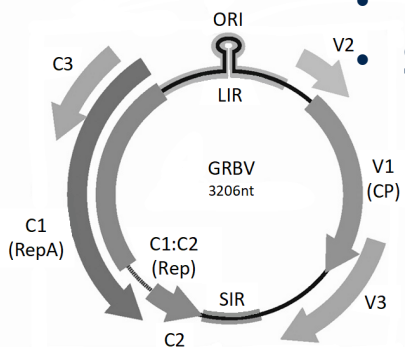


Impacts of Grapevine Red Blotch Virus (GRBV) on Grapes and Wine Composition

Anita Oberholster, S. Kaan Kurtural, Mysore (Sudhi) Sudarshana, A. Rumbaugh, C. Medina Plaza, R. Girardello, A. Boghozian

Grapevine Red Blotch Virus

- *Vitis vinifera* - one of the most susceptible plant hosts to viral infection
 - > 70 viruses recorded that potentially impact grapevine performance
 - A 'newly' identified virus is Grapevine Red Blotch Virus (GRBV)
 - DNA virus which is relatively rare
 - Part of the *Geminiviridae* family of viruses
 - Symptoms:
 - Red blotches on leaf blades
 - Reddening of primary and secondary veins in red varieties
 - Symptoms similar to late season potassium deficiency and marginal necrosis in white varieties



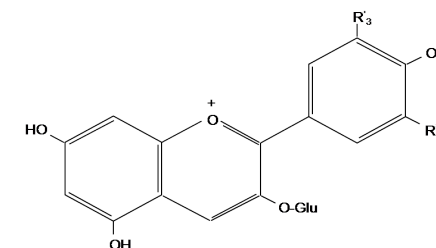
Definitions

- GRBV causal agent of GRBD
- All studies utilized symptomatic vines = RB (+)
- Symptomatic vines (healthy) = RB (-)
- Symptomatic vines = GRBV pos
 - Testing of petioles in early fall

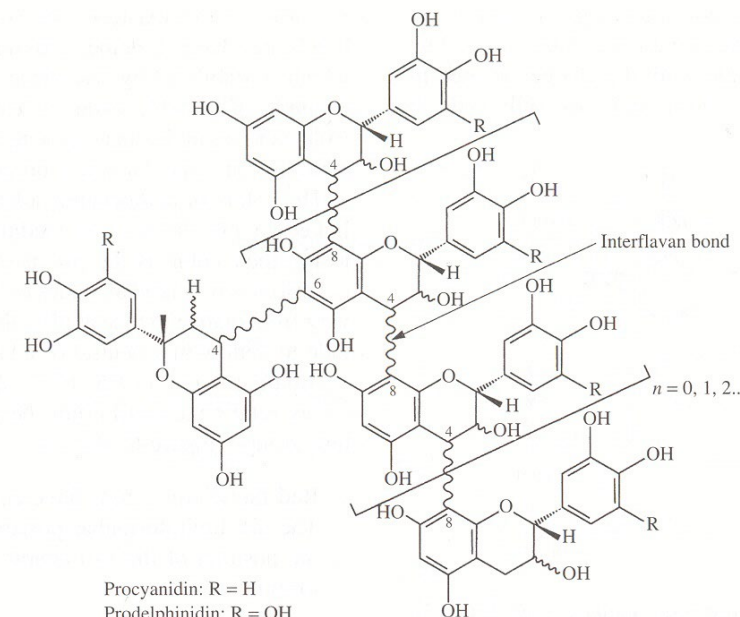


Background: Phenols in Grapes & Wines

- Main phenols (flavonoids) in grapes/wines
 - Anthocyanins responsible for red color
 - Flavan-3-ols (ex. catechin, epicatechin, epigallocatechin, epicatechin gallate)
 - Oligomers and polymers of flavan-3-ols, so called proanthocyanidins (PA) or condensed tannins
 - Responsible for bitterness and astringency (mouthfeel characteristics of wine)
 - Flavonols (protect against UV radiation)
 - Mild bitter taste and can co-pigment with anthocyanins to increase, stabilize color
 - Hydroxycinnamic acids
 - Mild acidic taste, responsible for color in white wine, easily oxidize and can cause browning
 - Can also act as co-pigments



Anthocyanin



Polymeric phenol (Tannins)

Research progression

- Initially investigated the impact of GRBD (grapevine red blotch disease) on the composition of grapes at harvest and the resulting wines
 - Potential sensory and quality differences between wines made from GRBV positive and negative grapes
 - Screening of CH, ME and CS vineyard over two years (2014-2015)



Grape Analyses

- Secondary metabolites

Total Phenols

Anthocyanins

Tannins

Flavan-3-ols

Flavonols

Polymeric Pigments

Aroma compounds



Primary metabolites

Brix

Sugar Loading

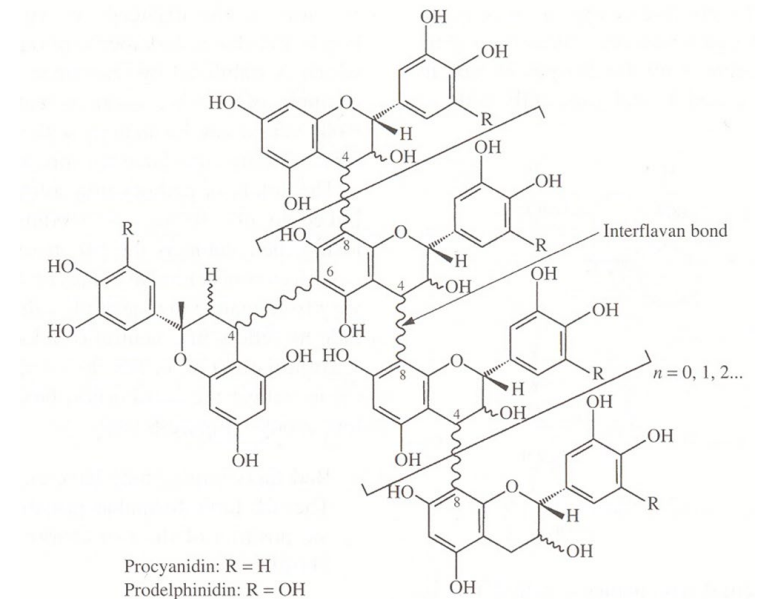
pH

TA (titratable acidity)

Malic acid

Impact of RB disease on grape composition

- Main findings
 - Both primary and secondary metabolites impacted by GRBV
 - In general, ↓ sugar accumulation
 - CH ↓ 0 – 6% Brix
 - ME ↓ 6 – 16% Brix
 - CS ↓ 4 – 20% Brix
 - RB (+) ↑ amino acids and organic acids
 - Trend of increased skin tannin in RB (+) grapes
 - Increased light exposure
 - Plant response to pathogen – defence mechanism

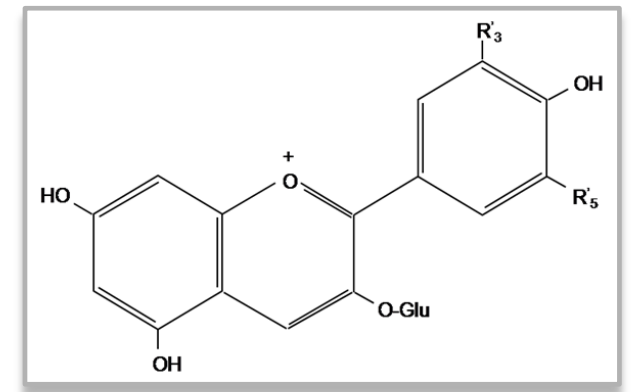


Girardello et al. (2019) *J. Agric. Food Chem.* 67, 2437-2448. DOI: 10.1021/acs.jafc.8b05555

Impact of RB disease on grape composition

- RB (+) trend of ↓ anthocyanin (red pigment) concentrations in red varieties
- Volatile (aroma) compounds impacted
- Grapevine red blotch disease (GRBD) impact varied greatly by site and season within a variety
 - Compounded by vineyard variability

Girardello et al. (2019) *J. Agric. Food Chem.* 67, 2437-2448. DOI: 10.1021/acs.jafc.8b05555



Omics (transcriptomics & metabolomics)

- GRBV inhibited or delayed ripening events (Blanco-Ulate et al. 2017)
 - Down regulation of the phenylpropanoid pathway (flavonoid synthesis)
- Multi-seasonal study using CS on rootstocks 110R and 420A (Rumbaugh et al. 2022)
 - GRBV impact on amino acid and malate acid levels
 - ↑ Volatile aroma compounds derived from lipoxygenase pathway (C6-compounds – ‘green’ aromas like leaves and fresh cut grass)
 - ↓ Anthocyanin synthesize from phenylpropanoid pathway
 - Induction of plant-pathogen interactions at pre-veraison
 - Shift from metabolic synthesis and energy metabolism to transcription and translation processes associated with virus-induced gene silencing
 - This plant derived defense was significantly upregulated at veraison across seasons and genotypes

Blanco-Ulate et al (2017) J. Exp. Bot. 68 (5): 1225-1258; Rumbaugh et al. (2022) Int J. Molecular Sci. 23: 13248 <https://doi.org/10.3390/ijms2321132483>

Wine Analyses

Primary/Secondary Metabolites

Alcohol

Residual Sugars (RS)

pH

TA

Anthocyanins

Tannins

Flavonols

Hydroxycinnamic acid

Flavan-3-ols

Total Phenols

Polymeric Pigments

Aroma compounds

Descriptive analysis



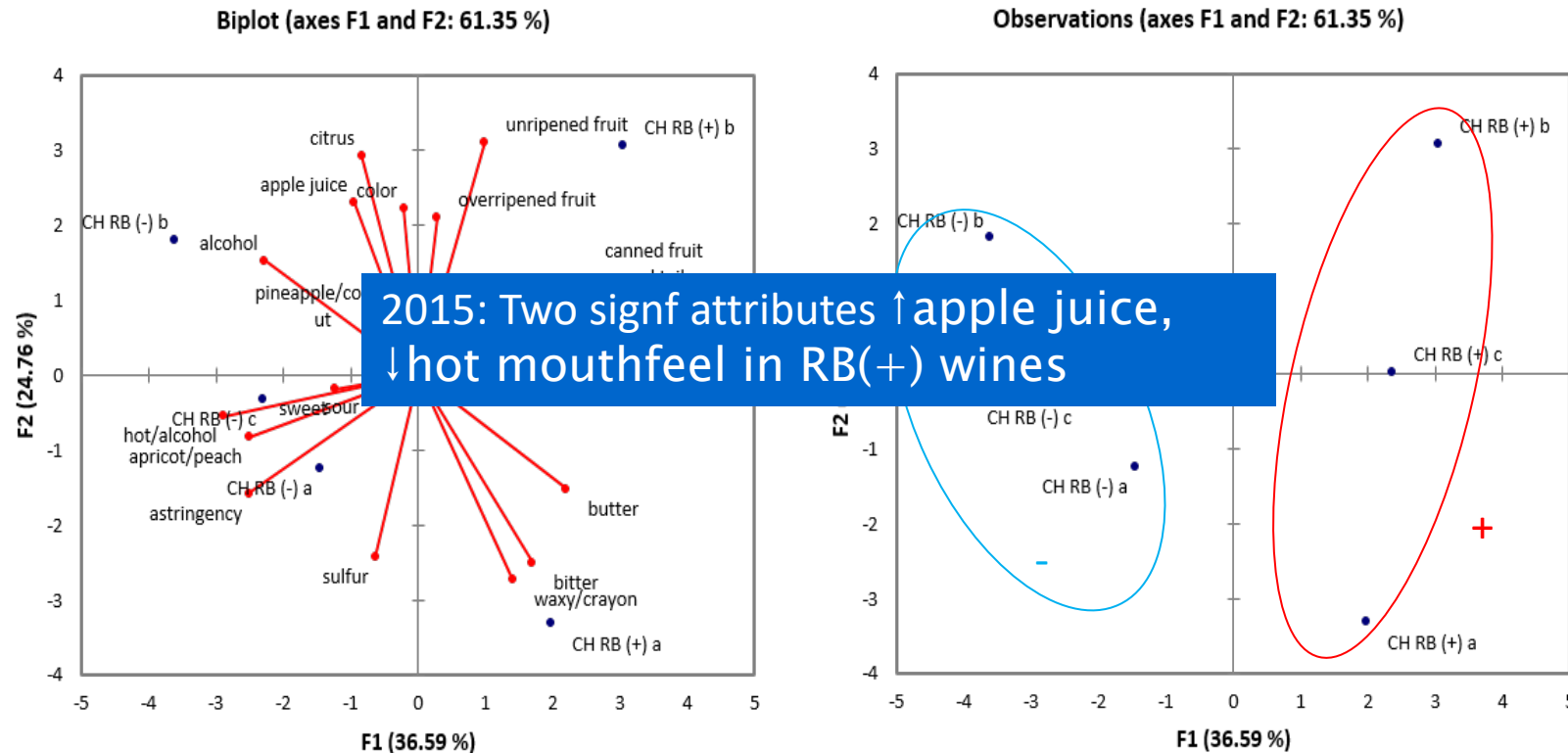
Impact of RB disease on wine composition

- Differences between RB (+) and RB (-) grapes were mostly carried over into the resulting wines
 - Both primary and secondary metabolite differences
 - Phenolic content
 - % EtOH



Girardello et al. (2020) Journal of the Science of Food and Agriculture, 100:4, 1436-1447, <https://doi.org/10.1002/jsfa.10147>.

White wine sensory data 2014



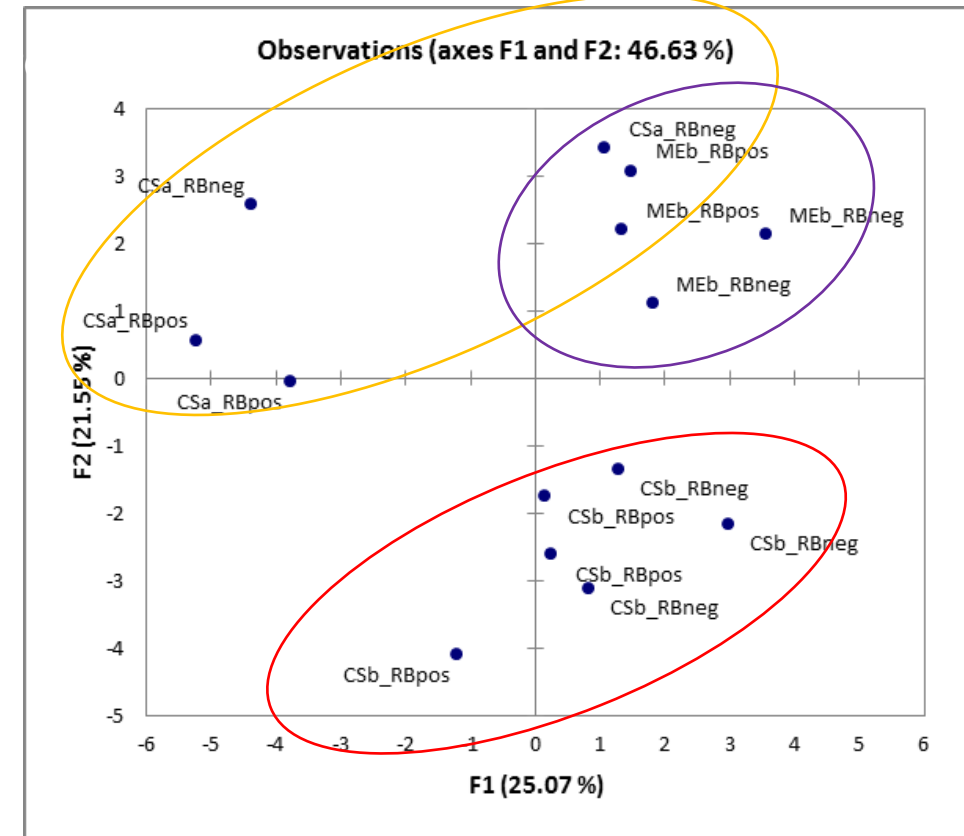
PCA scores and loading plot

- PCA separation of the wines although very little different
 - Only 1 out of 18 attributes significantly different

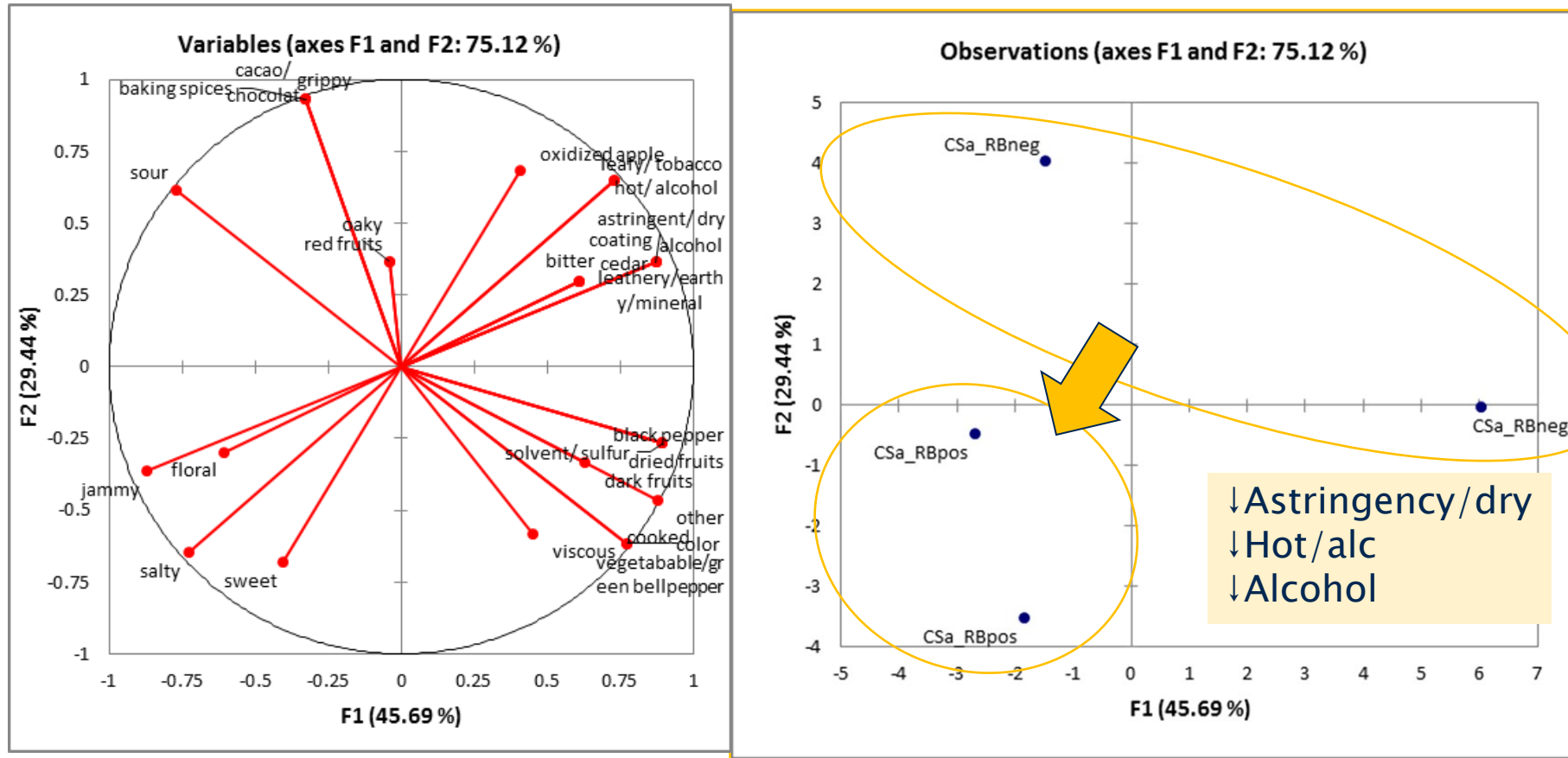
Girardello et al. (2020) Journal of the Science of Food and Agriculture, 100:4, 1436-1447, <https://doi.org/10.1002/jsfa.10147>.

Corrected F values for red DA attributes – 2014 data

Attributes	F value wine	Significant
red fruits	1.184	no
dark fruits	1.393	no
dried fruits	2.744	yes**
oxidized apple	0.484	no
jammy	0.654	no
cooked vegetables/green bellpepper	1.551	no
leafy/tobacco	2.382	no
ceder	1.085	no
leathery/earthy/mineral	0.874	no
okay	0.970	no
alcohol	3.405	yes***
solvent/sulfur	0.520	no
baking spices	0.586	no
black pepper	0.805	no
cacao/chocolate	1.666	no
floral	1.135	no
sweet	1.994	yes
sour	3.798	yes
salty	1.418	no
bitter	1.753	no
coating	2.205	yes*
viscous	0.579	no
astringent/dry	6.484	yes***
grippy	2.205	yes*
hot/alcohol	2.587	yes**
color	1.630	no



PCA: Descriptive analysis of CS (1)a



- Phenolic analyses: RB (+) ↓ [anthocyanin], [pol pigments], [pol phenols] and % Alc

Girardello et al. (2020) Journal of the Science of Food and Agriculture, 100:4, 1436-1447, <https://doi.org/10.1002/jsfa.10147>.

How to deal with RB infection in the vineyard?

- Selective harvesting?
- Making wine with 0, 5, 15, 25 and 100% RB(+) fruit included
 - Chemical (volatile and non-volatile) and sensory profiling



0%
RB



5%
RB



15%
RB

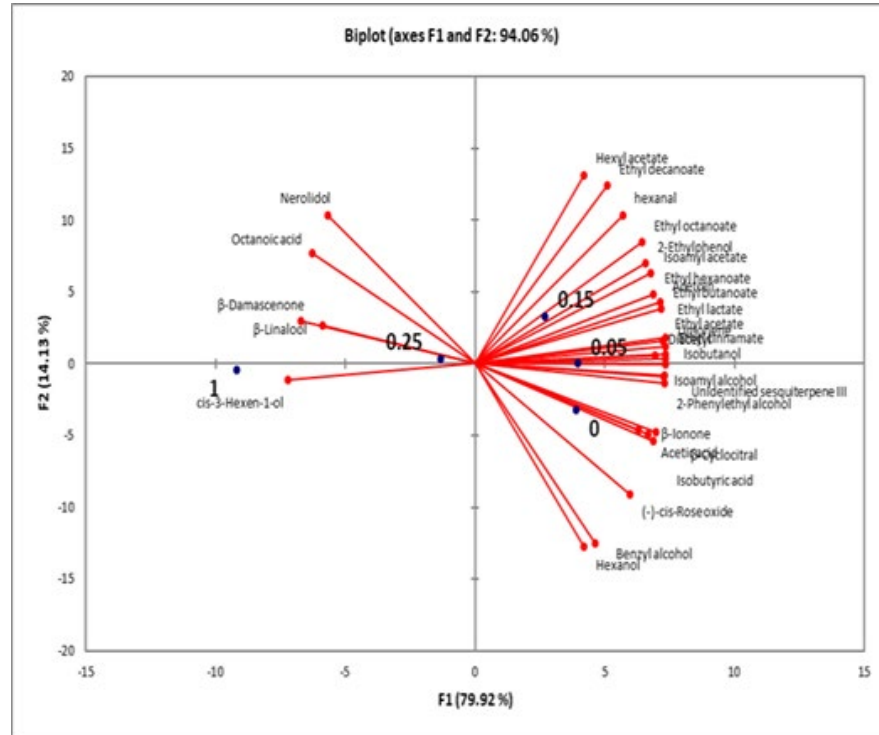


25%
RB

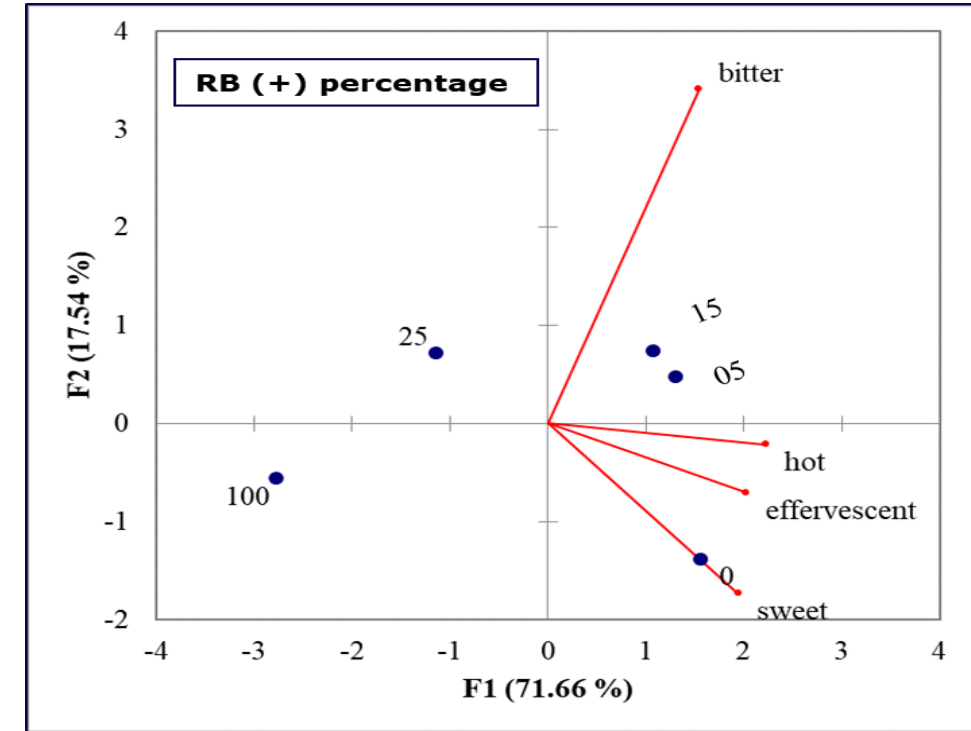


100%
RB

Volatile Compounds (GC-MS)



Descriptive analysis

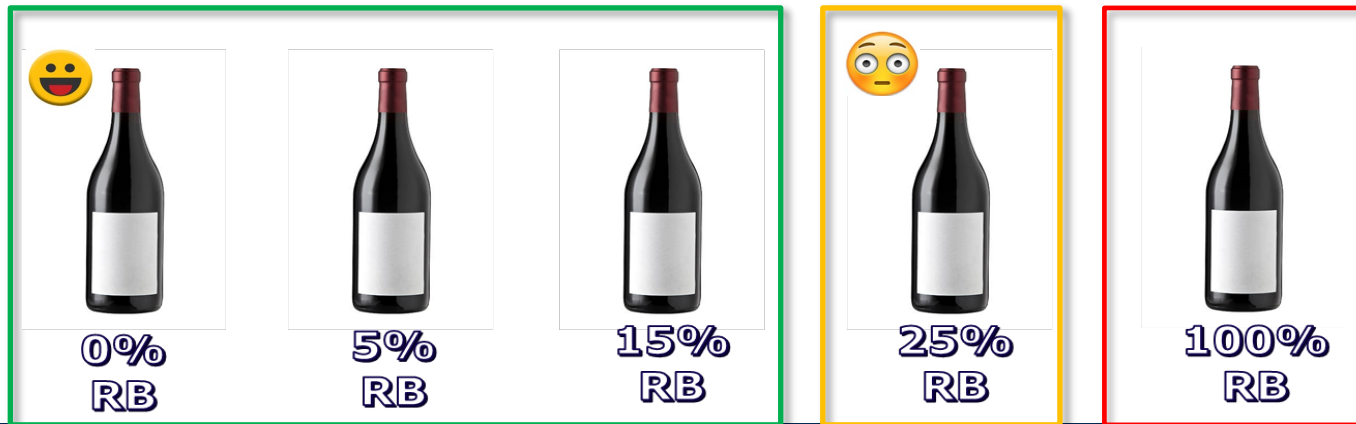


AH-assay performed on wines (n=3)

	Total Phenolics (mg/L)	Anthocyanin (mg/L)	Tannin (mg/L)
100% RB+	1226±14 a	432±1 c	376±1 a
25% RB+	1217±7 a	487±8 b	357±1 a
15% RB+	1261±17 a	535±1 a	346±3 a
5% RB+	1248±9 a	533±6 a	342±3 a
0% RB+	1115±9 b	504±3 a	295±3 b

Selective harvesting

- For this specific site and season
 - 3.6 Brix difference between RB (+) and RB (-)
- 25% RB (+) fruit included in fermentation could have significant impact
- Selective harvesting recommended at >15% incidence in vineyard
 - Recommend separate chemical analysis for healthy and diseased vines
- Make decision based on chemical difference

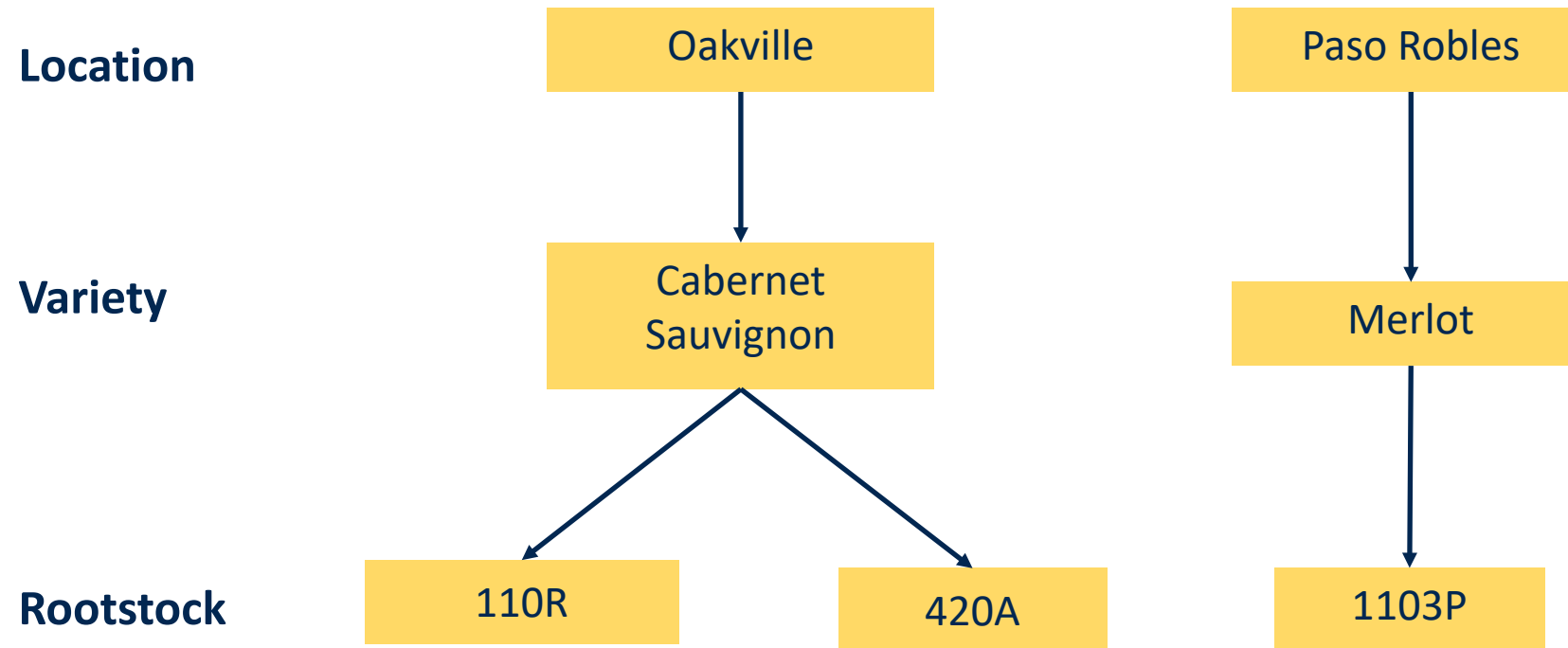


GRBV Research – Part II (2016.....)

- Investigating sequential harvesting



Red Blotch study: 2016-2017



Girardello et al. (2023) Journal of the Science of Food and Agriculture <https://doi.org/10.1002/jsfa.12983>

Grape chemical composition - 2016

Sample	GRBV Status	Harvest Date	°Brix	pH	TA (g/L)	
Merlot	-	15-Sep-16	25.2	4.2	4.2	
	+ ¹	15-Sep-16	22.1	3.8	3.4	↓12%
	+ ²	28-Sep-16	24.5	4.0	3.3	
Cab Sauv	+	20-Sep-16	25.7	3.6	3.8	
110R	+ ¹	20-Sep-16	21.8	3.5	4.8	↓15%
	+ ²	28-Sep-16	23.8	3.6	4.5	
Cab Sauv	+	20-Sep-16	24.3	3.5	4.2	
420A	+ ¹	20-Sep-16	22.2	3.5	4.5	↓9%
	+ ²	28-Sep-16	23.8	3.5	4.6	

- ↓°Brix 12% GRBV (+) ME and 9-15% in CS grapes
- Small differences in pH
- Variable TA impact of GRBV in grapes

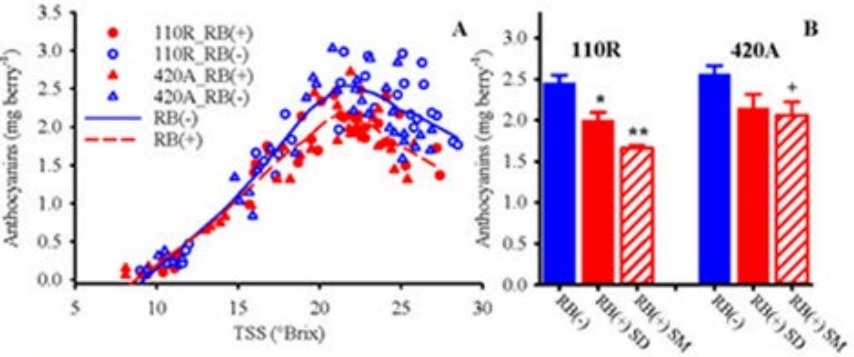
Girardello et al. (2023) Journal of the Science of Food and Agriculture <https://doi.org/10.1002/jsfa.12983>

Main Cab Sauv findings



GRBV(+) grape canopy

Poor color accumulation in GRBV (+) grape berries

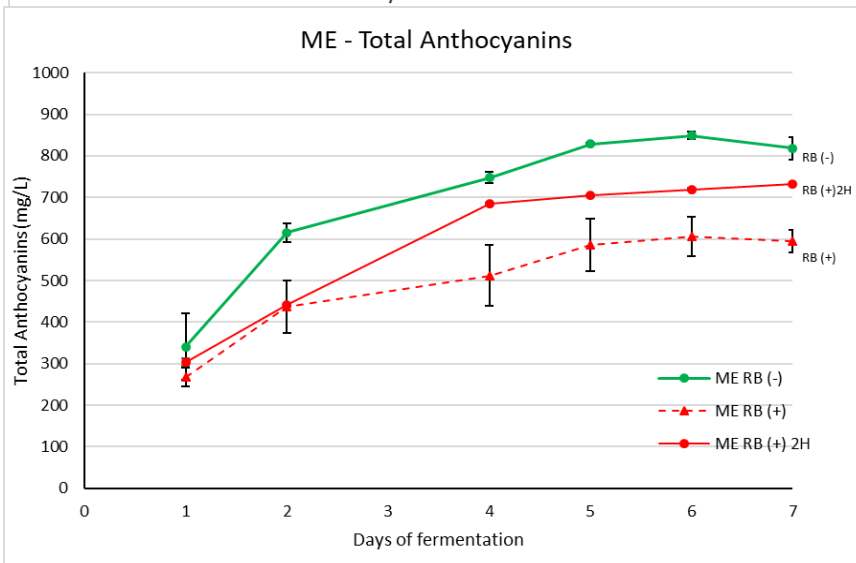
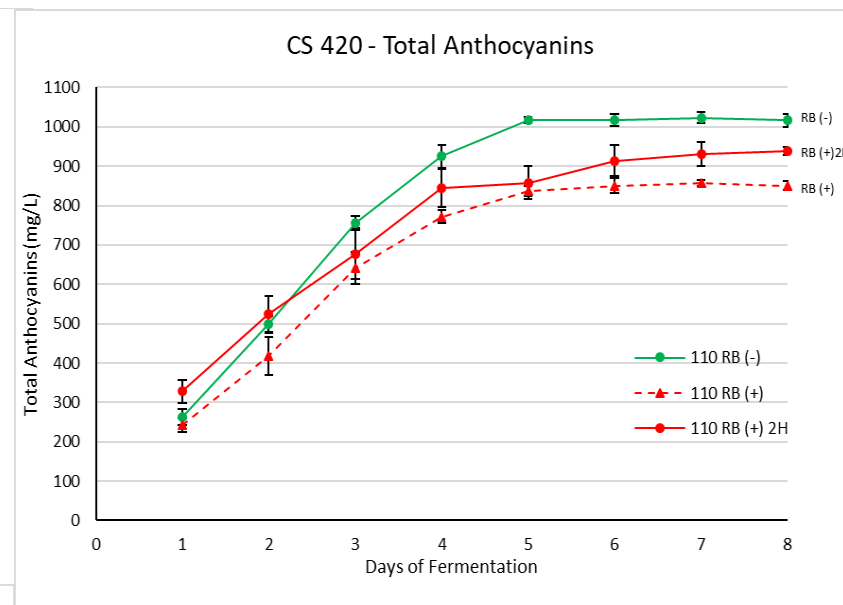
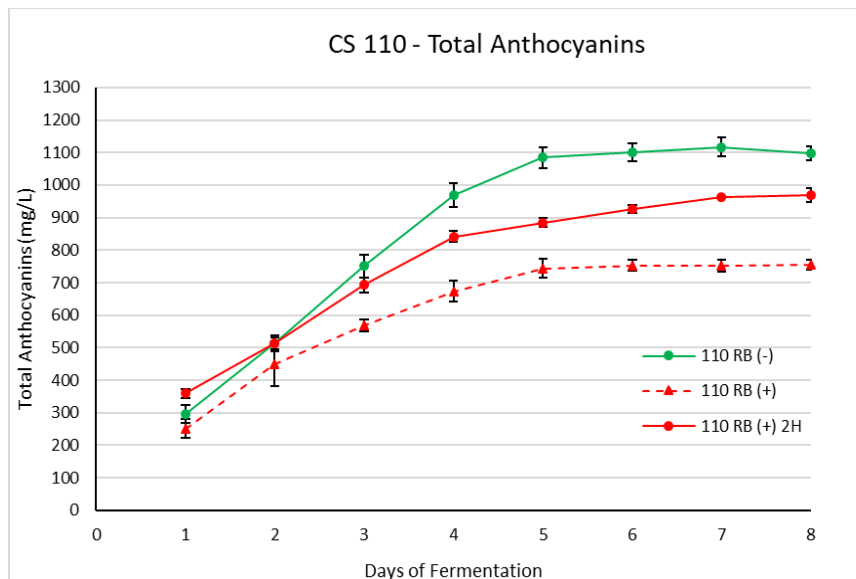


Relationship between skin anthocyanins and total soluble solids (A), comparison between anthocyanins (B) of treatments harvested on same date or same maturity

	Virus Status	
	GRBV (-)	GRBV (+)
Berry weight	↑	↓
Yield.vine ⁻¹	=	=
Ψ _{stem}	↓	↑
A _{net} (μmol CO ₂ .m ⁻² .s ⁻¹)	↑	↓
g _s (mmol H ₂ O.m ⁻² .s ⁻¹)	↑	↓
Leaf soluble sugars (mg.g ⁻¹)	↓	↑
TSS (Brix)	↑	↓
TA (g.L ⁻¹)	↓	↑
pH	↓	↑
Σ Anthocyanins mg.berry ⁻¹	↑	↓
Malvidin derivatives	↓	↑
Σ Proanthocyanidins (mg.berry ⁻¹)	↓	↑
mDP of proanthocyanidins	=	=

Martínez-Lüscher et al. (2019) *J. Agric. Food Chem.* 67, 2437-2448. DOI: 10.1021/acs.jafc.8b05555; Rumbaugh et al. (2021) *Plants* 10 (8), 1683.

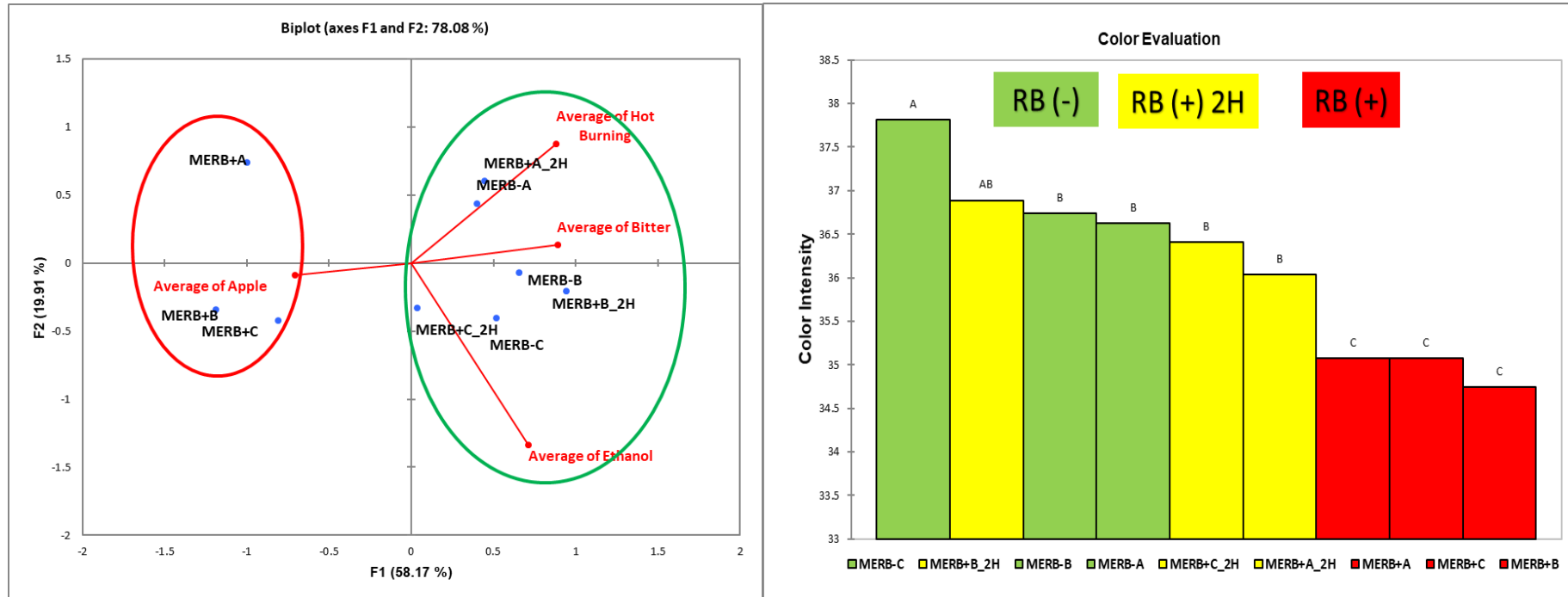
Extraction of phenolics during fermentation 2016



- Fermentation extraction profiles for anthocyanins
- Other phenolics performed similarly except for flavan-3-ols where there was no difference

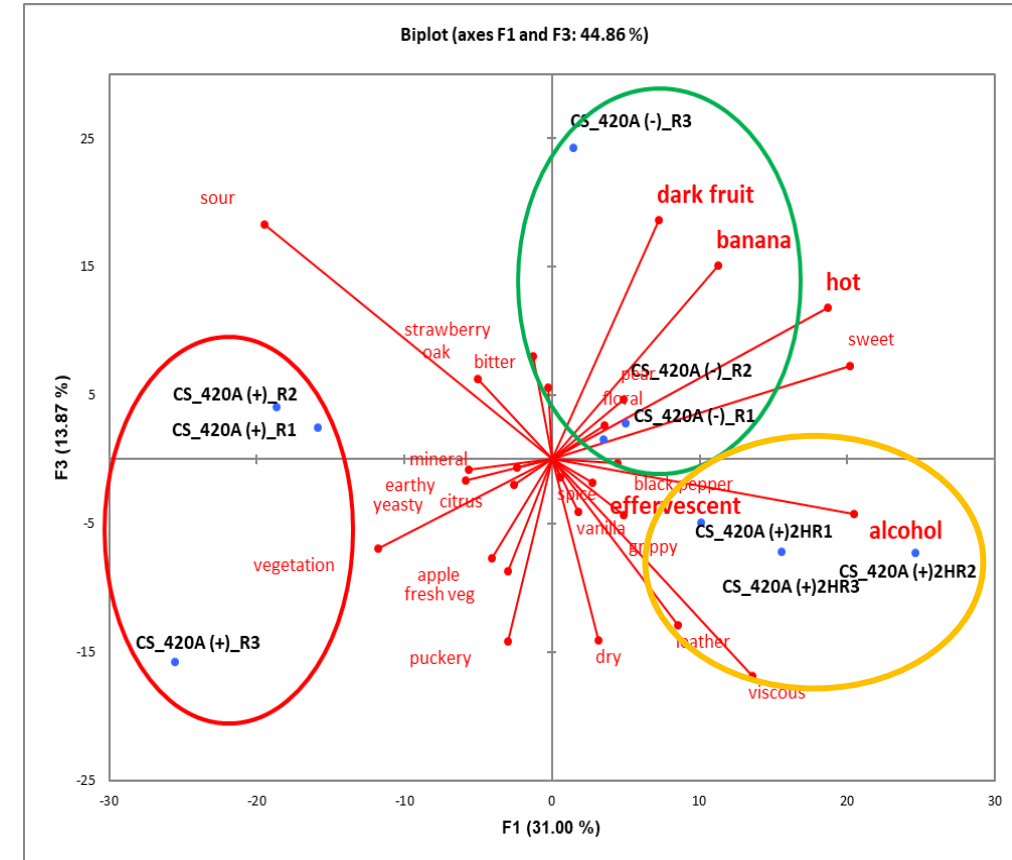
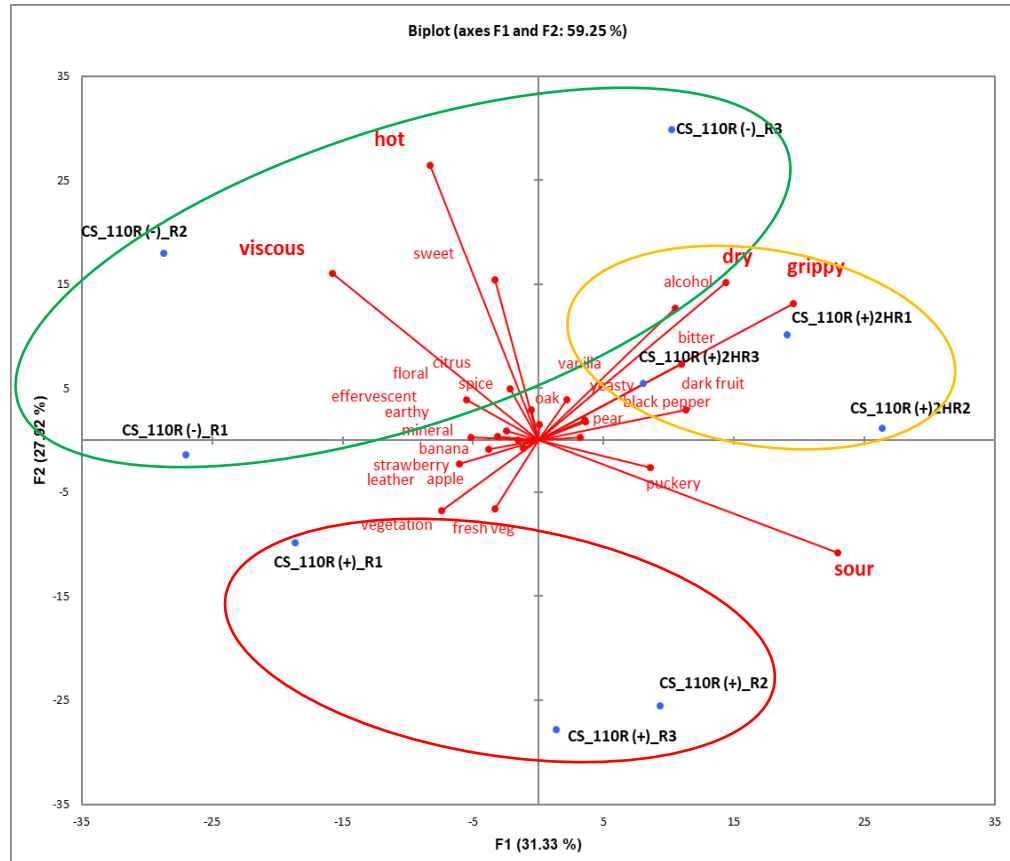
Rumbaugh et al. (2021) Beverages, 7, 76; <https://doi.org/10.3390/beverages7040076>

Sensory results: Merlot_DA



Rumbaugh et al. (2021) Beverages, 7, 76; <https://doi.org/10.3390/beverages7040076>

DA of CS wines - 2016



- RB(+) strong correlation with unripe fruit aromas and sour taste

CS 110 = Cabernet Sauvignon, Rootstock 110R CS 420 = Cabernet Sauvignon, Rootstock 420A

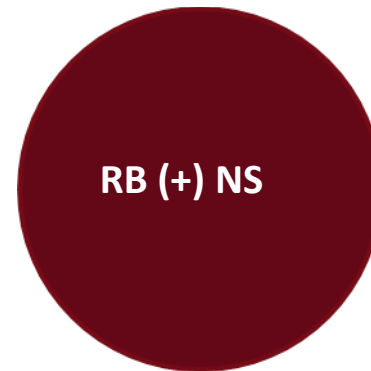
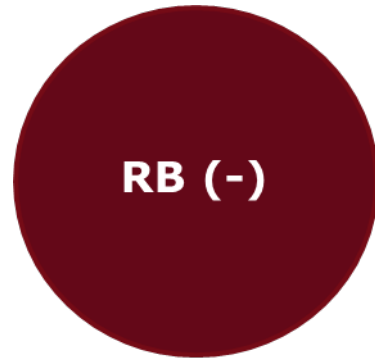
RB(-) = neg

RB(+) = pos

- RB(+)_2H = pos 2nd harvest

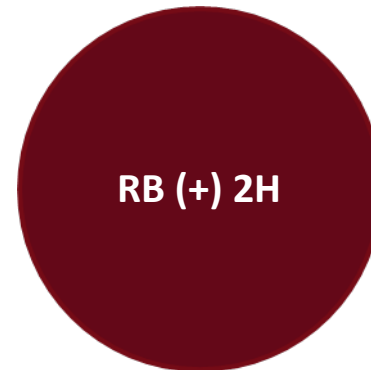
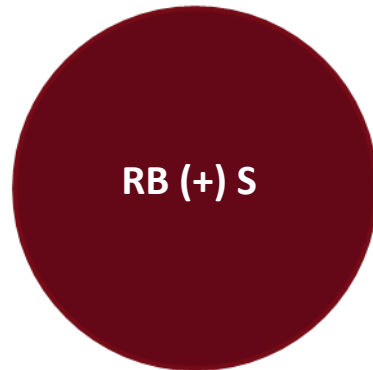
Wine treatments 2017

- Wine made from healthy fruit at 25°Brix



- Wine made from Red Blotch fruit harvested at same time as healthy fruit

- Wine made from Red Blotch fruit chaptalized to 25°Brix



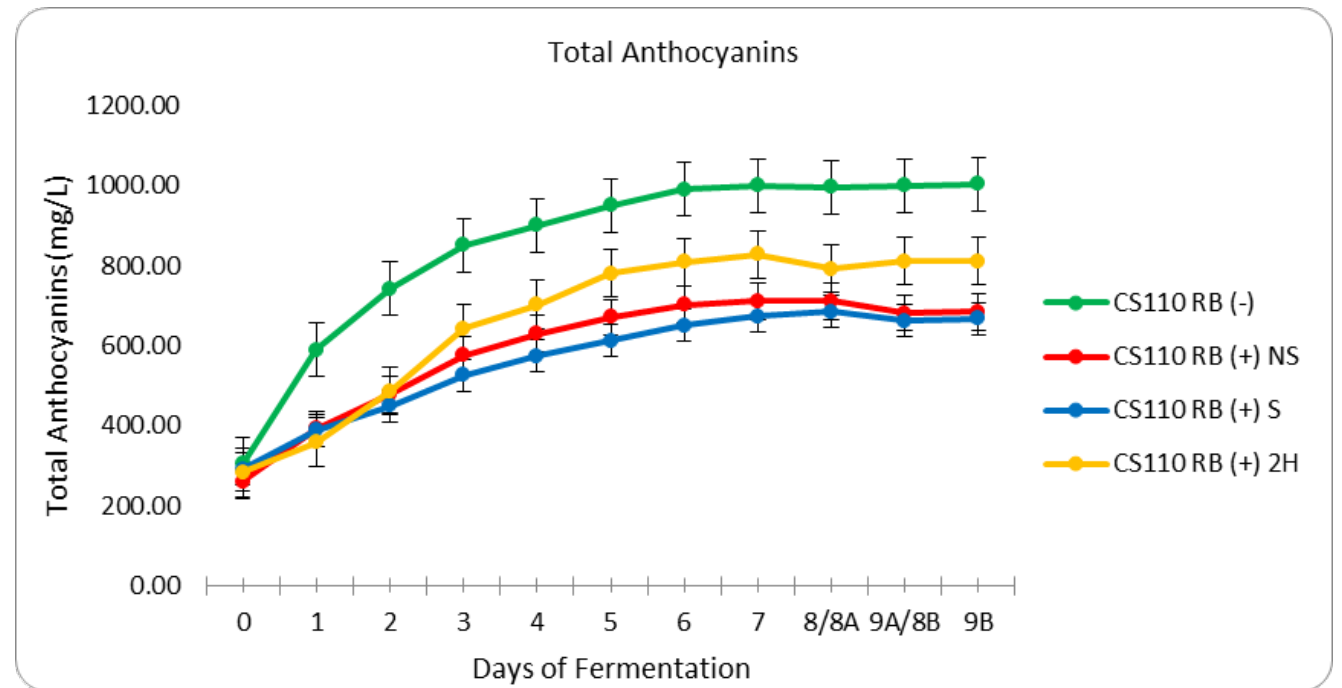
- Wine made from 2nd harvest Red Blotch fruit at 25°Brix

Rumbaugh et al. (2021) Beverages, 7, 76; <https://doi.org/10.3390/beverages7040076>.

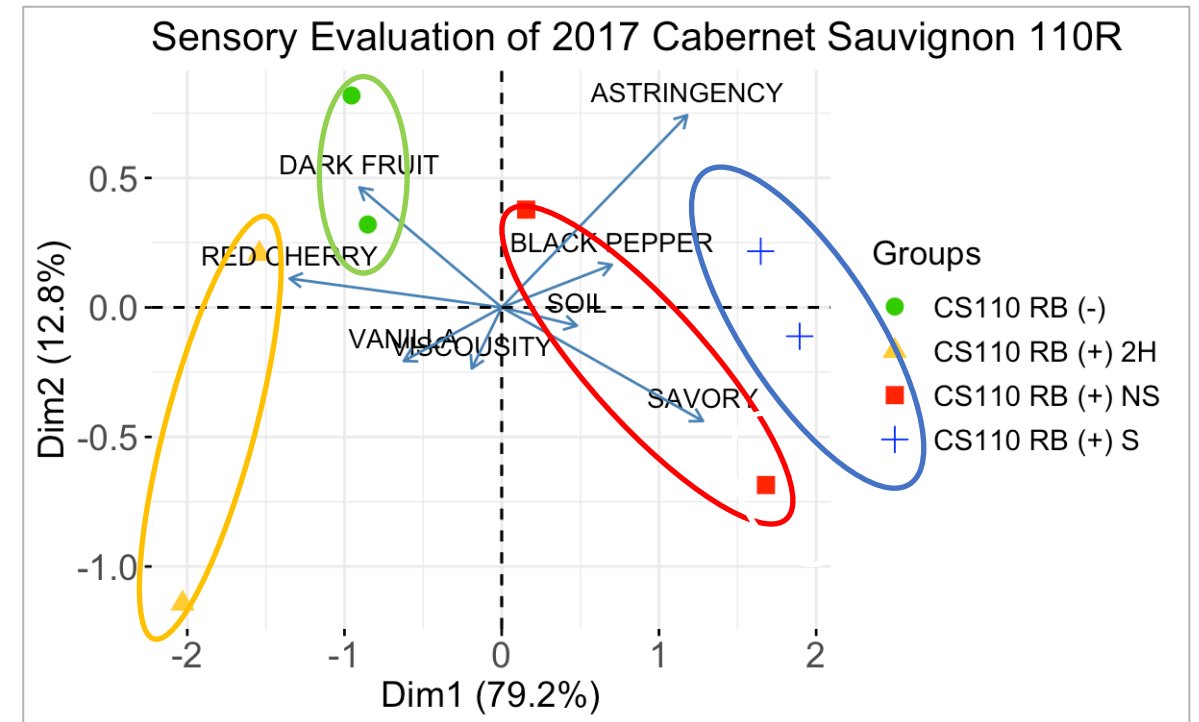
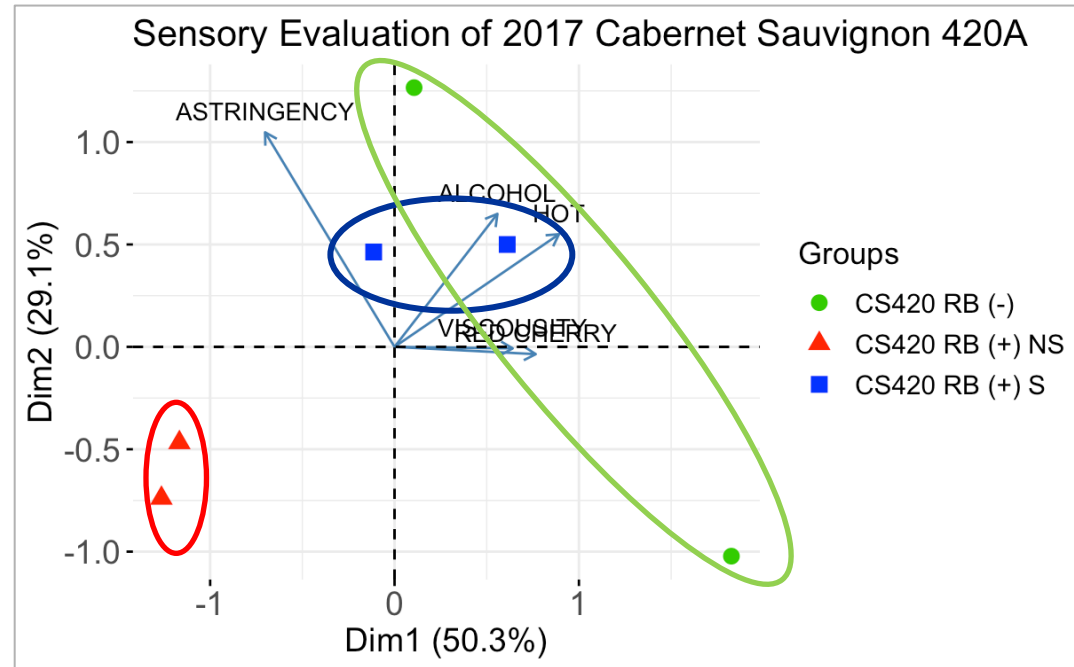
Harvest 2017

- Make wines from RB (+) and (-) grapes with the same sugar content
 - Sequential harvesting (RB (+) 2H)
 - Chaptalization (RB (+) S)
- $\uparrow \text{EtOH} \neq \uparrow \text{phenol extractability}$
 - Cell wall composition altered by GRBV?

Rumbaugh et al. (2021) Beverages, 7, 76; <https://doi.org/10.3390/beverages7040076>.



Descriptive analysis of 2017 CS wines



Red Blotch Disease (RBD) Impact

- ↓ alcohol content in final wines
- ↓ color (anthocyanins) in final wines
- ↓ tannin (astringency/bitterness) in final wines
- RB (+) wines are more sour, green aromas, and watery
- Sensory evaluations of RB(-) and RB(+) wines indicate they can be distinguished from each other based on alcohol and mouthfeel attributes
- If $>2^{\circ}$ Brix impact, consider sequential harvesting
- $>15\%$ incidence of GRBV
- Variable impact depending on site and year



Winery mitigation of GRBD Impacts

- Longer hang time decreased differences between RB (+) and RB (-) wines
 - Dehydration
 - Increased primary metabolites
 - Higher extractability
 - Due to cell wall changes?
 - Chaptalization could decrease difference due to volatile profiles in wines
 - Impact on volatility due to alcohol differences
 - Little impact on phenolic extraction
- Can vinification techniques remove extractability differences between healthy and GRBD fruit?
 - Maceration enzymes – no impact
 - Extended maceration – slight impact on body due to tannin extraction



GRBV Impact on Cell Wall Composition

- How does GRBV impact cell wall composition and integrity?
 - In ripening grapes, pectolytic enzyme degradation increase anth extraction
 - Grapes with higher [anth] and [skin tannin] $\approx$ higher [anth] and [skin tannin] in wines $\approx$ better ratings

Merlot data vines,
5 biological
replicates for both
RB (-) and RB (+)



Pre-veraison



Veraison



Post-veraison



Harvest

25

27

GRB disease impact on CW composition

- Transcriptional induction of cell wall loosening and solubilization processes at harvest in GRBV fruit did not correlate with cell wall composition
 - Enrichment of pathogenesis related protein synthesis did correlate with increased levels of soluble proteins in GRBV fruit
 - Increased levels of pectin and soluble proteins are potentially responsible for decreased extractability during winemaking



Rumbaugh et al. (2023) J. Sci. Food Agric. 103 (7):
3457-3467; [https://doi: 10.1002/jsfa.12481](https://doi.org/10.1002/jsfa.12481)

Ongoing research

- Identify soluble proteins and pectins in cell walls and how this may impact extractability for the development of mitigation actions
- Investigate the impact of virus titer on symptomology
- Investigate potential relationship between years of infection and symptoms
- Determine economic impact of GRBV for cost analysis of mitigation/remediation actions



Vineyard management



- Discing ground cover in the early growing season reduced *Spissistilus festinus* (three-cornered alfalfa leaf hopper) activity and abundance
- Vineyards with active disease scouting, removal of girdled shoots and rouging controlled spread <5%/yr, those with no rouging saw 30x more spread over 3 yrs
- However, if vineyard surrounded by GRBV infected vineyards, spread will continue even with active removal
- Removing asymptomatic vines alongside symptomatic vines did not improve outcomes

Achala et al. (2022) AJEV 73: 116-124; Tanner et al. (2022) <http://dx.doi.org/10.2139/ssrn.4112960>

Vineyard management

- GRBV impedes carbon translocation mechanisms – delay ripening in grapes (Martinez-Lüscher et al. 2019)
- Vineyard management
 - No hormonal or nutrient sprays have impeded GRBV symptoms
 - Investigation with potassium fertilization is ongoing
 - K^+ does not improve sugar translocation – it dehydrated berries, increasing Brix at harvest
 - Manipulation of source:sink ratio even by 3x did not improve disease outcomes (Tanner et al. 2022)
 - Irrigation regimes did not have a major impact
 - Some indication that additional water stress can aggravate symptoms
 - Replacing 100% of evapotranspiration loss can potentially be beneficial



Martinez-Lüscher et al. (2019) J. Agric. Food Chem 67 (9): 2437-2448; Blanco-Ulate et al (2017) J. Exp. Bot. 68 (5): 1225-1258; Rumbaugh et al. (2022) Int J. Molecular Sci. 23: 13248 <https://doi.org/10.3390/ijms2321132483>

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- Brazilian Government (CAPES)
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