identified in **La Crescent** crushed berries in relation to measured brix from veraison (August 13, 2013) to harvest (August 31, 2013) in Ames, Iowa. "Other" represents the sum of 32 other volatile compounds not listed in the legend. Total abundance of volatiles increases with stages of development. Composition of individual volatiles in berries is highly variable.

Simultaneous chemical and sensory analysis resulted in detection of several flavor compounds. Organoleptic (aroma and taste perception) properties of compounds found in La Crescent include: **fruity** (butanoic acid, ethyl ester, CAS 105-54-4), **lemon** (limonene, CAS 138-86-3), **geranium** (2, 6-Octadien-1-ol, 3, 7-dimethyl-, (E)-, CAS 106-24-1), **clove** (caryophyllene, CAS 87-44-5), and many others.

Summary of volatiles in St. Croix crushed berries is presented in **Figure 3**. Volatile organic compounds evolve with time. However, the overall pattern was different compared with the volatiles in La Crescent. Few predominant compounds tend to decrease over time while several others tend to increase. Eighteen other compounds (compounded in 'other' series in **Figure 3**) tend to be detected at much lower and highly variable concentrations.

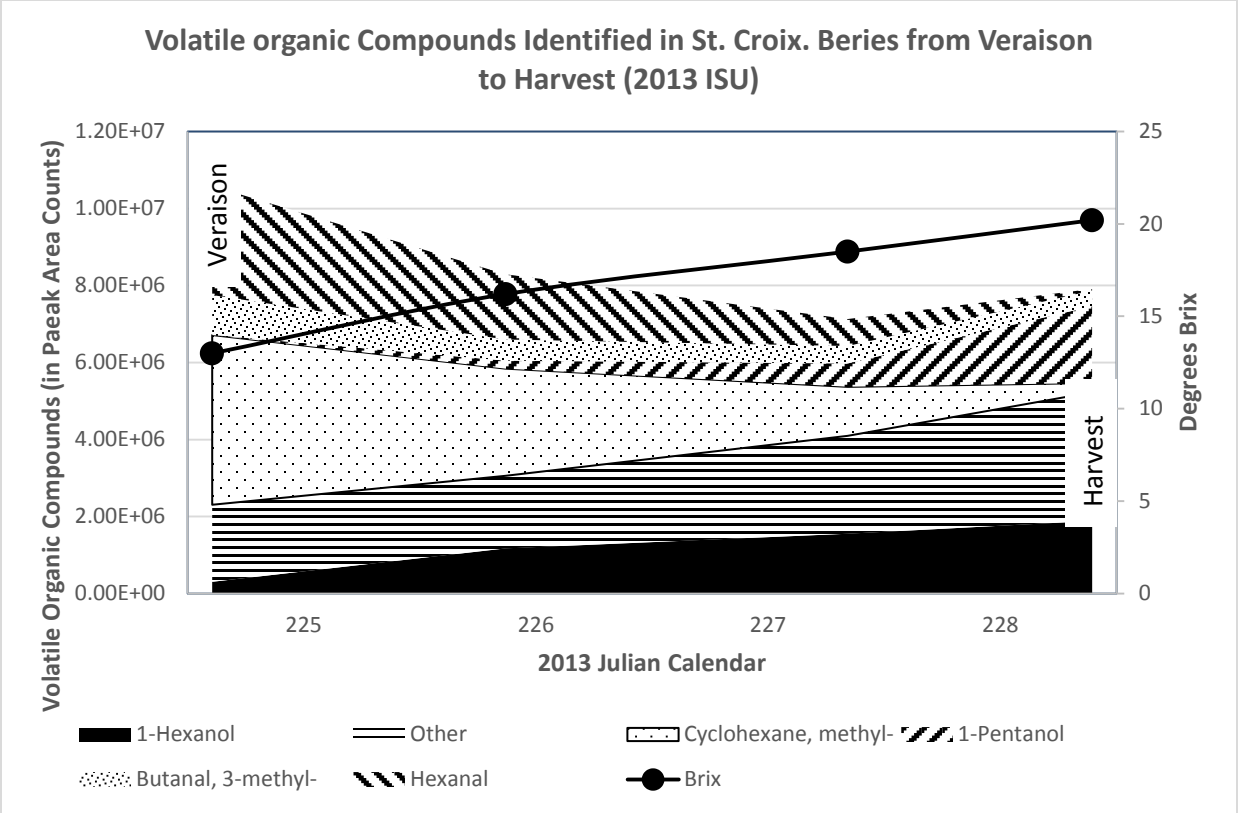

Figure 3. Evolution of volatile organic compounds identified in **St. Croix** crushed berries in relation to measured brix from veraison (August 13, 2013) to harvest (August 31, 2013) in Ames, Iowa. "Other" represents the sum of 17 other volatile compounds not listed in the legend. Total abundance of volatiles increases with stages of development. Composition of individual volatiles in berries is highly variable.

Organoleptic properties of compounds found in St. Croix include: **almond** (benzaldehyde, CAS 100-52-7), **rose** (2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-, CAS 23726-93-4), **honey** (phenylethyl alcohol, CAS 60-12-8), **citrus** (1,6-Octadien-3-ol, 3,7-dimethyl-, CAS 78-70-6), and many others.

What the results mean:

- Results from in-situ sampling with the glass vials were inconclusive. Variation among replicates is preventing compilation of a comprehensive volatile metabolite profile for VOCs emitted in-vivo. At the time of submission of this report, we are investigating if this is a flaw in methodology or an inherent property of the berries. We plan to address this by using longer sampling time (> 30 min) and improved sample preservation method to address the apparent low abundance of volatiles detected in Year 2.
- As many as 37 volatile organic compounds were identified in crushed La Crescent berries from veraison to harvest at varying concentrations.
- Organoleptic (aroma and taste perception) properties of compounds found in La Crescent include: **fruity lemon, geranium**, **clove**, and many others.
- 18 volatile organic compounds were identified in crushed St. Croix berries from veraison to harvest at varying concentrations, using the same identification criteria set for La Crescent.
- Organoleptic properties of compounds found in St. Croix include: **almond**, **rose**, **honey**, **citrus**, and others.
- It is apparent that some desirable flavor compounds found can be found in crushed berries as the berries mature from veraison to harvest.