Viticulture, enology and marketing for cold-hardy grapes

From Vine to Glass: Understanding the Flavors and Aromas of Cold-Hardy Grapes and Wine

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The Northern Grapes Project is funded by the USDA's Specialty Crops Research Initiative Program of the National Institute for Food and Agriculture, Project #2011-51181-30850 www.northerngrapesproject.org

Comprehensively track what genes are activated, what metabolites are produced, and what sensory descriptors develop (SDSU, UMN, ISU)

Frontenac, Marquette, Frontenac Gris, Brianna

Analyses:

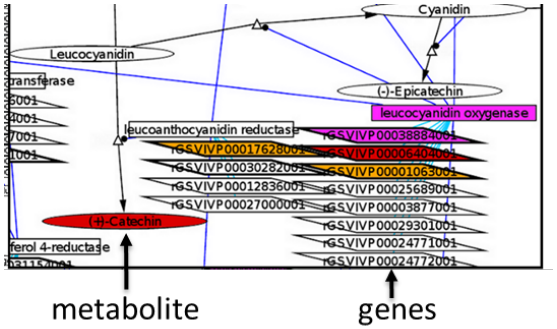
gene expression (transcriptome)

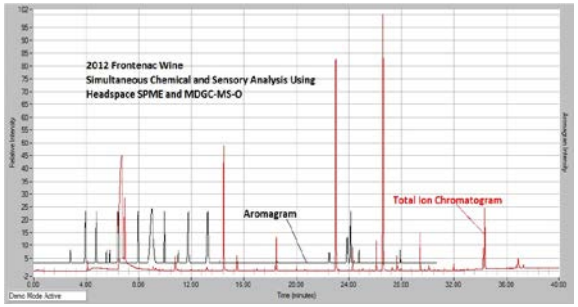
volatile compounds (aroma)

primary metabolites (sugars, and organic and amino acids)

secondary metabolites (tannins, flavonoids, anthocyanins, stilbenes, other phenolics, and terpenoids)

Sensory (berry and wine)

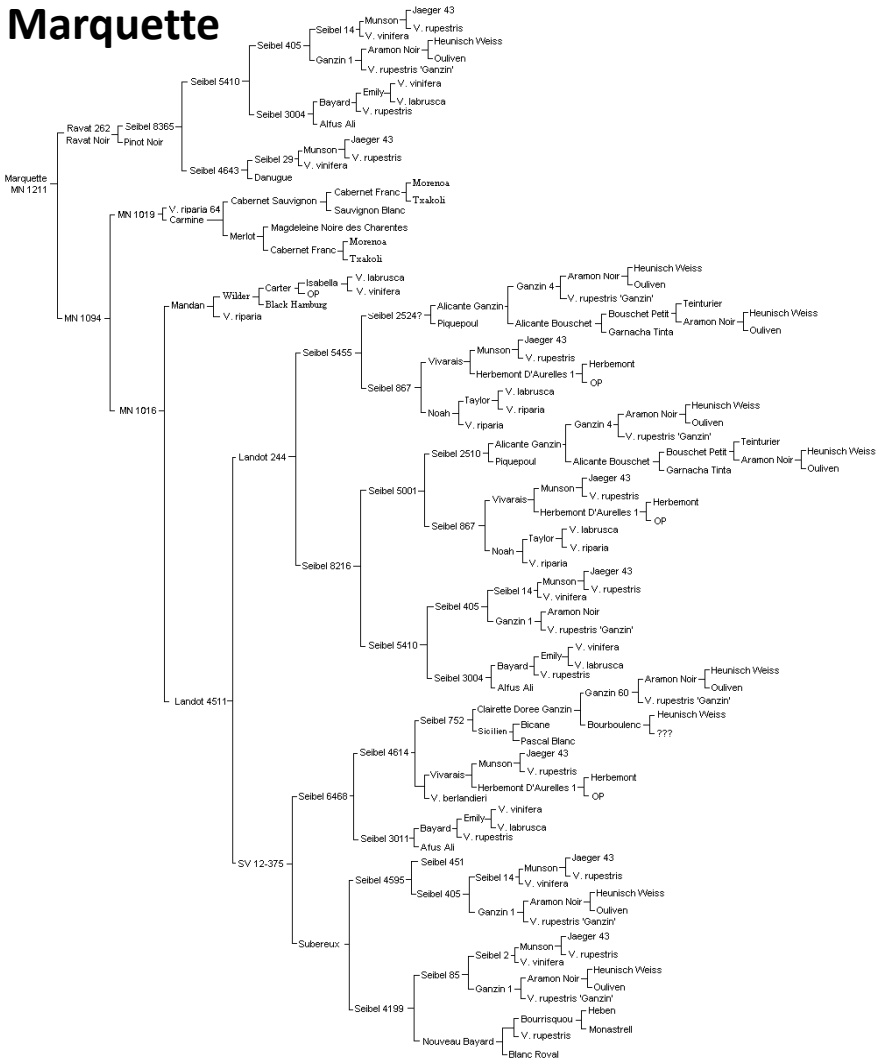




Marquette and Frontenac

- **Marquette and Frontenac have some of their pedigree in common**

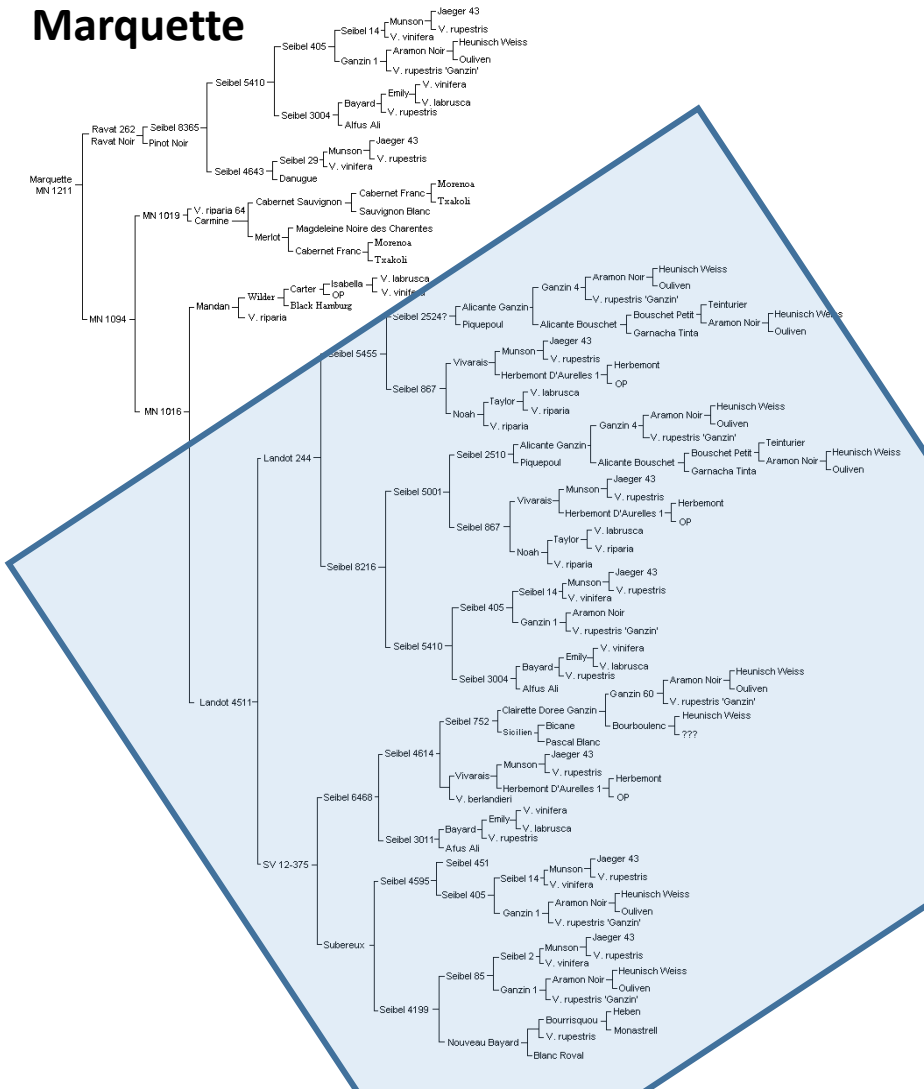
Marquette



Marquette and Frontenac

- **Marquette**
- **Frontenac (*V. riparia* x Landot 4511)**

Pedigree in common between Frontenac (parent) and Marquette (great grandparent)



From the Genome to Your Senses

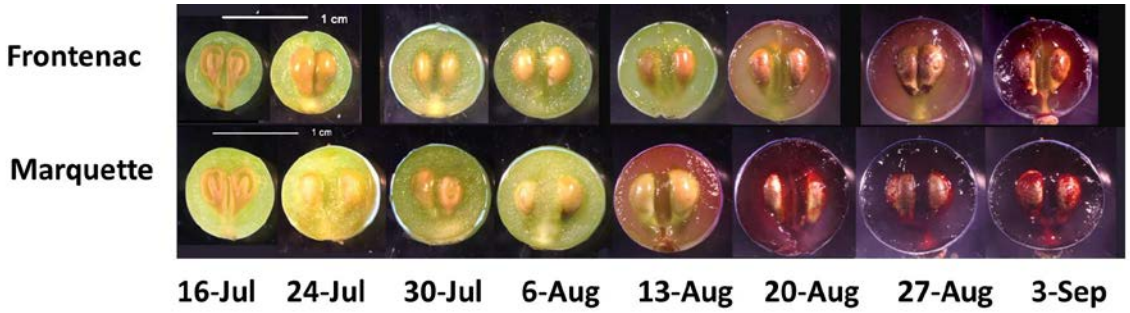


Fig. 1 Change in berry size, seed maturation and pulp pigmentation during ripening in Frontenac and Marquette.

From veraison to harvest:

1. What genes are activated?
2. What metabolites (chemicals) are produced?
3. What sensory descriptors develop?
4. How do genes, metabolites and sensory descriptors correlate?

Marquette and Frontenac

Physical and molecular characterization of berry development and ripening

- Veraison
- 20˚Brix
- 22˚Brix*
- 24˚Brix*
- 26˚Brix*

*Sensory analysis of berry & wine

- Berries collected weekly- Brix, pH, TA
- Whole berries- sensory and aroma analysis
- Berries separated into skin and pulp – gene expression and metabolite analysis
- Wine

Berry Samples
20 to 26°Brix

↓

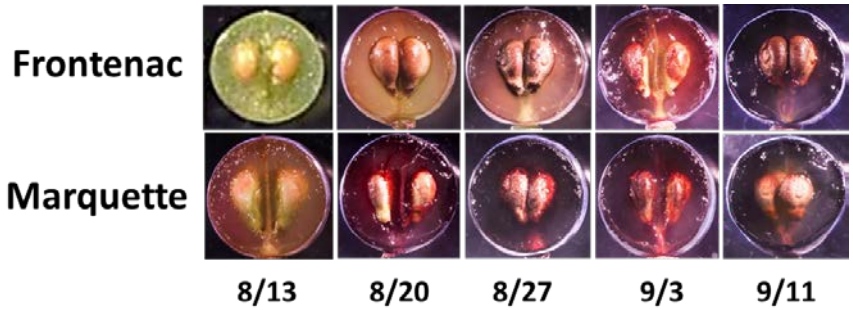
Transcriptome
(gene expression)

↓

Metabolome
(sugars, acids, flavors, aromas anthocyanins, tannins)

↓

Sensory descriptors



Berry Samples
20 to 26°Brix

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Transcriptome
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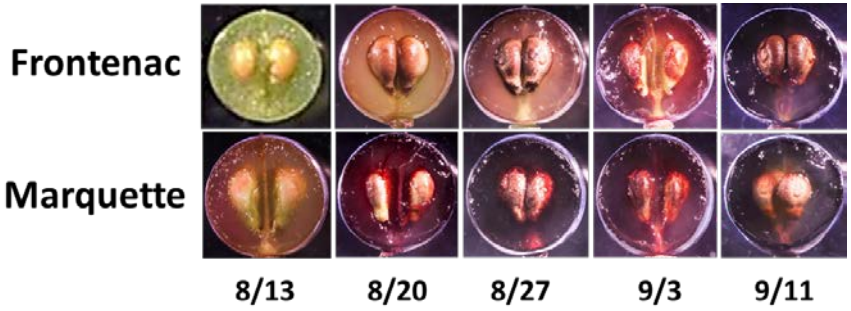
Sensory descriptors



- **Continuous changes in genes expressed during ripening.**
- **Greater number of genes with increasing or decreasing expression in the berry skin than in the pulp**

Berry Samples
20 to 26°Brix

↓

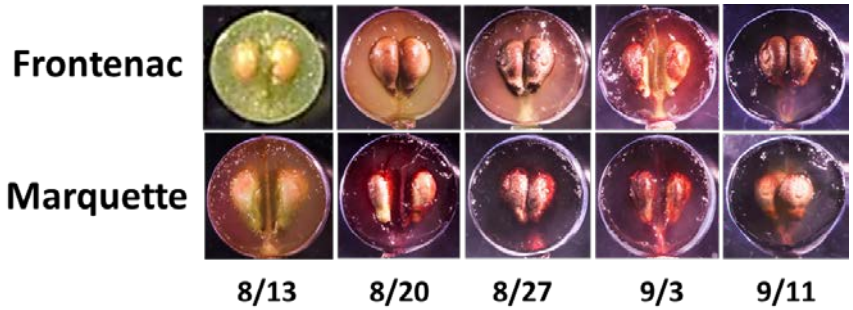
Transcriptome
(genes)

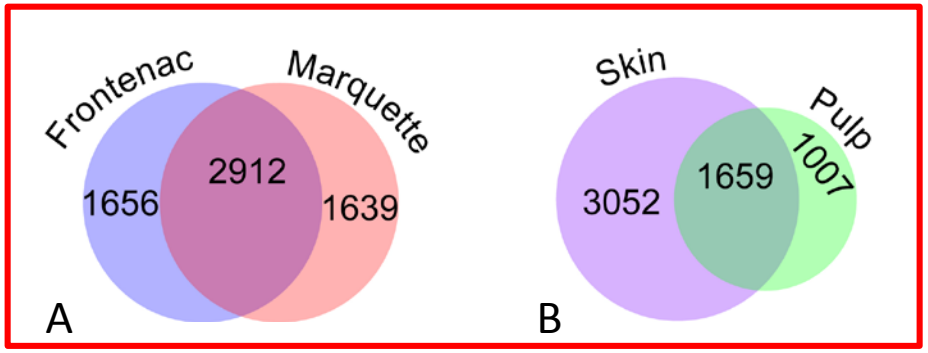
↓

Metabolome
(sugars, acids, flavors, aromas anthocyanins, tannins)

↓

Sensory descriptors





A. **Number of genes expressed differently between skin and pulp of Marquette or Frontenac at harvest**
B. **Number of gene expressed differently between skin and pulp across both cultivars at harvest**

Berry Samples
20 to 26°Brix

↓

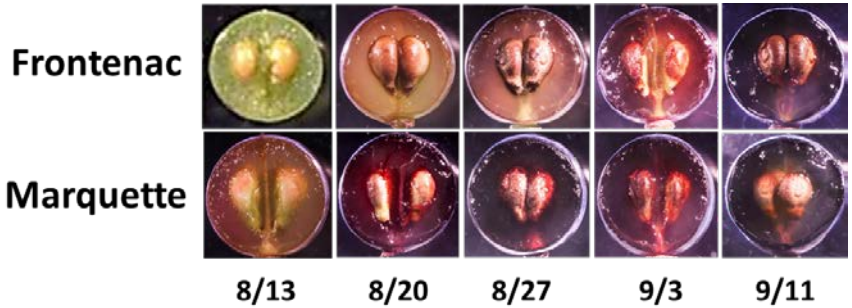
Transcriptome
(genes)

↓

Metabolome
(sugars, acids, flavors, aromas anthocyanins, tannins)

↓

Sensory descriptors



- **Color development: anthocyanin biosynthesis**
- **Aroma development: terpenoid biosynthesis; terpenes contribute to scent, flavor and color**

Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

Do gene expression differences conincide with existing knowledge?

- Anthocyanin biosynthesis genes preferentially expressed in berry skins
- Expression of anthocyanin biosynthesis genes significantly greater expression in Frontenac than Marquette

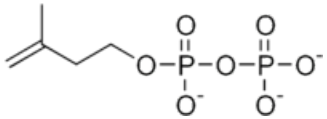
	Front Skin	Marq Skin
VIT_05s0062g00720	36	
VIT_12s0034g00080	47	
VIT_02s0033g00450	159	42
VIT_02s0033g00390	165	84
VIT_05s0049g01020	429	120
VIT_02s0033g00380	531	219

Several anthocyanin biosynthesis genes highly expressed in Frontenac in comparison to Marquette

Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

Are differences in gene expression related to aroma or flavor?

- > number of gene expression differences in the berry skins than the berry pulp
- Cultivar specific expression patterns for terpenoid (aroma) biosynthesis genes



Terpenoid biosynthesis gene expression

	Front Skin	Marq Skin
VIT_12s0134g00030	0.0	8.5
VIT_06s0004g06480	0.1	2.0
VIT_17s0000g05580	0.2	1.4
VIT_01s0010g02320	0.5	2.5
VIT_13s0067g00380	0.6	0.1
VIT_15s0046g03600	0.8	3.5
VIT_13s0067g00370	0.8	0.2
VIT_19s0135g00200	1.9	4.9
VIT_00s0253g00140	1.9	0.4
VIT_19s0135g00190	2.4	5.2
VIT_05s0049g00400	3.6	1.4
VIT_15s0046g03570	3.7	1.2
VIT_08s0032g00240	5.0	2.4
VIT_15s0021g01060	6.7	3.2
VIT_11s0016g01290	7.4	1.2
VIT_19s0015g02500	9.1	1.9
VIT_17s0000g09610	13.3	1.7
VIT_15s0048g01490	22.3	4.8
VIT_02s0025g04880	119.5	35.3

Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

Are differences in gene expression related to aroma or flavor?

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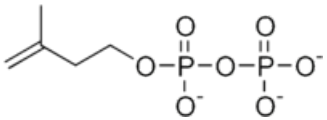

Terpenoid biosynthesis gene expression

Marquette E-beta-ocimene synthase: Terpy, woody green, vegetable nuances

VIT_13s0067g00380	0.6	0.1
VIT_15s0046g03600	0.8	3.5
VIT_13s0067g00370	0.8	0.2
VIT_19s0135g00200	1.9	4.9
VIT_00s0253g00140	1.9	0.4
VIT_19s0135g00190	2.4	5.2
VIT_05s0049g00400	3.6	1.4

Frontenac Pinene synthase: eucalyptus and camphoraceous note with a spicy peppery and nutmeg nuance

VIT_02s0025g04880	119.5	35.3

Genomics and Fruit Composition:

- **Distinct cultivar differences exist in gene expression patterns**
- **Differences in expression of genes related to aroma and flavor are found between Marquette and Frontenac.**
- **Results will be correlated with volatile compound and other metabolites.**

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Somchai Rice, Jacek Koziel, Murli Dharmadhikari, Devin Maurer – ISU

Emily Del Bel, Soon Li Teh, Bety Rostandy, Jenna Brady, Zata Vickers, Adrian Hegeman, Jim Luby – UMN

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Somchai Rice
Agricultural and Biosystems Engineering
Toxicology
Iowa State University

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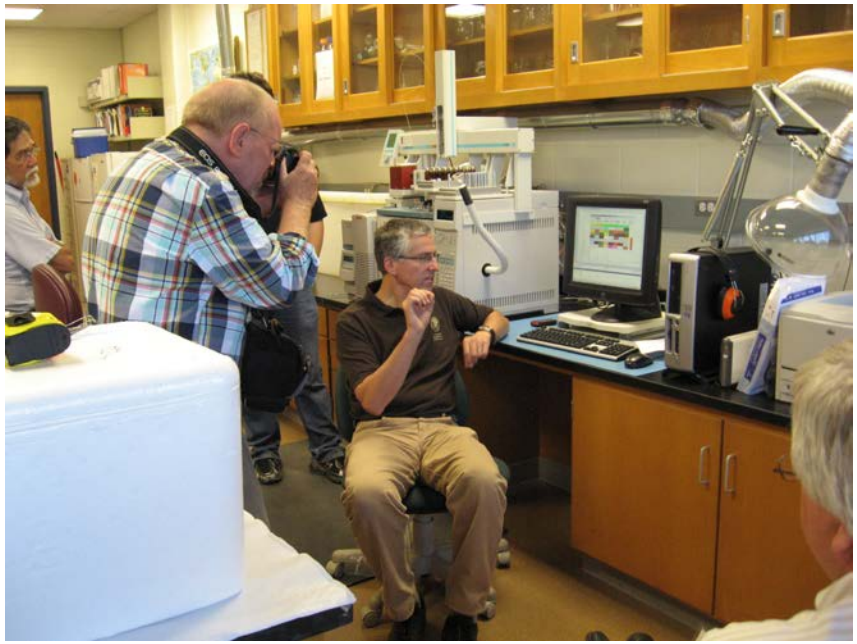
- 1 cm stationary phase fused to silica fiber
- Bonded to stainless steel plunger
- Holder operates like microliter syringe

- Analytes adsorb/absorb to the coating
- Fiber is then thermally desorbed into GC inlet, and is cleaned for re-use

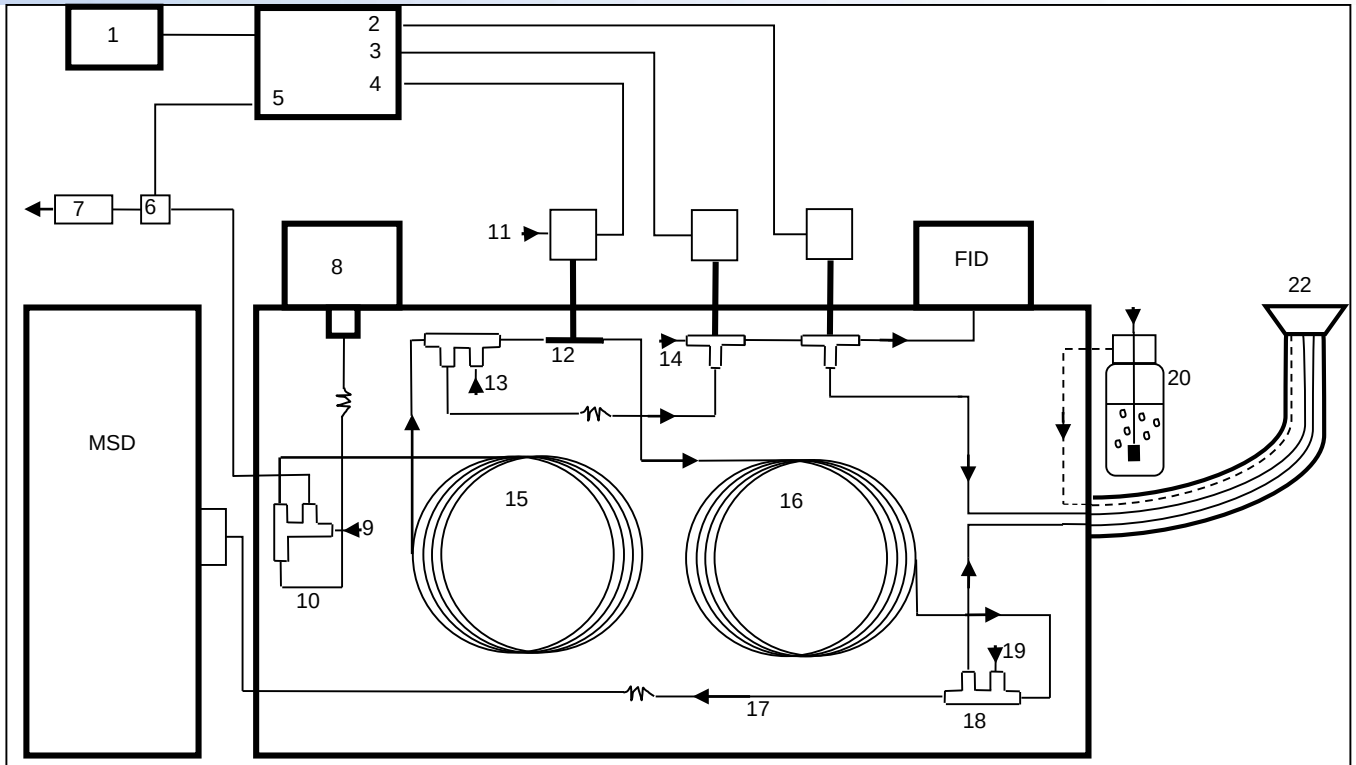
Solid phase microextraction (SPME) fiber

Chemical and Sensory profile analysis (ISU)



Multidimensional gas chromatography – mass spectrometry – olfactometry (MDGC-MS-O)
Left to Right: Paul Domoto (ISU), Timothy Martinson (Cornell), Jason Vallone (ISU), Jacek Koziel (ISU). Photo by Somchai Rice

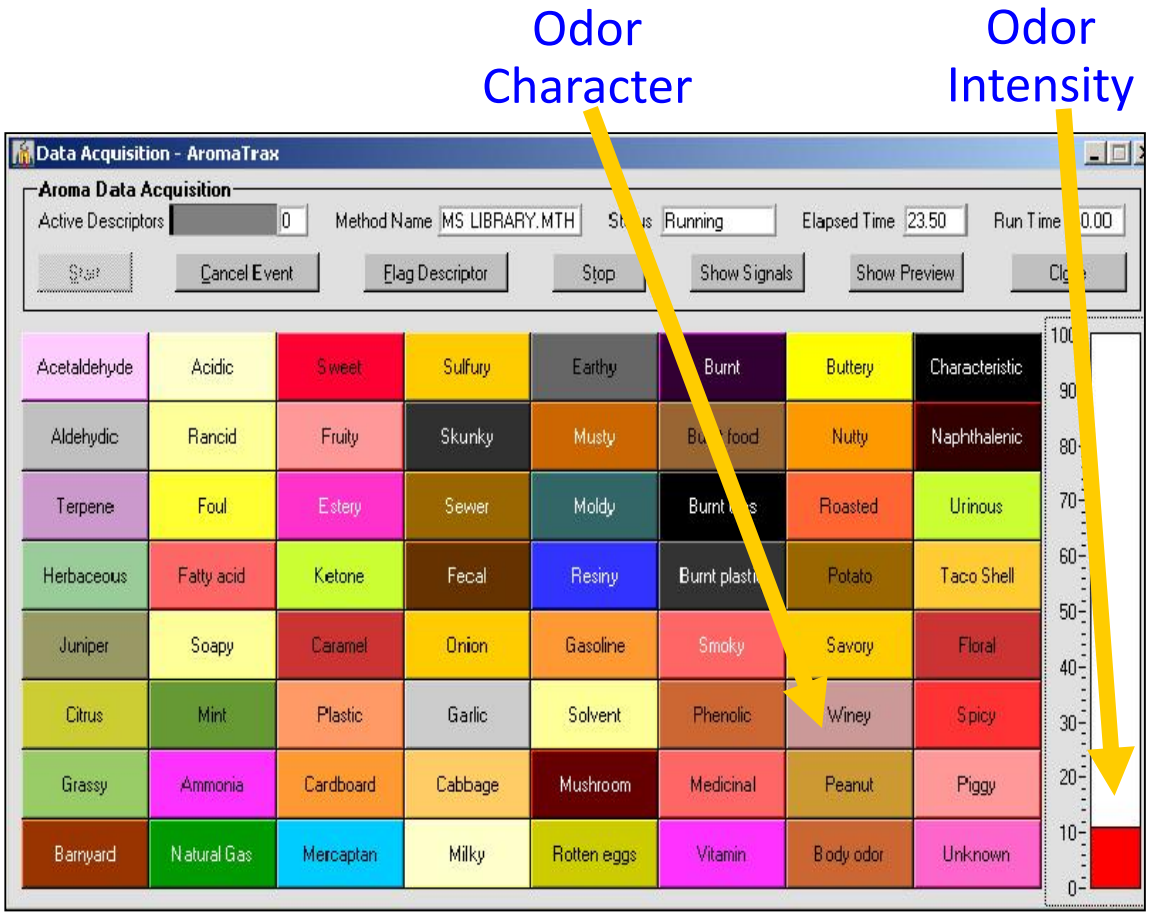
Chemical and Sensory profile analysis (ISU)



Notes:
1: MultiTrax Controller
2: Precolumn Sniff Port Selector
3: Heartcut Valve
4: CO_2 Cryotrap
5: Precolumn Backflush
6: Solenoid
7: Filter
8: Injector
9: Backflush Sweep
10: Fixed Restrictor to Inlet
11: Liquid CO_2 Feed
12: CO_2 Cryotrap
13: Midpoint Pressure
14: Heartcut Sweep
15: Non-Polar column
16: Polar Column
17: Fixed Restrictor to MSD
18: Open Split Interface (OSI)
19: OSI Sweep
20: Humidifier
21: Air in
22: Sniff Port

Schematic of MDGC-MS-O (MOCON, Round Rock, TX)

Chemical and Sensory profile analysis (ISU)



Aroma descriptor of AromaTrax (MOCON, Round Rock, TX)

Chemical and Sensory profile analysis (ISU)

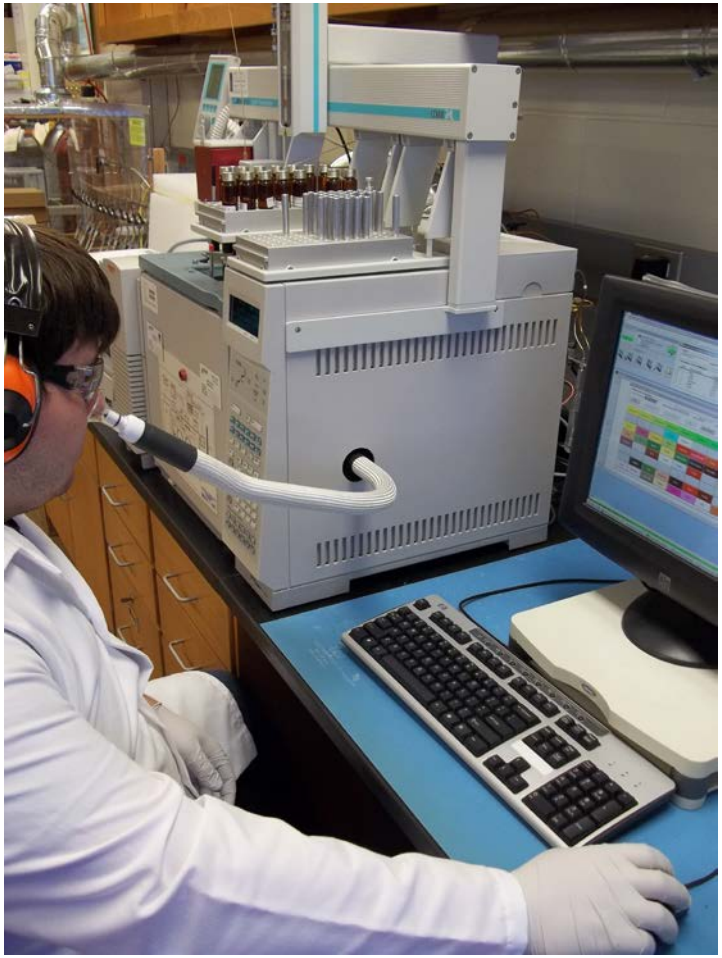
Marquette cluster in polyvinyl film (Tedlar) enclosure with stainless steel cage and solid phase microextraction (SPME) port. Photo by Somchai Rice.

Teflon SPME port with teflon backed septum. PDMS/DVB SPME extraction, exposed fiber, 30 minutes at ambient temperature. Photo by Somchai Rice.

Chemical and Sensory profile analysis (ISU)

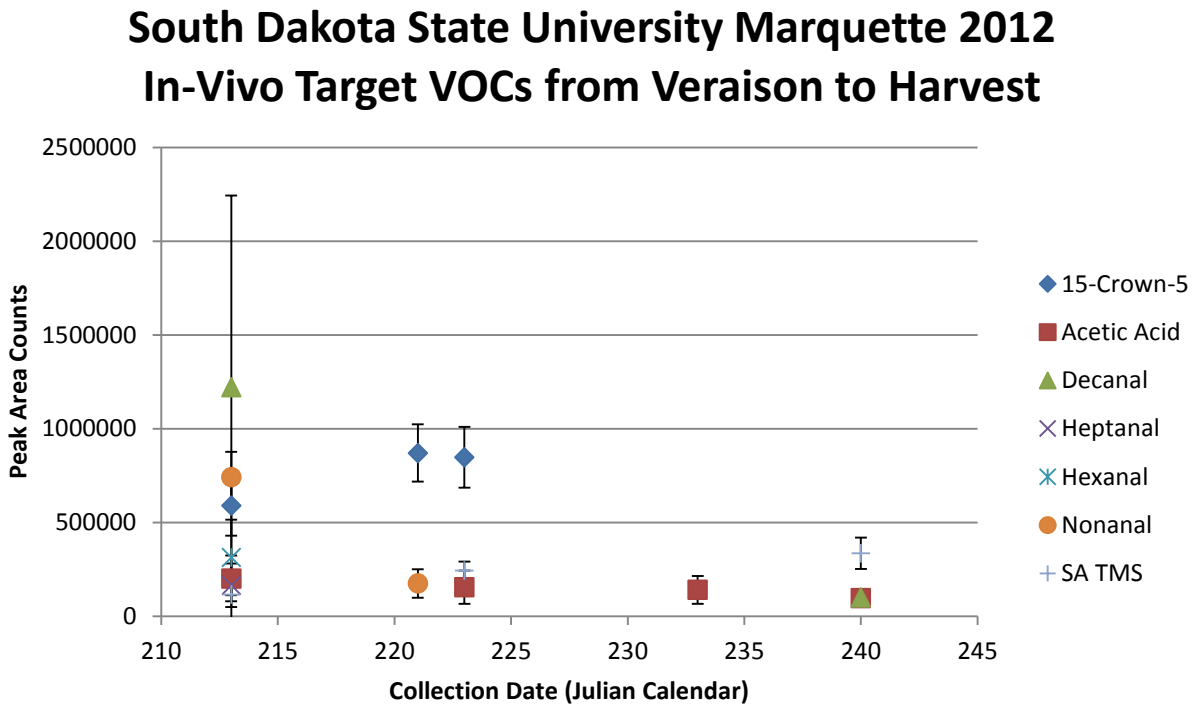
Modified 2 mL glass vial with septa, held by negative pressure. Photo by Somchai Rice

Chemical and Sensory profile analysis (ISU)



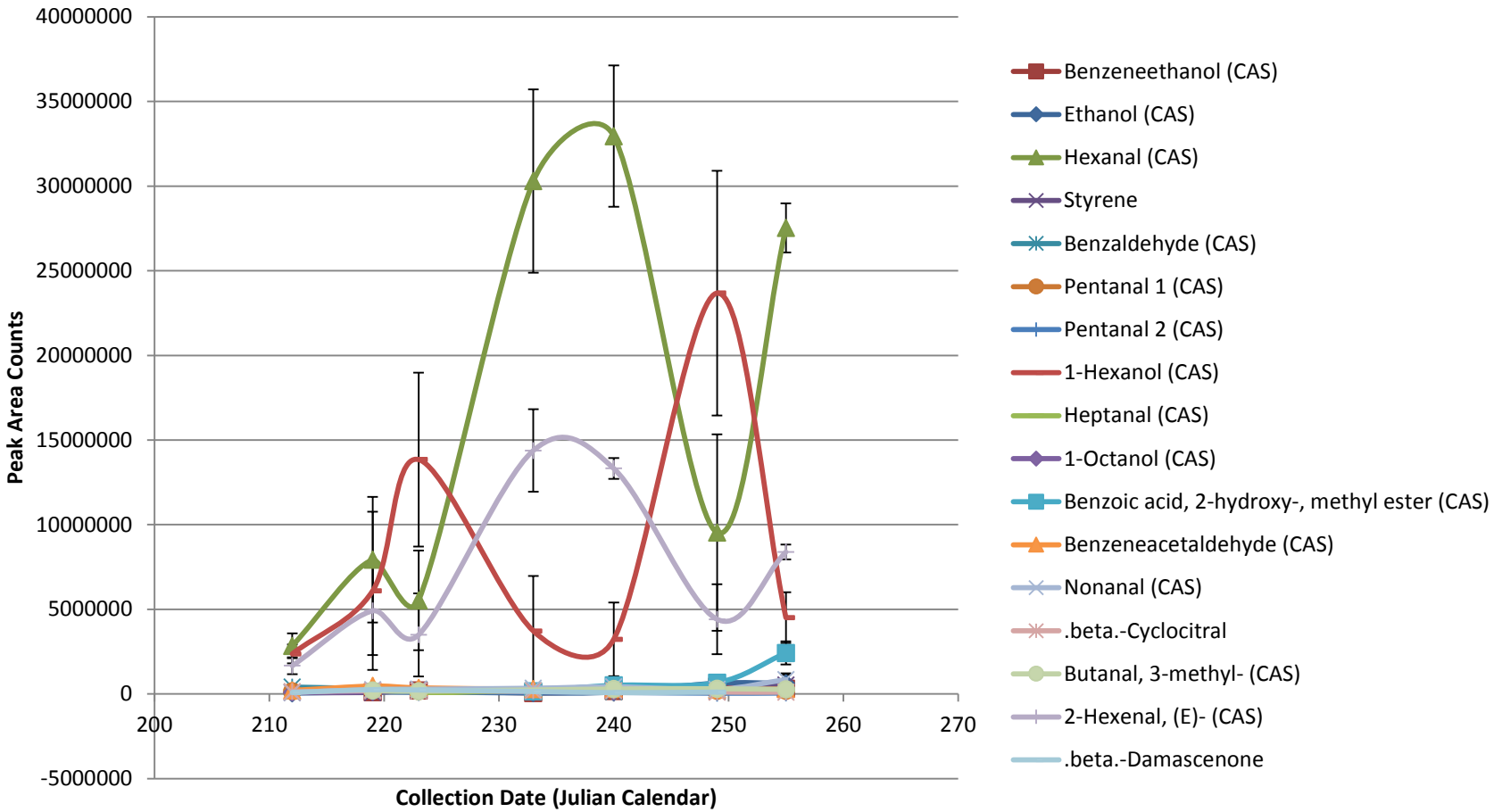
MDGC-MS-O Jason Vallone analyses in vivo gas emissions from Frontenac grape clusters at 24° Brix. Photo by Somchai Rice

Chemical and Sensory profile analysis (ISU)



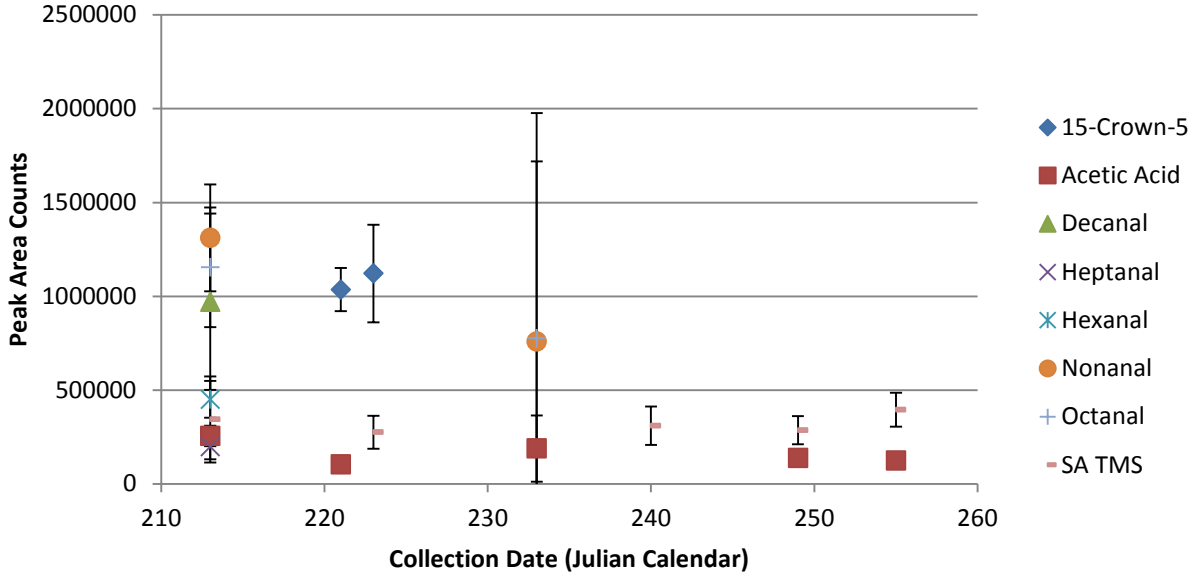
Chemical and Sensory profile analysis (ISU)

Total Target VOCs from crushed berries - SDSU Marquette 2012



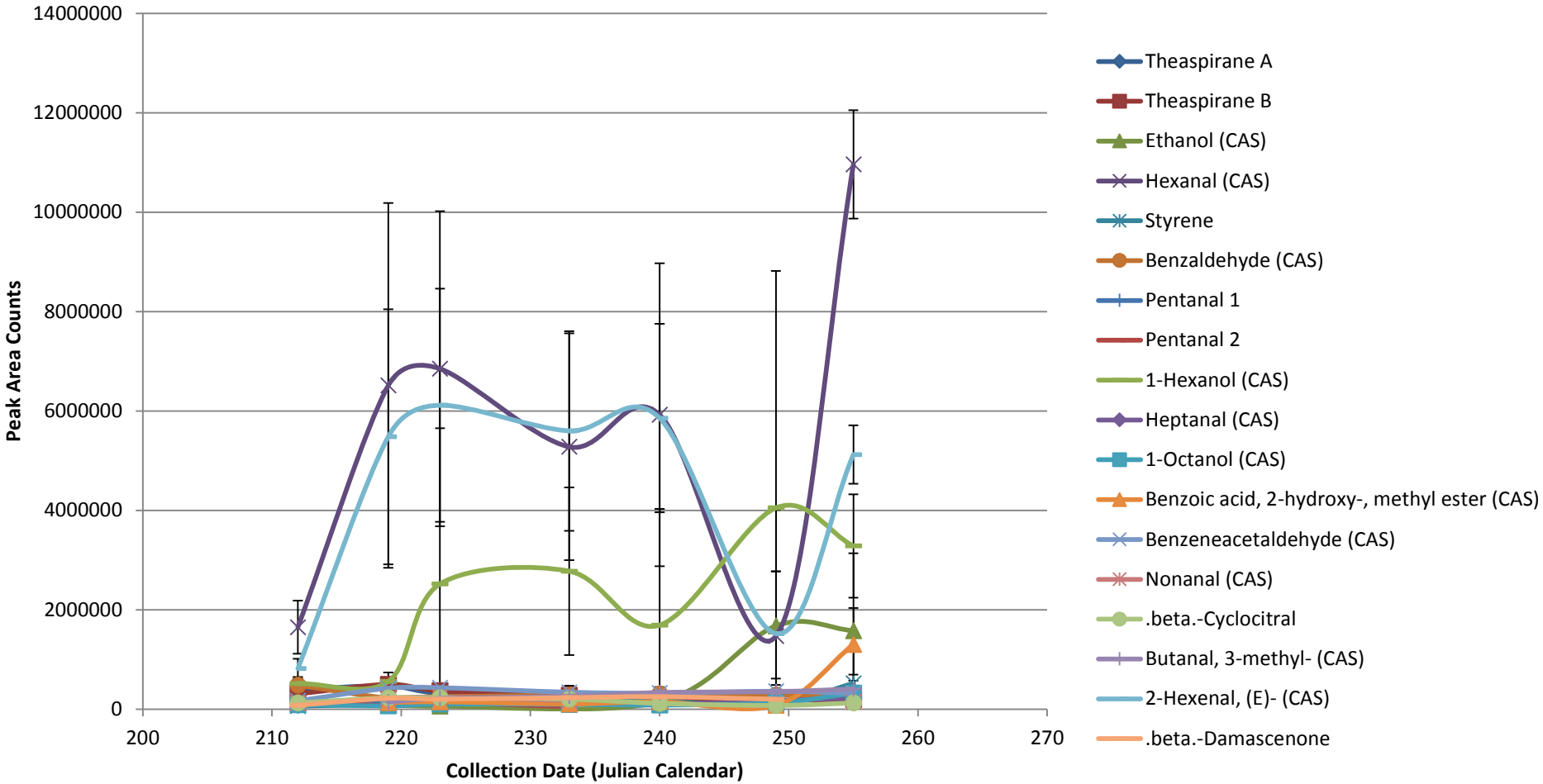
Chemical and Sensory profile analysis (ISU)

South Dakota State University Frontenac 2012
In-Vivo Target VOCs from Veraison to Harvest



Chemical and Sensory profile analysis (ISU)

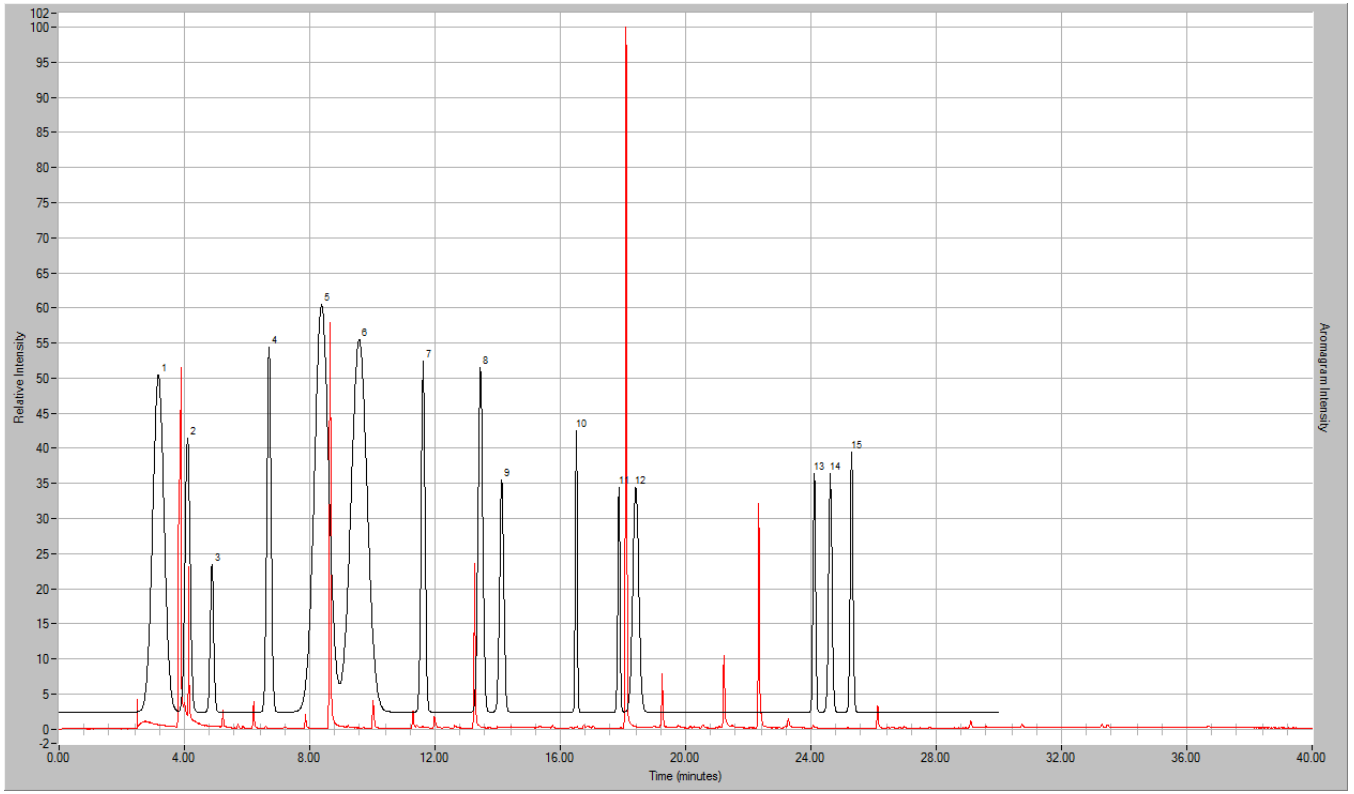
Total Target VOCs from crushed berries - SDSU Frontenac 2012



Chemical and Sensory profile analysis (ISU)

- Aroma dilution analysis
- Successive dilutions of wine samples were analyzed to determine which compounds were most impactful in total aroma
- An automated SPME MDGC-MS-O method was developed

Chemical and Sensory profile analysis (ISU)



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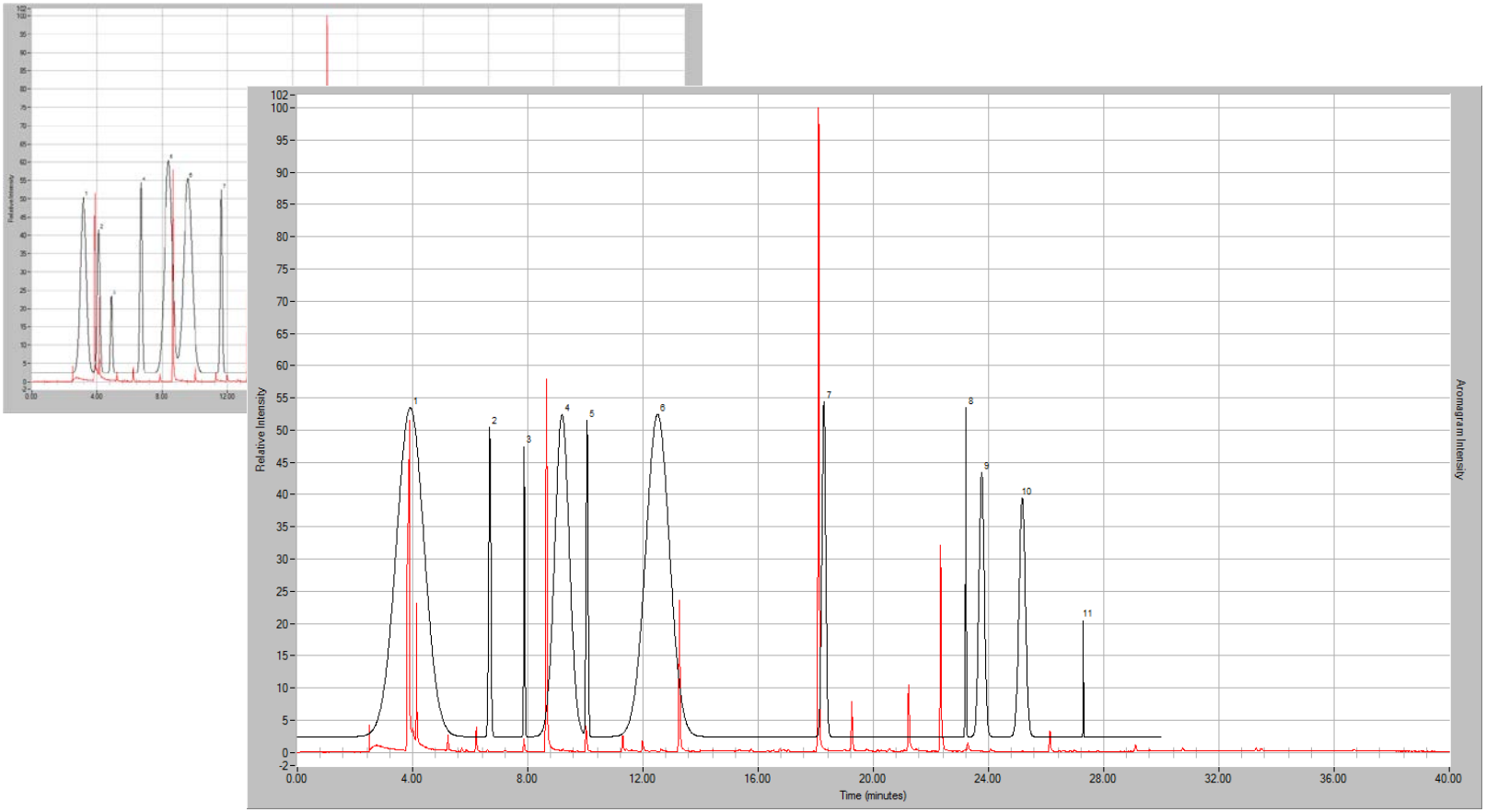
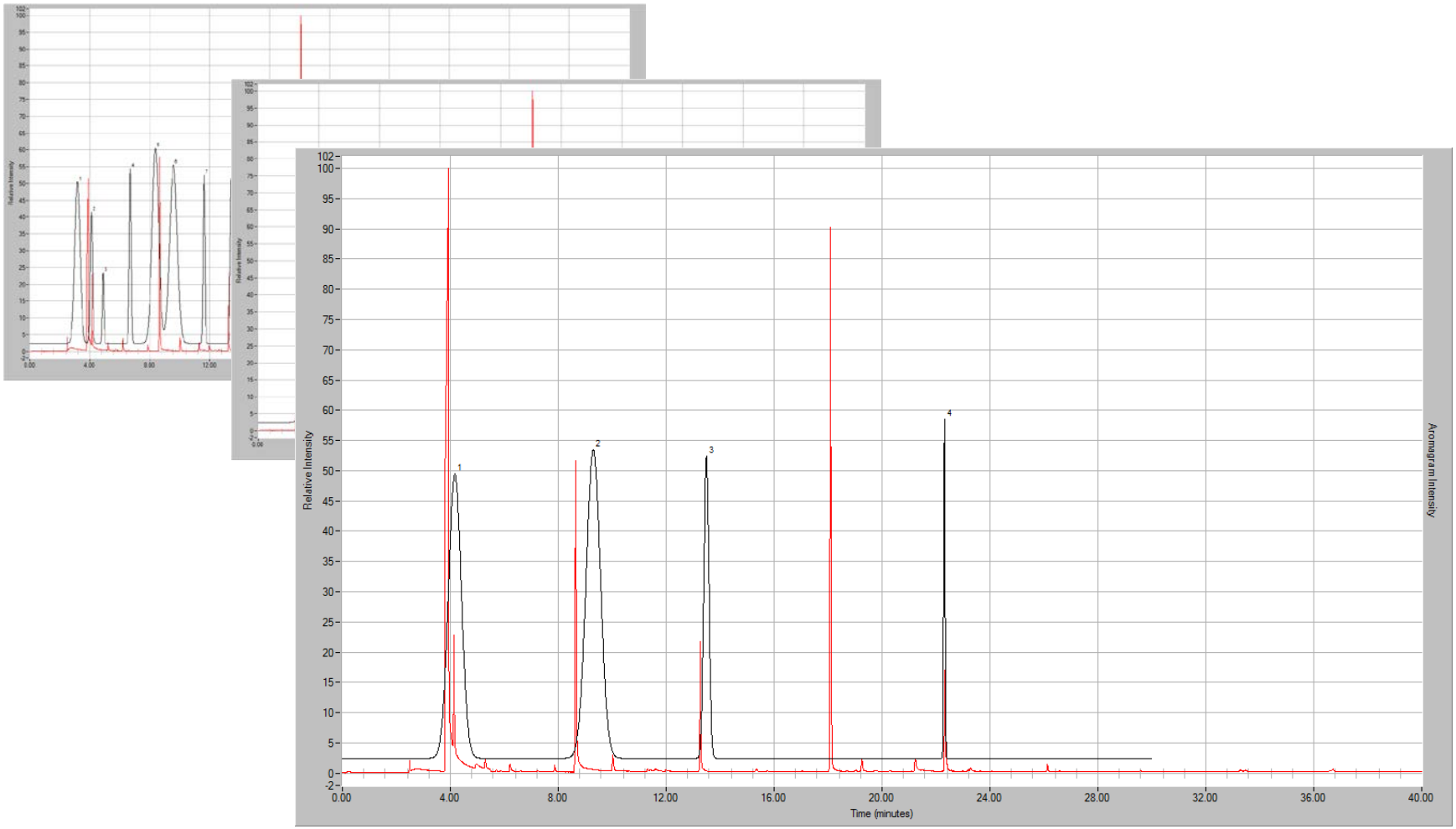
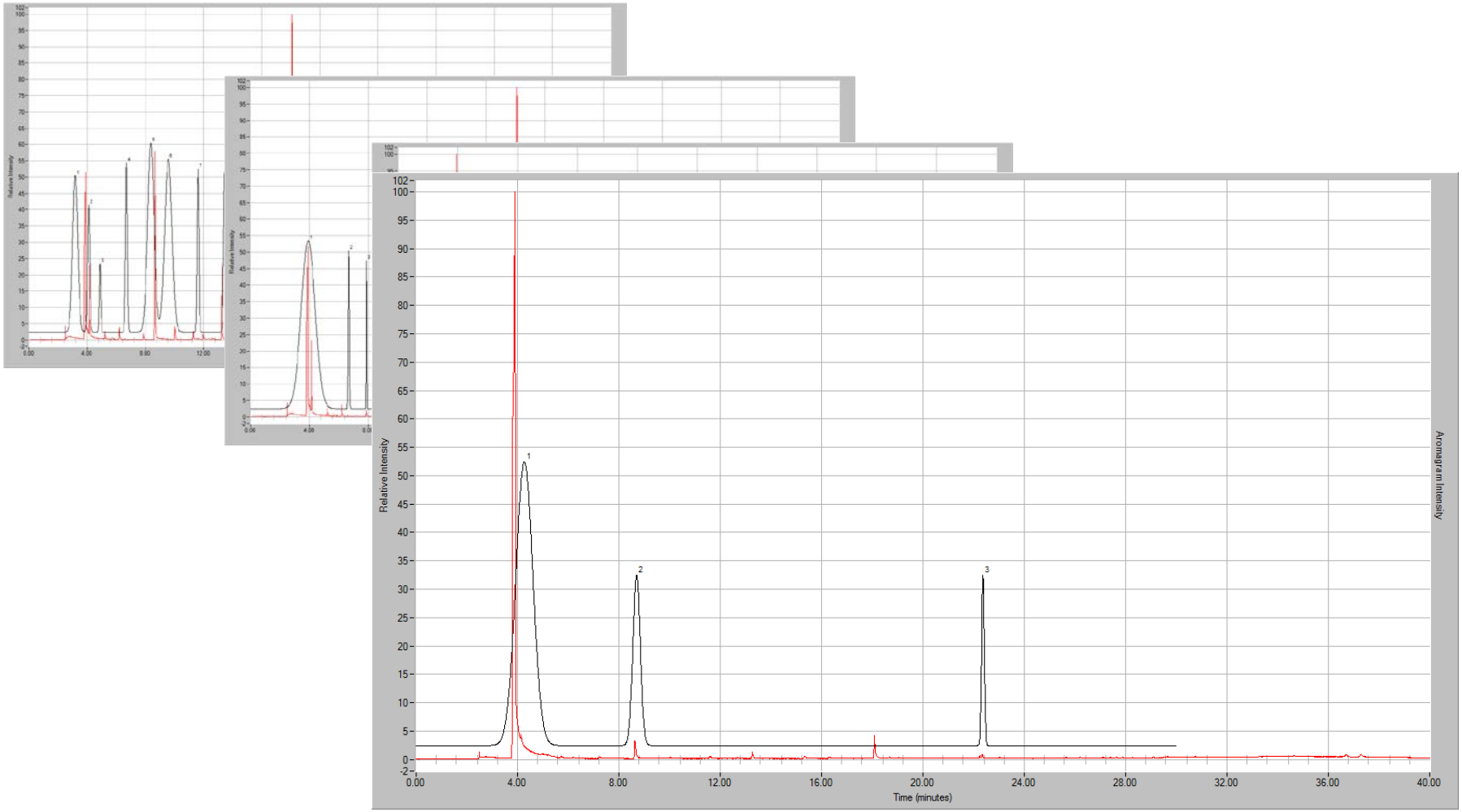


Chemical and Sensory profile analysis (ISU)



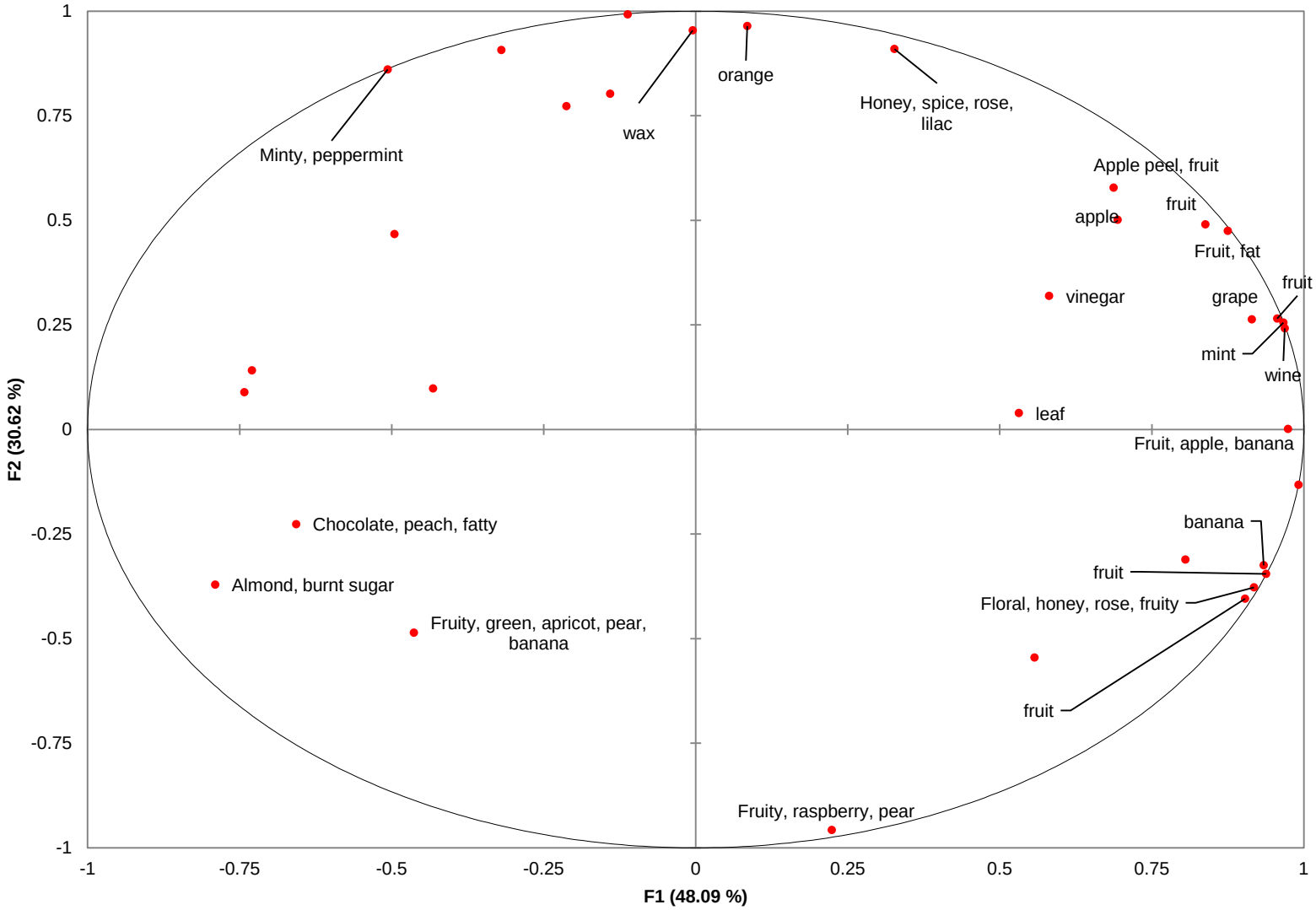
Chemical and Sensory profile analysis (ISU)



Chemical and Sensory profile analysis (ISU)

- Marquette wine aromas developed from ‘wine, apple and fruity’ to ‘cheesy, chocolate, and strawberry’.
- ‘Chocolate and molasses’ aroma intensified, and ‘jam’ aroma was developed in Frontenac wine as sugar levels increased.

Chemical and Sensory profile analysis (ISU)



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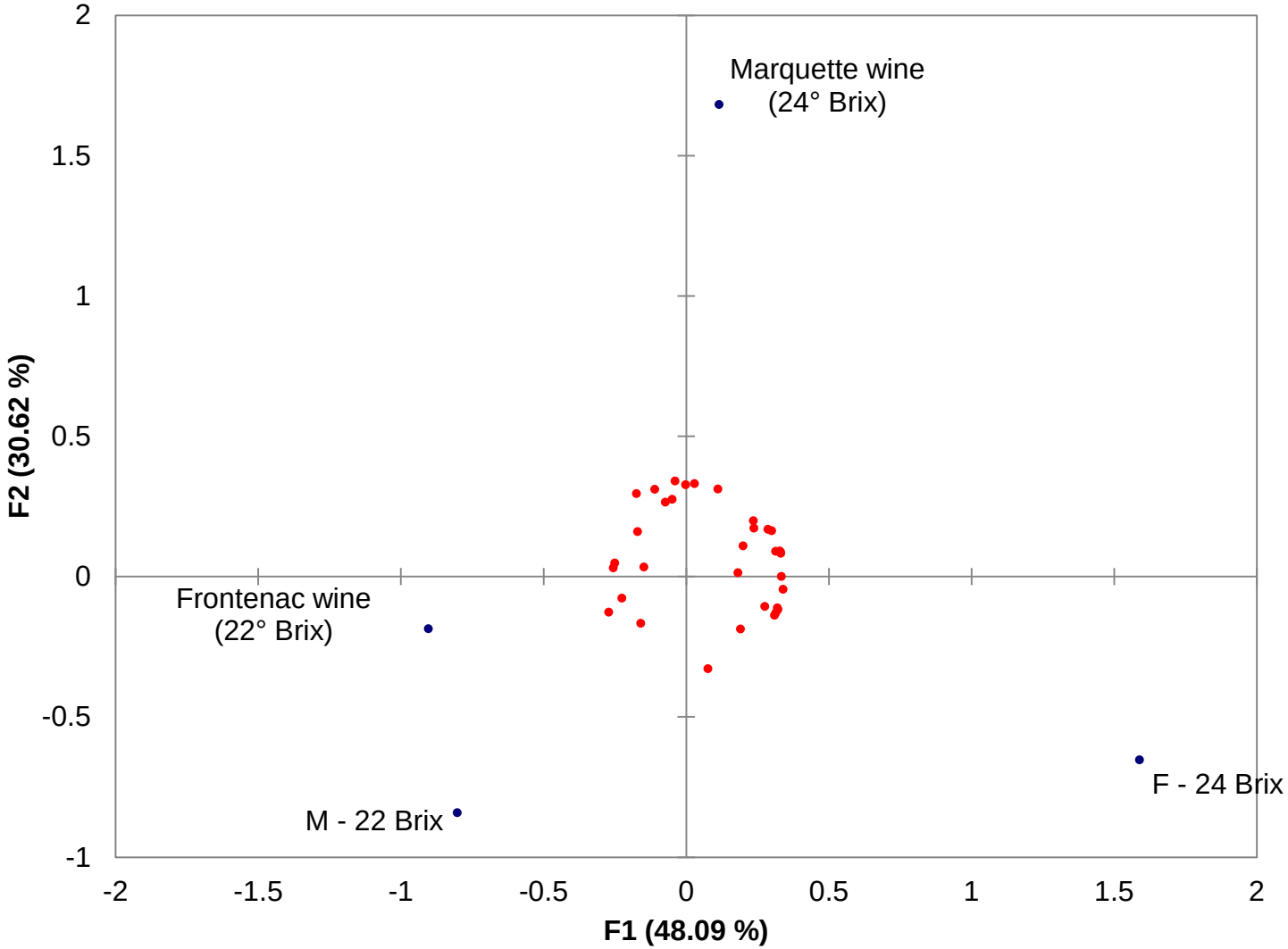

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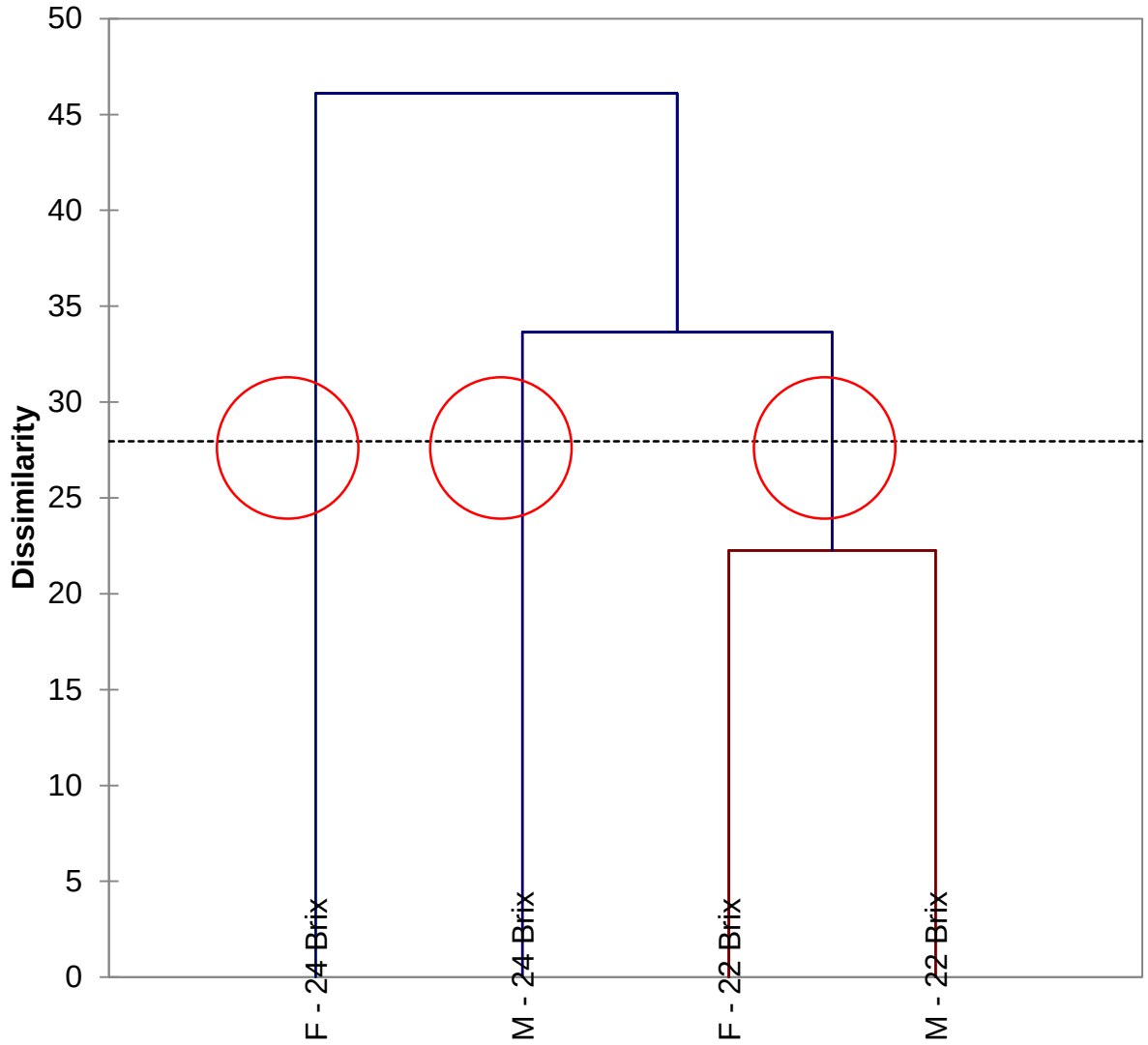

Chemical and Sensory profile analysis (ISU)

- The VOCs of Marquette and Frontenac wine made from berries harvested at 24° Brix are unique from each other and from Marquette and Frontenac wine made from berries harvested at 22° Brix.
- The VOCs of Marquette and Frontenac wine made from berries harvested at 22° Brix are most similar to each other.
- Similar work is being done on skin contact studies of La Crescent and Edelweiss wines, and harvest times of Brianna and Frontenac Gris wines.

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Viticulture, enology and marketing for cold-hardy grapes

From Vine to Glass: Understanding the Flavors and Aromas of Cold-Hardy Grapes and Wine … (Part 3!)

Adrian Hegeman

Departments of Horticultural Science and Plant Biology

University of Minnesota

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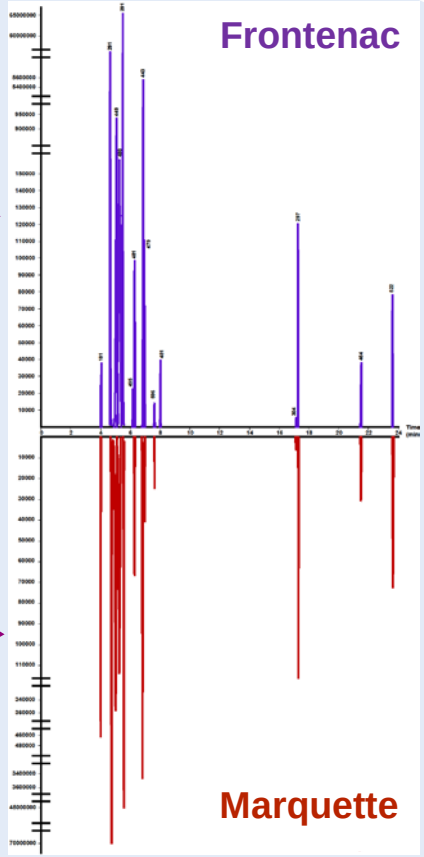
www.northerngrapesproject.org

Fruit Composition & Metabolomics

Soon Li Teh, Bety Rostandy,
Adrian Hegeman & Jim Luby – **UMN**

Goal:
Identify genes that can be used as markers for selection of desirable aroma, pigmentation, organoleptic trait metabolites (and removal of undesirable traits) via marker assisted breeding

Cultivars



Ripening profiles

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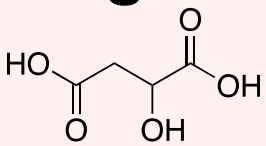
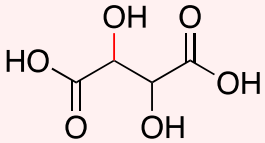
Plant Metabolomics Laboratory
Dept. Horticultural Science
University of Minnesota

Grape Berry Metabolic Profiling by LC-MS:

Organic acids:

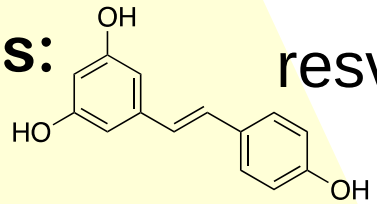


malic acid



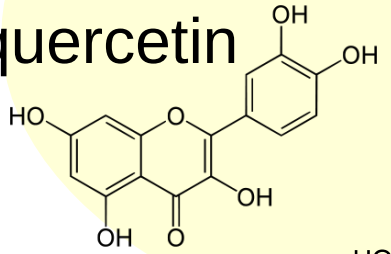
tartaric acid

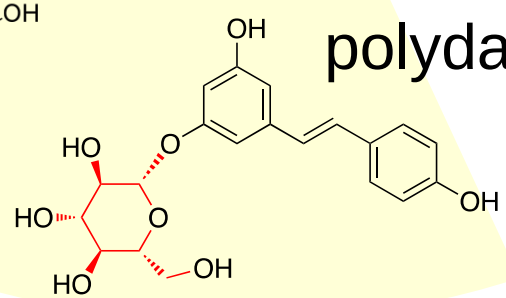
Polyphenolics:



resveratrol

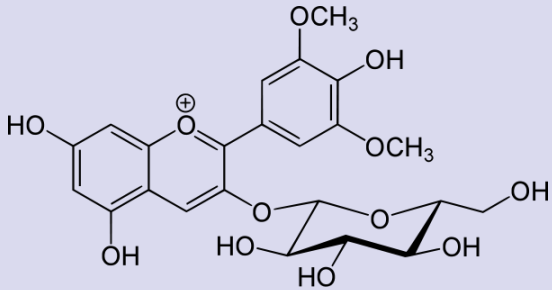
quercetin



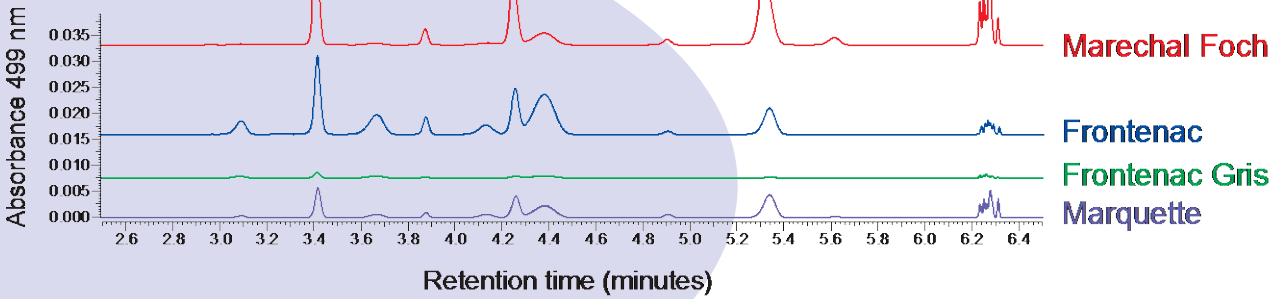


polydatin

Anthocyanins:



malvidin

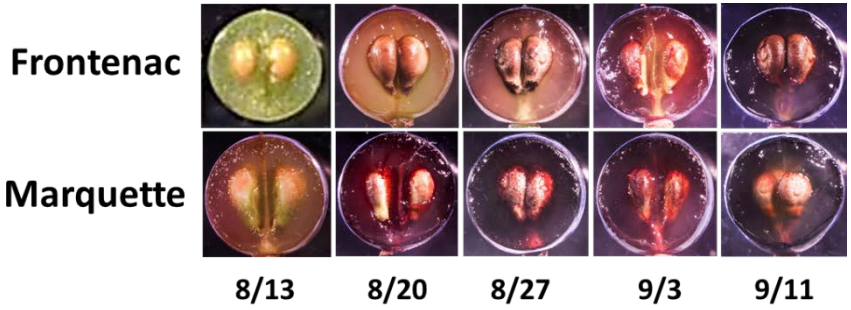


Berry Samples
20 to 26° Brix

Transcriptome
(gene expression)

Metabolome
(sugars, acids, flavors, aromas anthocyanins, tannins)

Sensory descriptors



Workflow


http://license.umn.edu/

Skin

Pulp & seed → Extract with ethanol

Homogenize

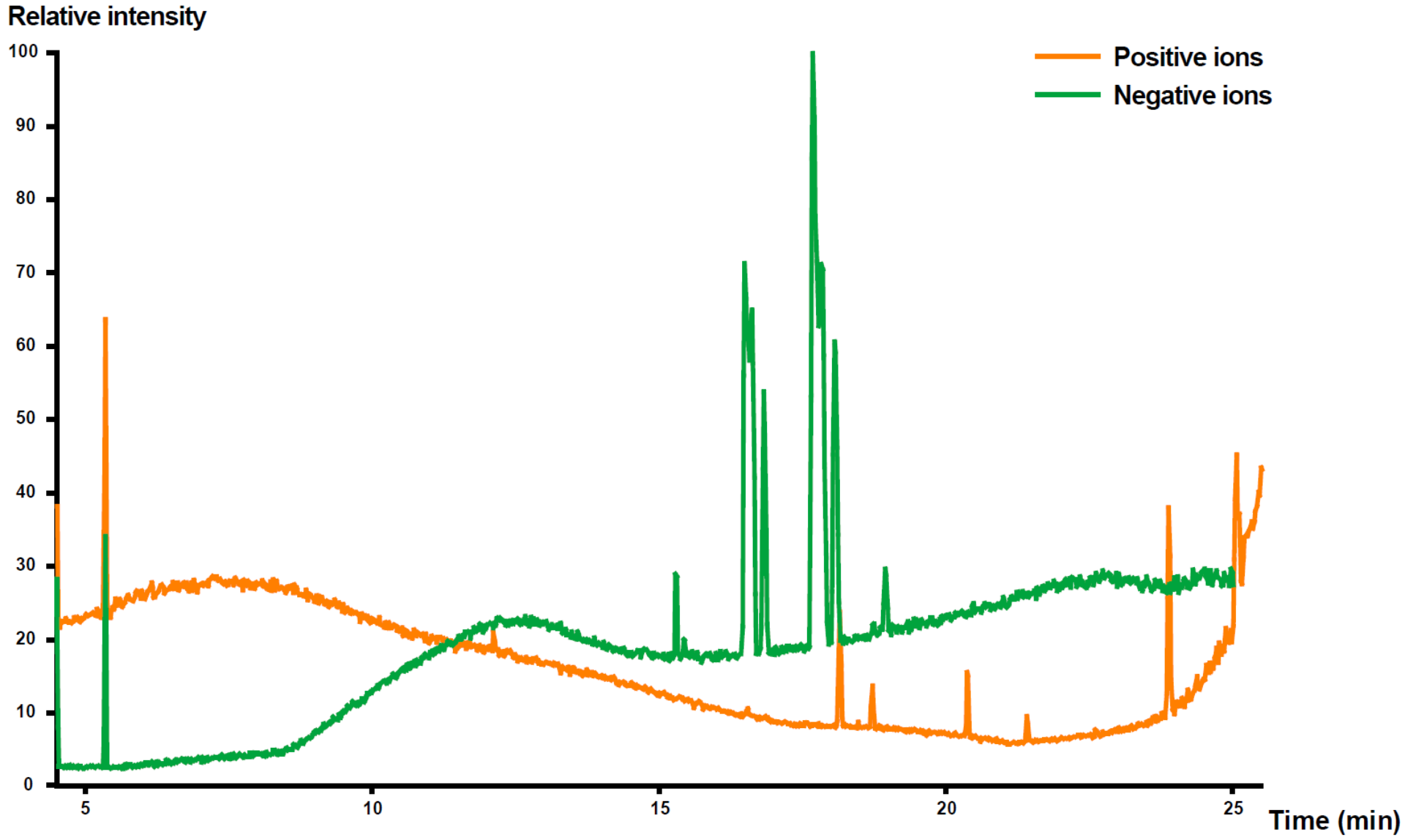
Centrifuge

Retain supernatant

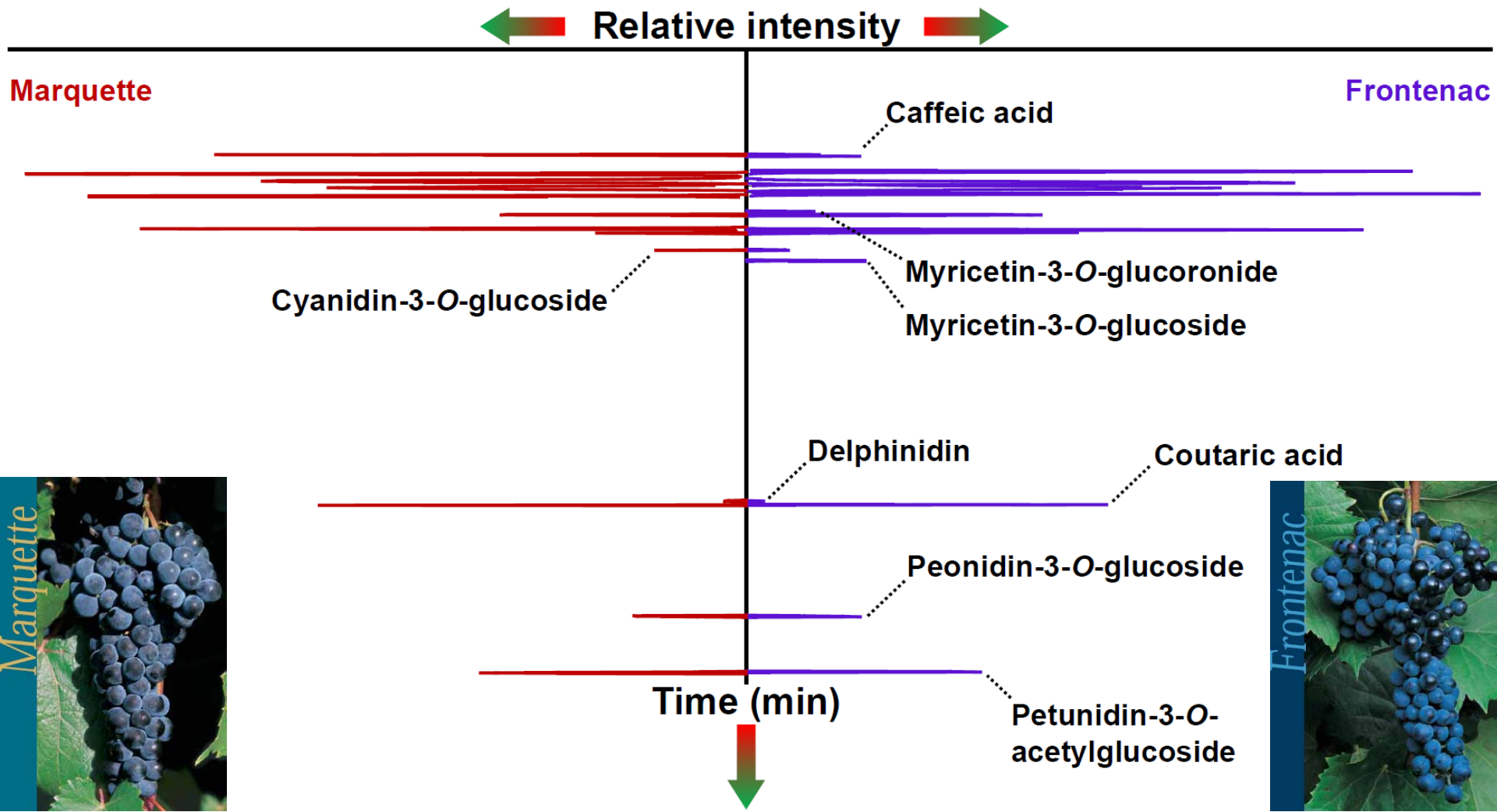
Analyze by LC–MS (liquid chromatography–mass spectrometry)


http://www.thermoscientific.com/

Chromatogram



Comparisons of Extracted Ion Chromatograms



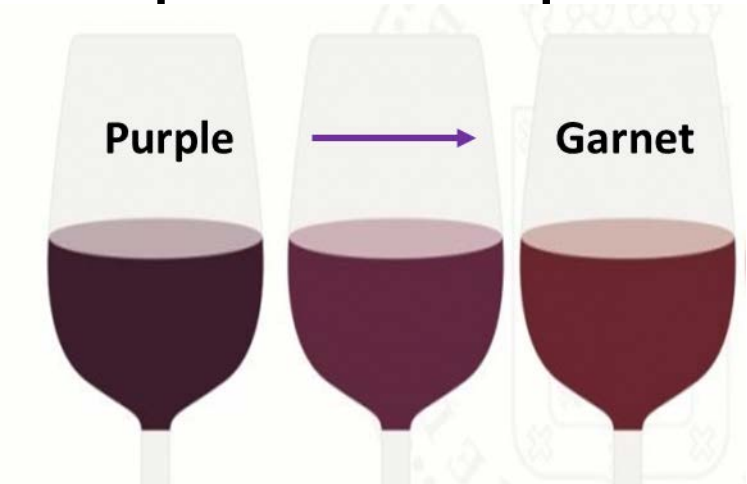
Genomics and Fruit Composition: Link Genetics to Sensory (SDSU, UMN, IA State)

Do gene expression differences coincide with existing knowledge?

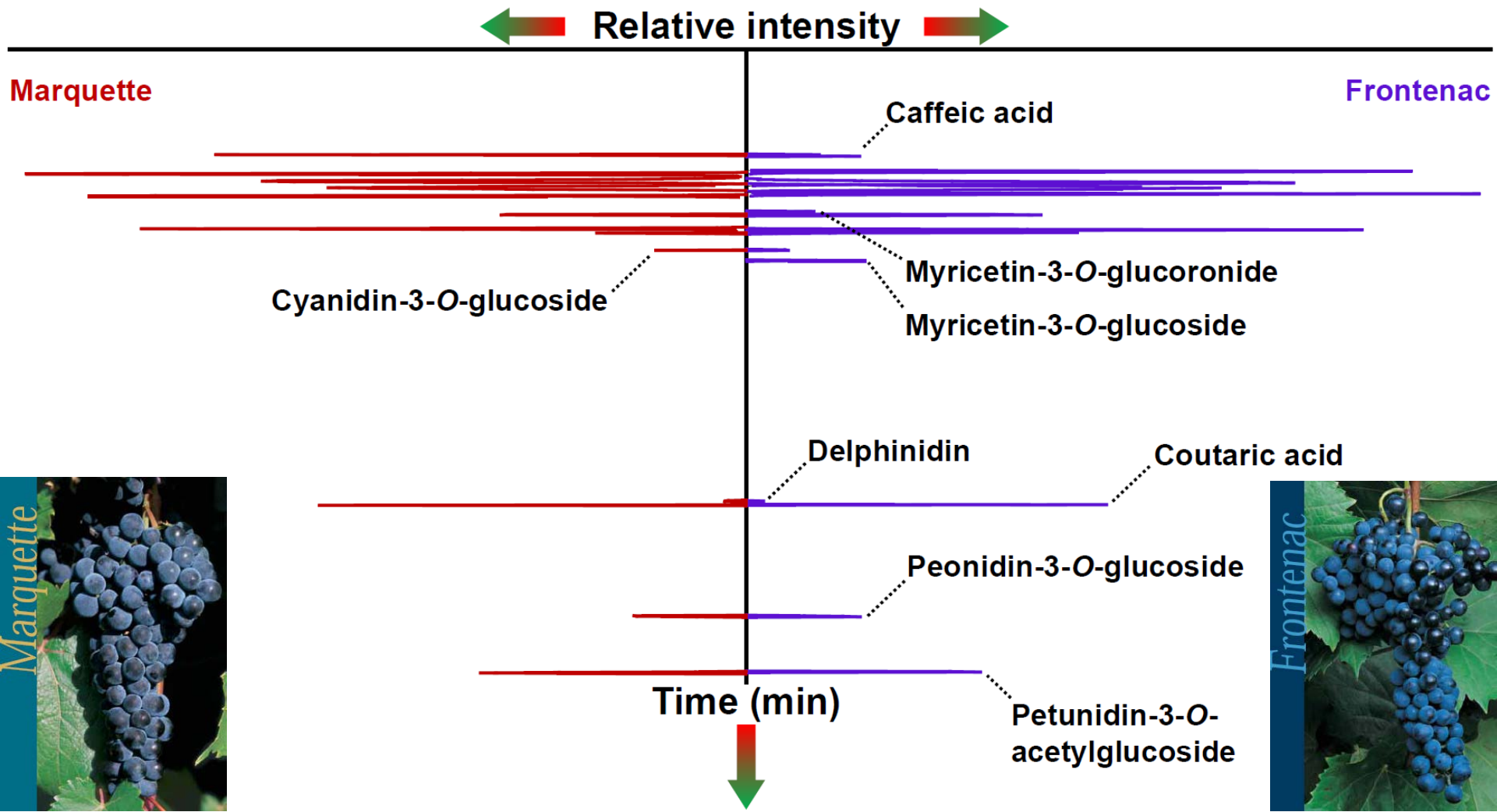
- **Anthocyanin biosynthesis genes preferentially expressed in berry skins**
- **Expression of anthocyanin biosynthesis genes significantly greater expression in Frontenac than Marquette**

	Front Skin	Marq Skin
VIT_05s0062g00720	36	
VIT_12s0034g00080	47	
VIT_02s0033g00450	159	42
VIT_02s0033g00390	165	84
VIT_05s0049g01020	429	120
VIT_02s0033g00380	531	219

Several anthocyanin biosynthesis genes highly expressed in Frontenac in comparison to Marquette

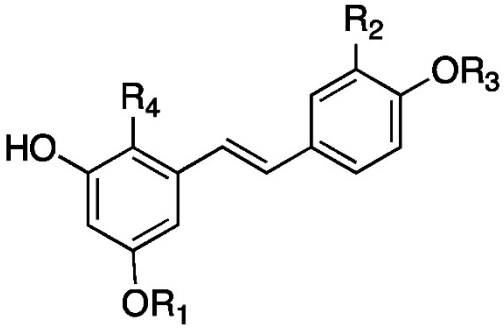


Comparisons of Extracted Ion Chromatograms



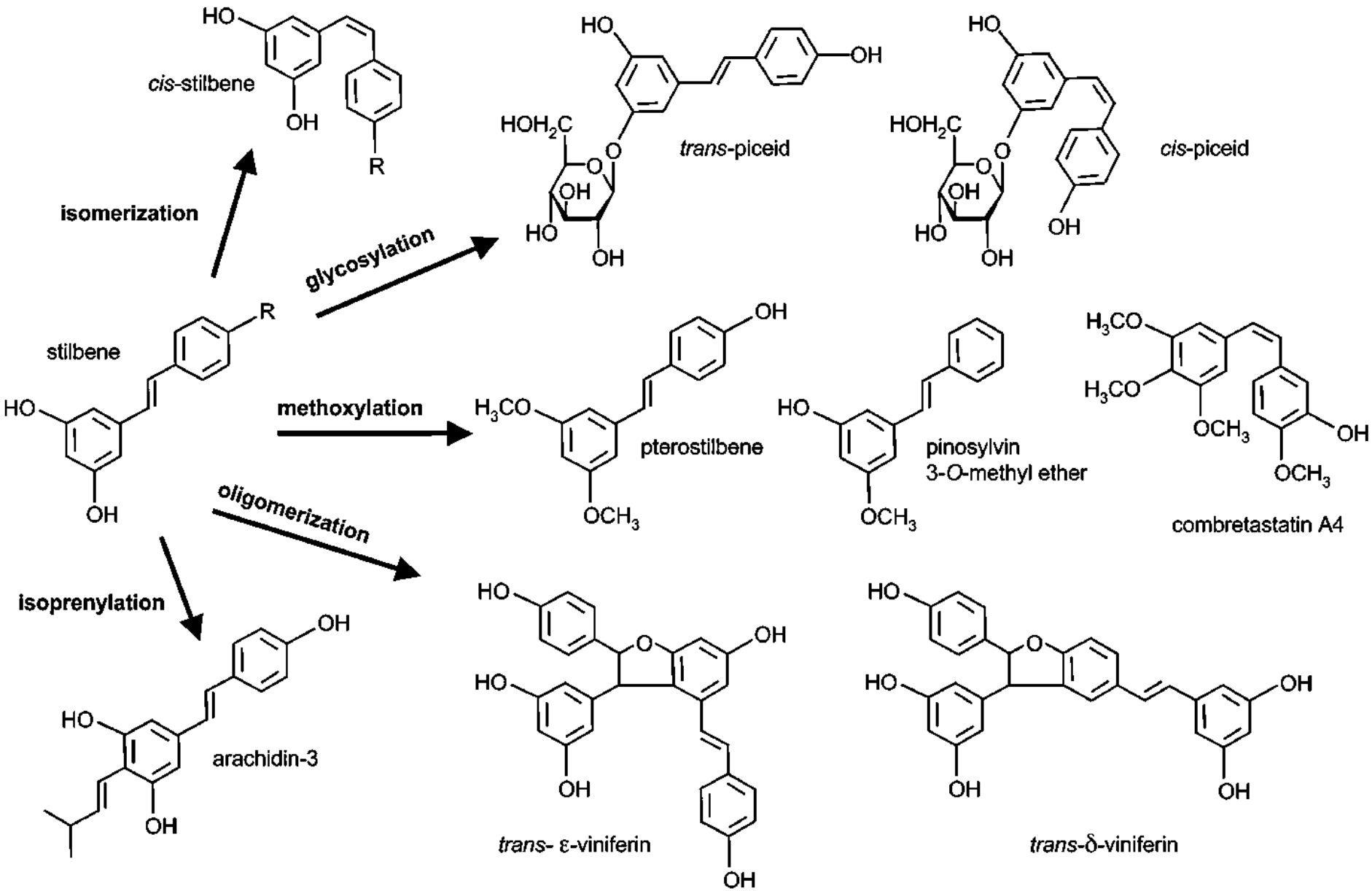
Stilbenoids

- A class of polyphenolic secondary metabolites
- Receive much attention due to:
  - Health-promoting properties
  - Phytoalexins in *Vitaceae*



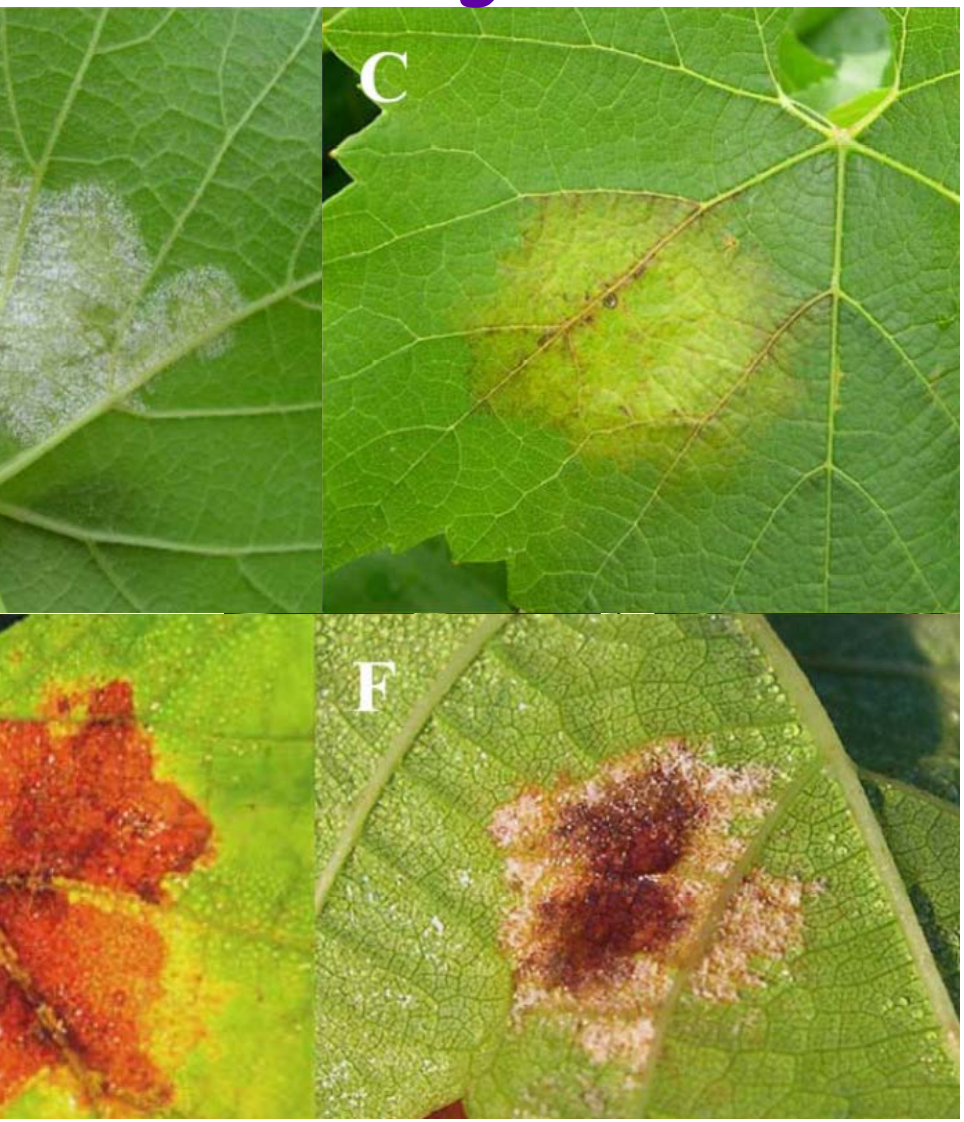
Resveratrol: $R_1,R_2,R_3 = H$, $R_4 = H$
Piceid: $R_1 = Glc$ $R_2,R_3,R_4 = H$
Piceatannol: $R_1,R_3,R_4 = H$, $R_2 = OH$
Resveratroloside: $R_1,R_2,R_4 = H$, $R_3 = Glc$

Pawlus *et al*. (2013). *Journal of agricultural and food chemistry*, *61*(3), 501-511.



Chong *et al*. (2009). *Plant Science*, *177*(3), 143-155.

Downy and Powdery Mildews



Kennelly *et al*. (2007). *Phytopathology*, *97*(4), 512-522.

PHOTO BY R. PEARSON

Fig. 3. Distortion of young leaves.

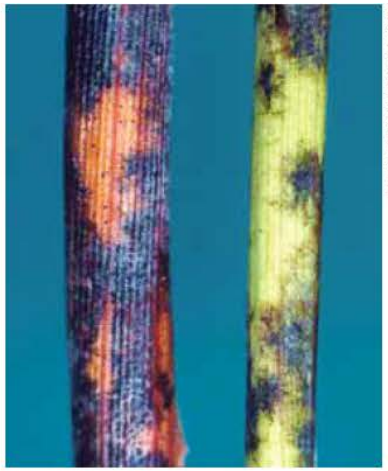


PHOTO BY R. PEARSON

Fig. 4. Dark lesions on green shoots.

PHOTO BY R. PEARSON

Fig. 7. Dark, dusty fruit infections.

PHOTO BY R. PEARSON

Fig. 8. Shriveled and cracked

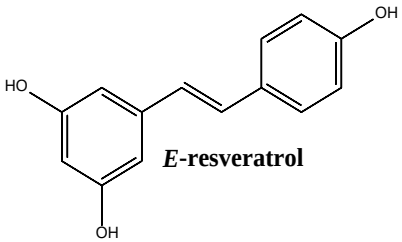
Disease Identification Sheet No. 102GFSG-D2 by NY State IPM

Stilbenoids *versus P. viticola*

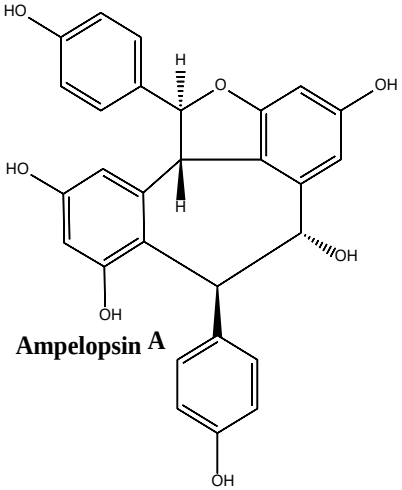
Study	Compounds	ED_{50} (µM)	
		Zoospore mobility	Disease expression
Pezet *et al*. 2004	*E*-resveratrol	192	145
	E-ε-viniferin	73	74
	E-δ-viniferin	15	15
	E-pterostilbene	28	13

Study	Compounds	IC_{50} (µM)	
		Zoospore mobility	Sporulation
Schnee *et al*. 2013	Ampelopsin A	124	282
	Hopeaphenol	17	26
	E-resveratrol	122	121
	Ampelopsin H	92	282
	E-ε-viniferin	66	63
	E-vitisin B	13	12

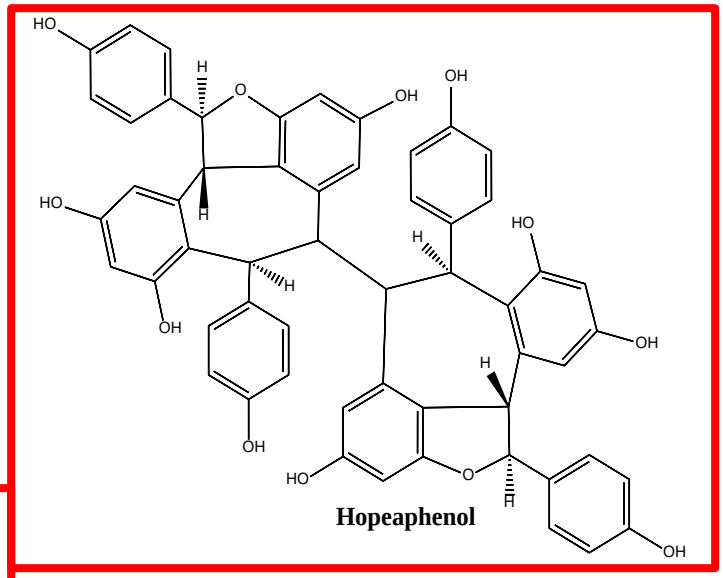
Effective dose (ED_{50}): Dose causing 50% of the maximum biological effect.
Inhibitory concentration (IC_{50}): Concentration causing 50% inhibition.



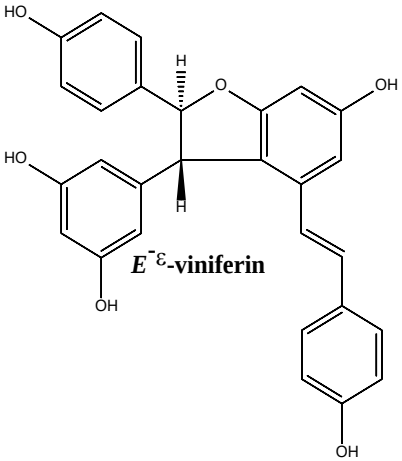
***E*-resveratrol**



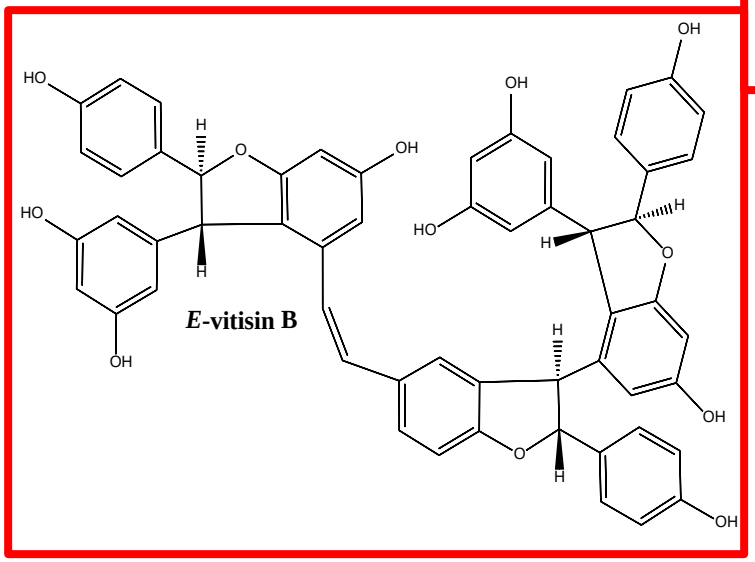
Ampelopsin A



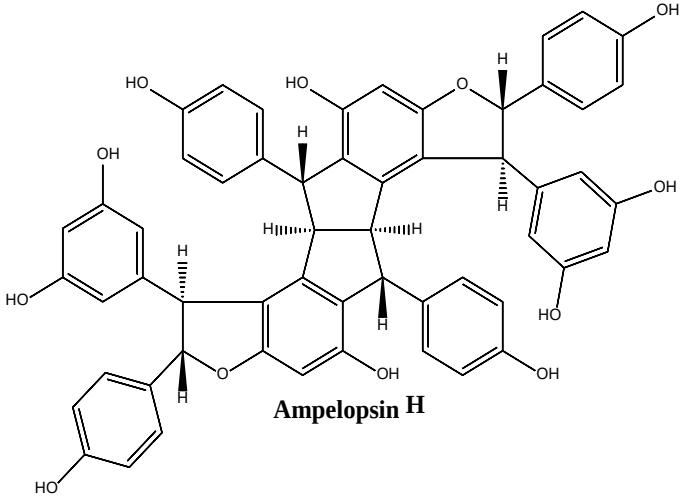
Hopeaphenol



***E*$^{-}$ε-viniferin**

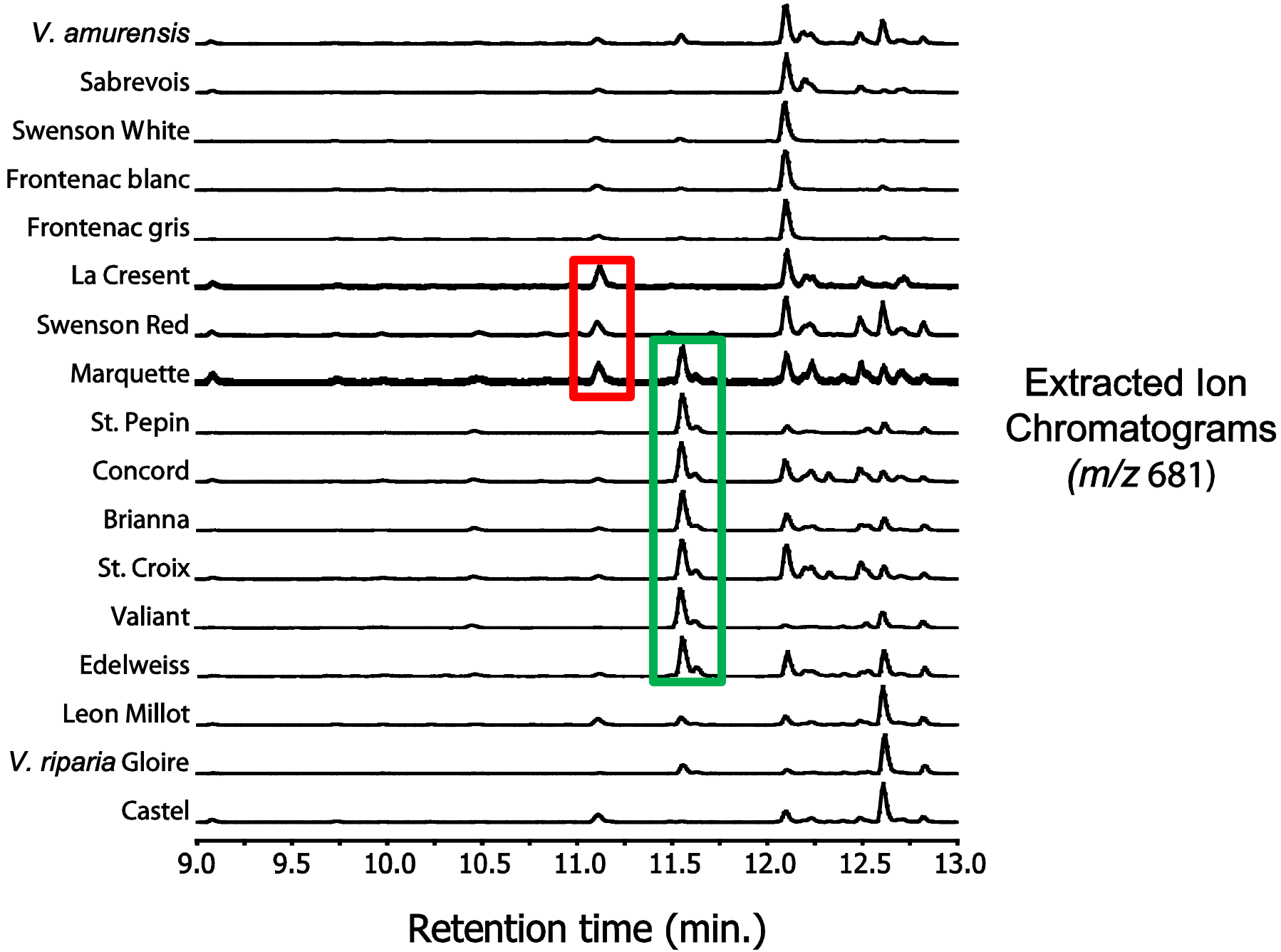


***E*-vitisin B**

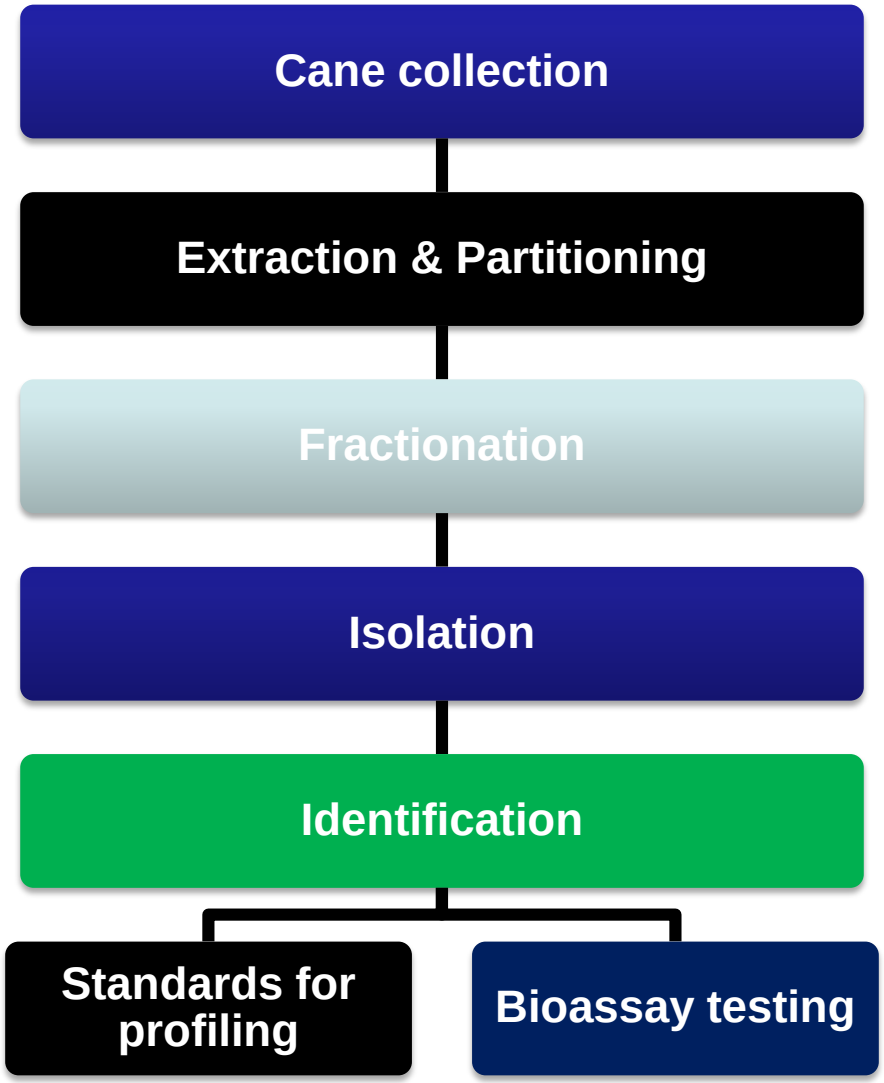


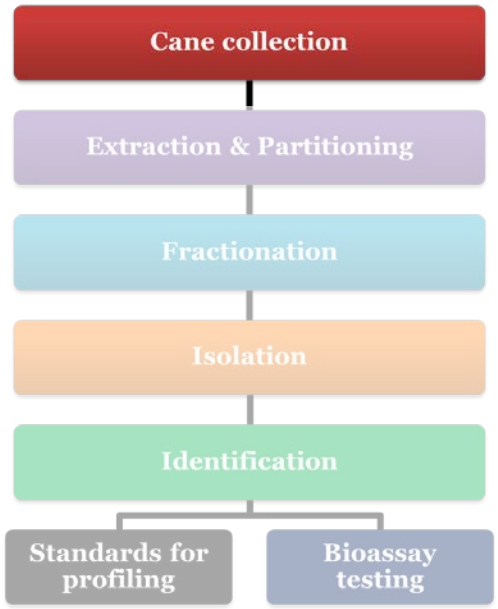
Ampelopsin H

Structure credit: Alison Pawlus

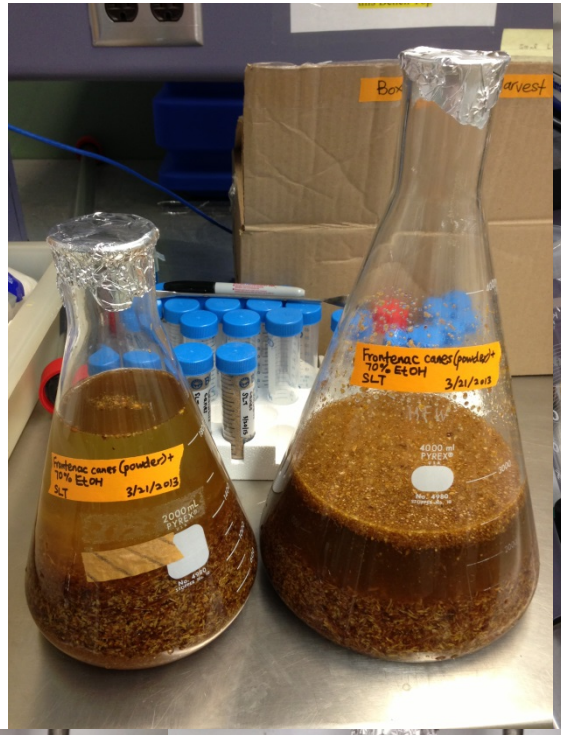

V. amurensis
Sabrevois
Swenson White
Frontenac blanc
Frontenac gris
La Cresent
Swenson Red
Marquette
St. Pepin
Concord
Brianna
St. Croix
Valiant
Edelweiss
Leon Millot
V. riparia Gloire
Castel
9.0
9.5
10.0
10.5
11.0
11.5
12.0
12.5
13.0
Retention time (min.)
Extracted Ion Chromatograms (m/z 681)

Isolation & Identification of Stilbenoids



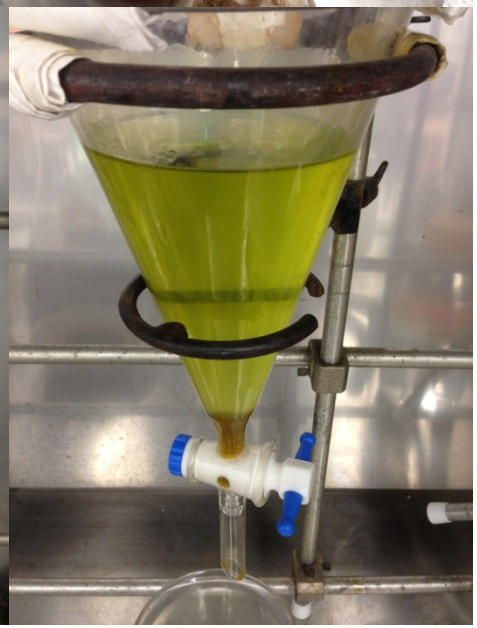


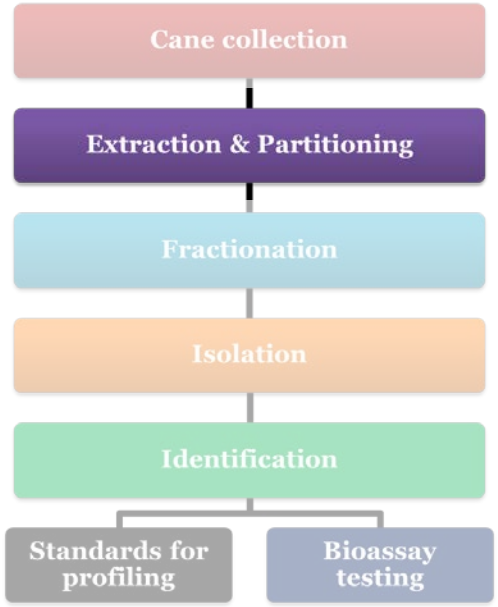
Cane credit:
John Thull

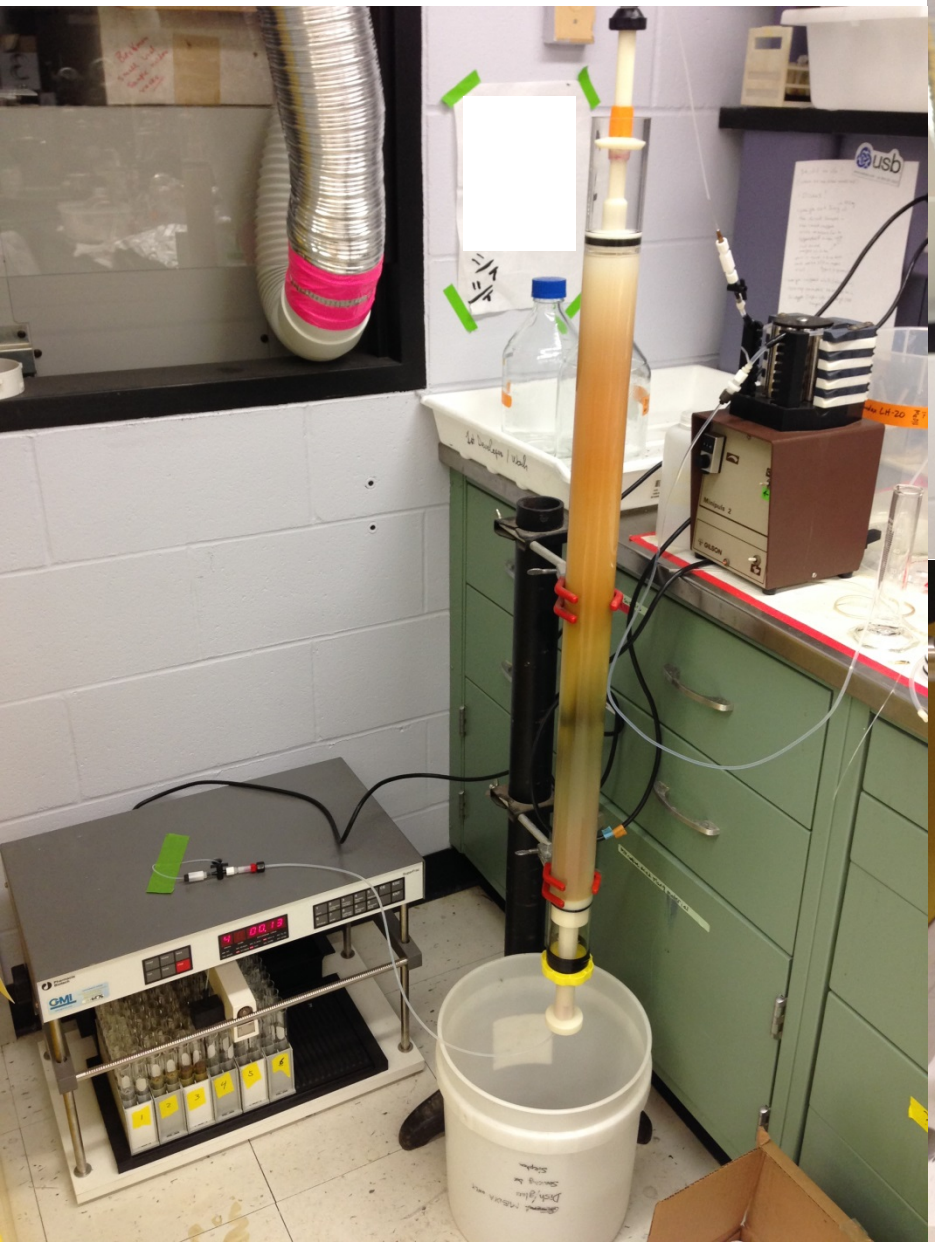

Frontenac canes (powder)+
70% EtOH
SLT
3/21/2013
2000 ml
PYREX
Frontenac canes (powder)+
70% EtOH
SLT
3/21/2013
4000 ml
PYREX

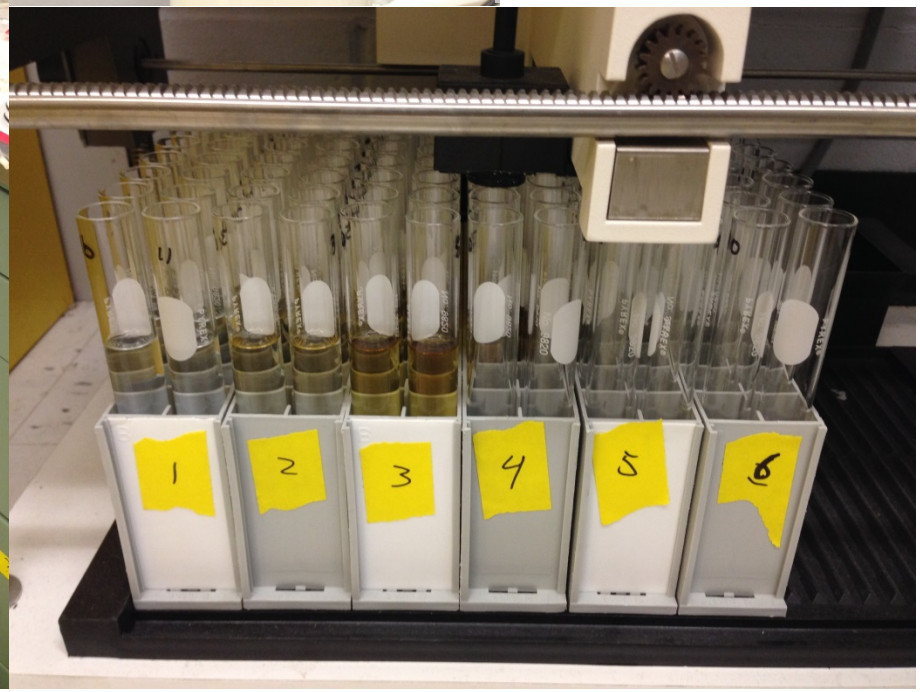

2000 ml
PYREX


2000 ml
PYREX




Cane collection
Extraction & Partitioning
Fractionation
Isolation
Identification
Standards for profiling
Bioassay testing





Cane collection

Extraction & Partitioning

Fractionation

Isolation

Identification

Standards for profiling

Bioassay testing

Viticulture, enology and marketing for cold-hardy grapes

Kalley Besler, Padmapriya Swaminathan, Anne Fennell – SDSU

Somchai Rice, Jacek Koziel, Murli Dharmadhikari, Devin Maurer – ISU

Emily Del Bel, Soon Li Teh, Bety Rostandy, Jenna Brady, Zata Vickers,

Adrian Hegeman, Jim Luby – UMN

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www.northerngrapesproject.org