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IMPACT OF RED BLOTCH DISEASE ON GRAPE AND WINE COMPOSITION

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Oregon Grape Day: Management of Trunk Disease, Grapevine Viruses and Fungicide Resistance
LaSells Stewart Center, OSU Campus
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Introduction

- Grapevine red blotch-associated virus (GRBaV)
  - Grapevine Red Blotch disease was first described in Cab Sauv, Zin and Cab Franc in New York and California (1)
  - A DNA virus (GRBaV) was shown to be the causal agent of grapevine red blotch disease (2)
  - Widespread in vineyards in USA and Canada

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(1) Al Rwahnih et al., (2013) Phytopath. 103: 1069-1076
(2) Fuchs (2013) http://lecture.ucanr.org/Mediasite/Play/7e6250539e5e4676ad4cd88051164c1d

Introduction

- Grapevine Red Blotch disease symptoms
  - RB disease shows symptoms similar to Leafroll disease
    - Unlike Leafroll – RB show red veins on leaf undersides and no rolling

Introduction

- **Red Blotch disease spread**
  - **Widespread occurrence of Red Blotch disease indicate primary spread through propagation (1)**
  - **Increase incidence in young healthy vines adjacent to infected vineyards suggest vector (2)**
  - **3-cornered alfalfa treehopper (*Spissistilus festinus)* have recently be shown to be able to spread the disease (3)**

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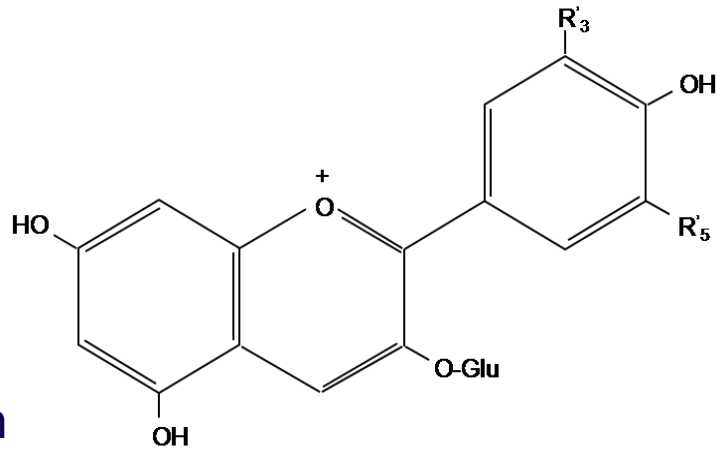
(1) Al Rwahnih et al., (2013) Phytopath. 103: 1069-1076
(2) Poojaric et al. (2013) PLosONE 8: e64194

Perceived impact of RB disease on grape composition

- ↓ Sugar accumulation
  - As much 4-5 °Brix less
  - Delay in ripening
- ↓ Color development
- ↑ TA
  - Current research - show not always true
  - ↑ Malic acid
    - True for CH and CS, not Zin
  - For CH, ↓yield

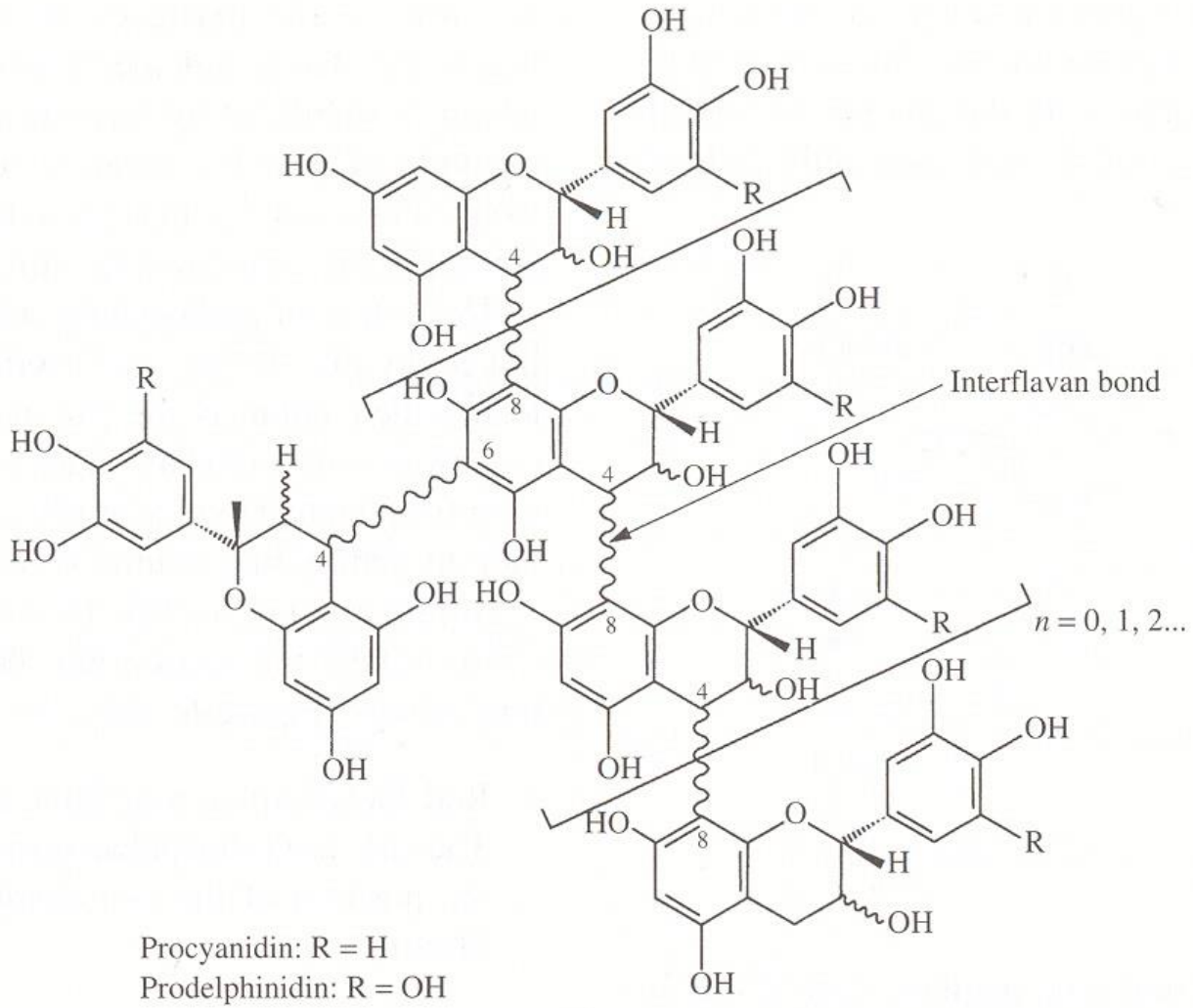
Background – phenols in wine

- **Main phenols (flavonoids) in red wine**
  - **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**



Anthocyanin

Proanthocyanidins



Extension units

Terminal unit

Impact of RB disease on grape & wine composition

- Much not known
  - Influence of cultivar and site?
  - Influence of stress?
  - Seasonal/climatic impact?
  - No well documented influence on grape development
  - Effect on wine composition and quality?
    - Wine ageability?

Progress….

- 2014
  - Funded project to determine the impact of GRBaV on the composition of grapes at harvest and the resulting wines
  - To investigate potential sensory and quality differences between wines made from GRBaV positive and negative grapes
- 2015
  - Unfunded – small investigation
- 2016
  - Funded again

Experimental layout

- Virus testing (GRBaV and GRLaV) of subset vines to determine GRBaV (+) and (-) sample plots
- Sample grapes at harvest
  - Basic chemical panels (Brix, pH, TA)
  - Metabolomics analysis (primary and secondary metabolite profile)
  - Phenolic profile (AH-assay, RP-HPLC)
  - Tannin composition (SPE isolation, phloroglucinolysis)

Experimental layout

- Winemaking from GRBaV (+) and (-) grapes
  - Chemical analyses similar to grapes (previous slide)
  - Descriptive sensory analysis
    - Correlate wine composition with sensory attributes
    - Impact of GRBaV on wine style/quality

Experimental layout 2014

Variety (site #)	Source County	Grape Sampling	Winemaking
Chardonnay 1a	Sonoma	Yes	Yes
Chardonnay 1b	Sonoma	Yes	No
Chardonnay 2	Sonoma	Yes	No
Merlot 1	Napa	Yes	No
Merlot 2	Napa	Yes	Yes
Cab Sauv 1	Napa	Yes	Yes
Cab Sauv 2	Napa	Yes	Yes

Results: Grape chemical composition (2014)

Sample	GRBaV Status	Harvest Date	°Brix	pH	TA (g/L)	
Chardonnay 1a	-	12-Sep-14	24.4	3.4	6.0	
	+	12-Sep-14	23.0	3.5	6.7	↓6%
Chardonnay 1b	-	11-Sep-14	23.0	3.4	6.6	
	+	11-Sep-14	22.5	3.6	6.9	↓2%
Chardonnay 2	-	16-Sep-14	24.1	3.3	7.8	
	+	16-Sep-14	24.2	3.5	8.9	0%

- ↓°Brix 0-6% GRBaV(+) CH grapes
- Small differences in pH
- ↑ TA in GRBaV(+) grapes

Results: CH 1a chemical composition

CH 1a	GRBaV Status	Harvest Date	°Brix	pH	TA (g/L)	
2014	-	12-Sep-14	24.4	3.4	6.0	
	+	12-Sep-14	23.0	3.5	6.7	↓6%
2015	-	9-Sep-15	25.7	3.5	5.3	
	+	9-Sep-15	23.6	3.6	6.3	↓8%
2016	-	12-Sep-16	23.7	3.4	6.1	
	+[1]	12-Sep-16	22.7	3.6	5.9	↓4%
	+[2]	19-Sep-16	23.7	3.7	5.6	

- For all 3 years a ↓°Brix 4-8% GRBaV(+) CH grapes
- Small differences in pH
- Variable TA impact of GRBaV in grapes

Results: Red grape chemical composition (2014)

Sample	GRBaV Status	Harvest Date	°Brix	pH	TA (g/L)	
Merlot 1	-	29-Aug-14	25.0	3.6	3.2	
	+	29-Aug-14	21.1	3.6	3.6	↓16%
Merlot 2	-	26-Sep-14	24.9	3.5	4.2	
	+	26-Sep-14	23.5	3.5	4.7	↓6%
Cab Sauv 1	-	18-Sep-14	25.7	3.3	7.8	
	+	18-Sep-14	20.6	3.5	8.6	↓20%
Cab Sauv 2	-	7-Oct-14	26.3	3.6	4.8	
	+	7-Oct-14	25.2	3.6	4.9	↓4%

- ↓°Brix 6-16% GRBaV(+) ME and 4-20% in CS grapes
- Small differences in pH
- ↑ TA in GRBaV(+) grapes

Results: Grape chemical composition

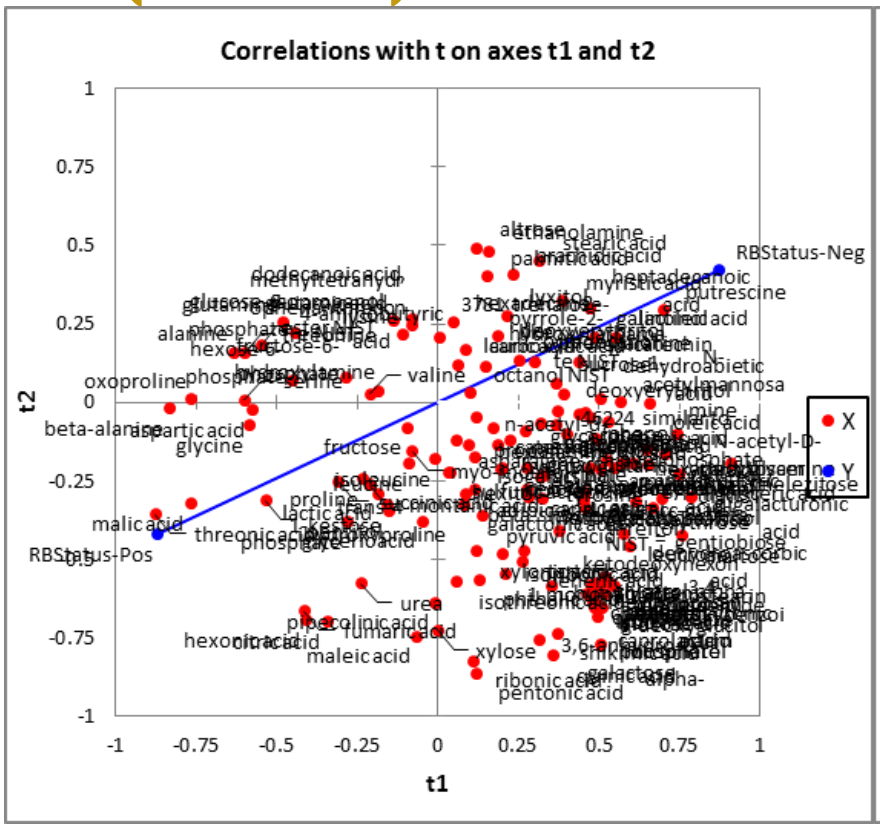
CS 2	GRBaV Status	Harvest Date	°Brix	pH	TA (g/L)
2014	-	7-Oct-14	26.3	3.6	4.8
	+	7-Oct-14	25.2	3.6	4.9
2015	-	21-Sep-15	26.0	3.7	4.3
	+	21-Sep-15	22.4	3.7	4.4

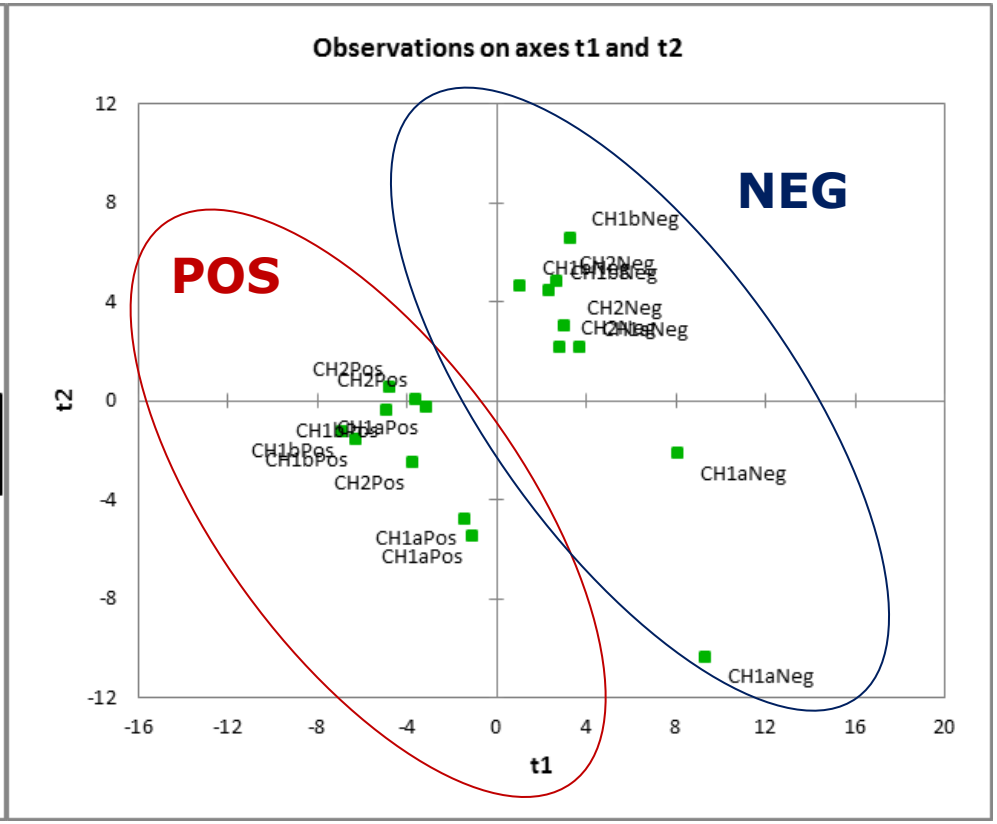
↓4%

↓14%

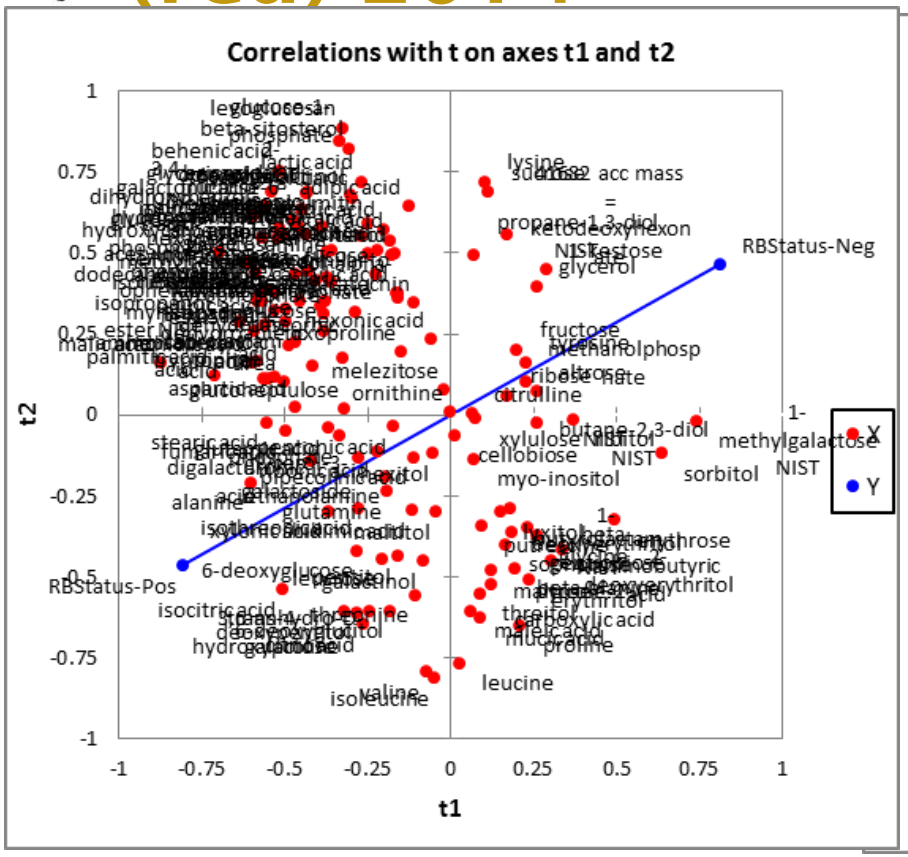
- Both years ↓°Brix 4-14% GRBaV (+)
- Small differences in pH
- ↑ TA in GRBaV(+) grapes

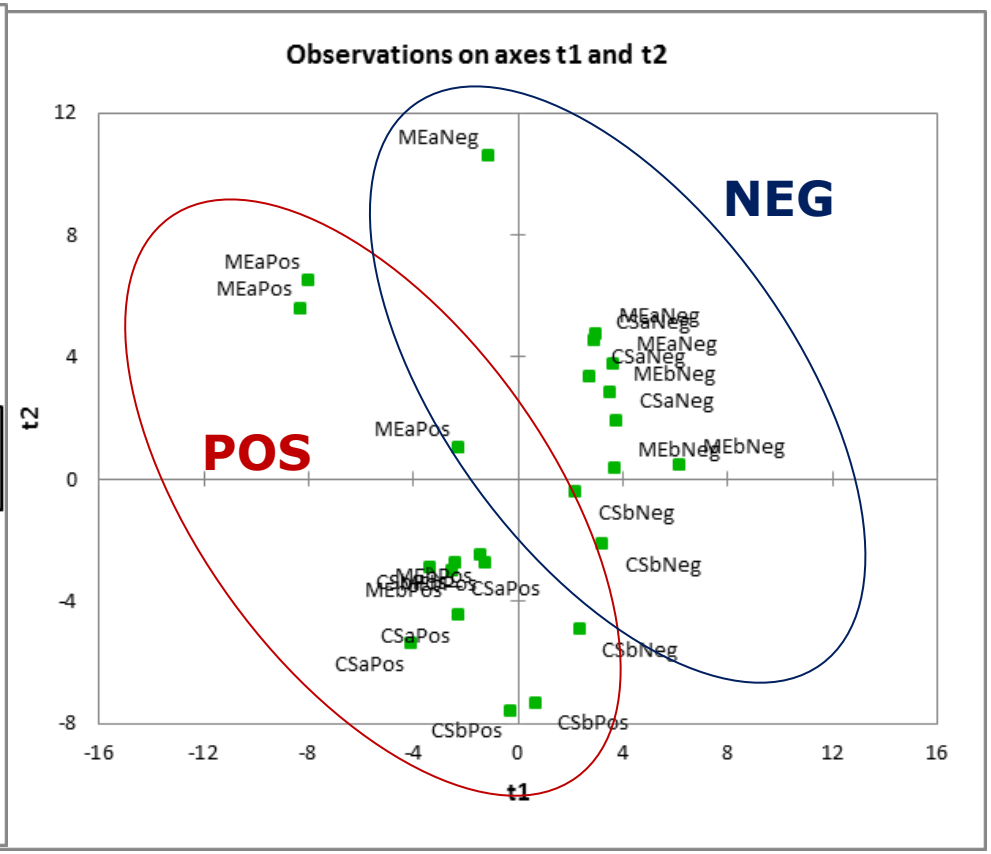
PLS-DA of metabolomics grape data (white) 2014





PLS-DA of metabolomics grape data (red) 2014





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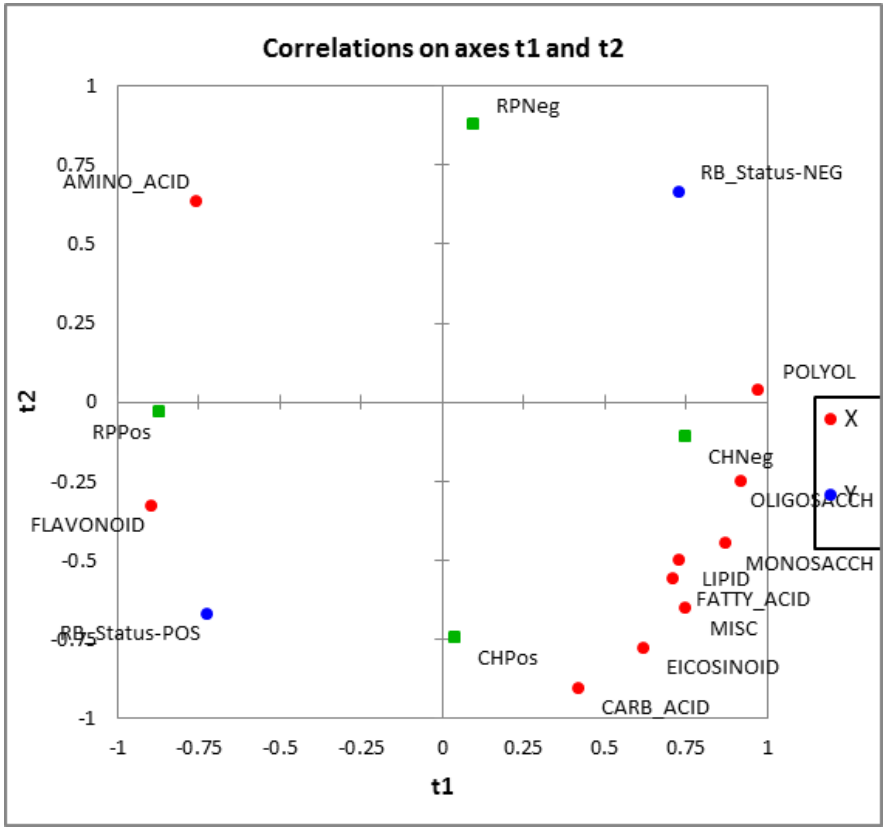
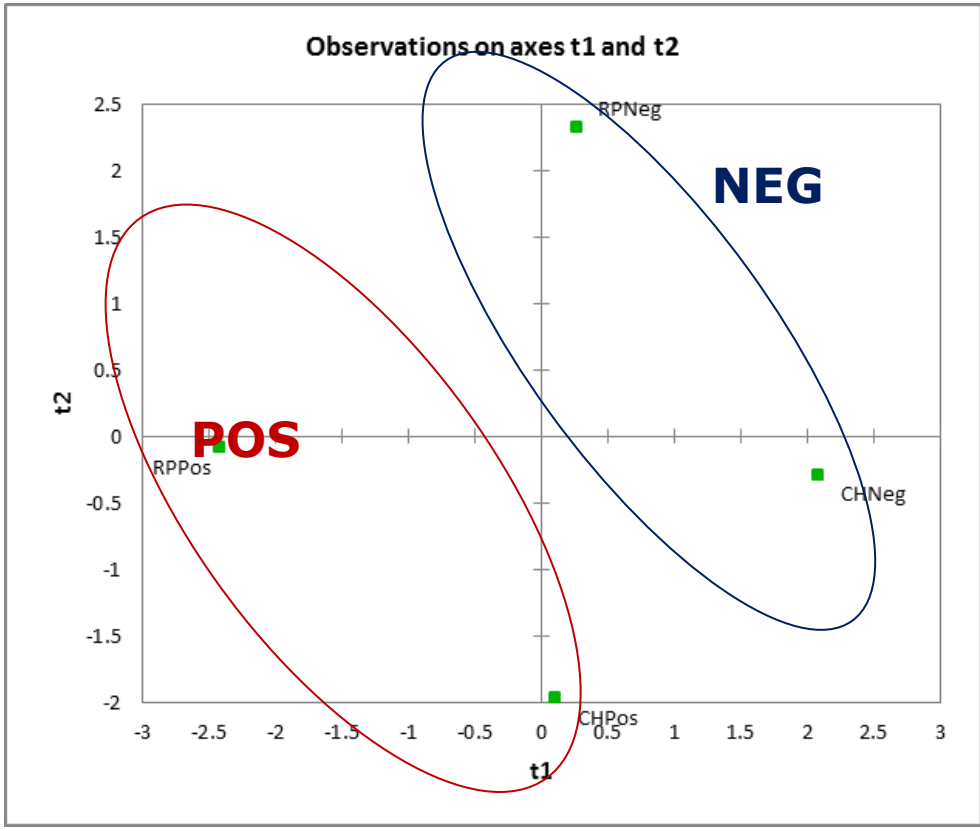
PLS-DA of metabolomics grape data 2015





Phenolic profile: Chardonnay

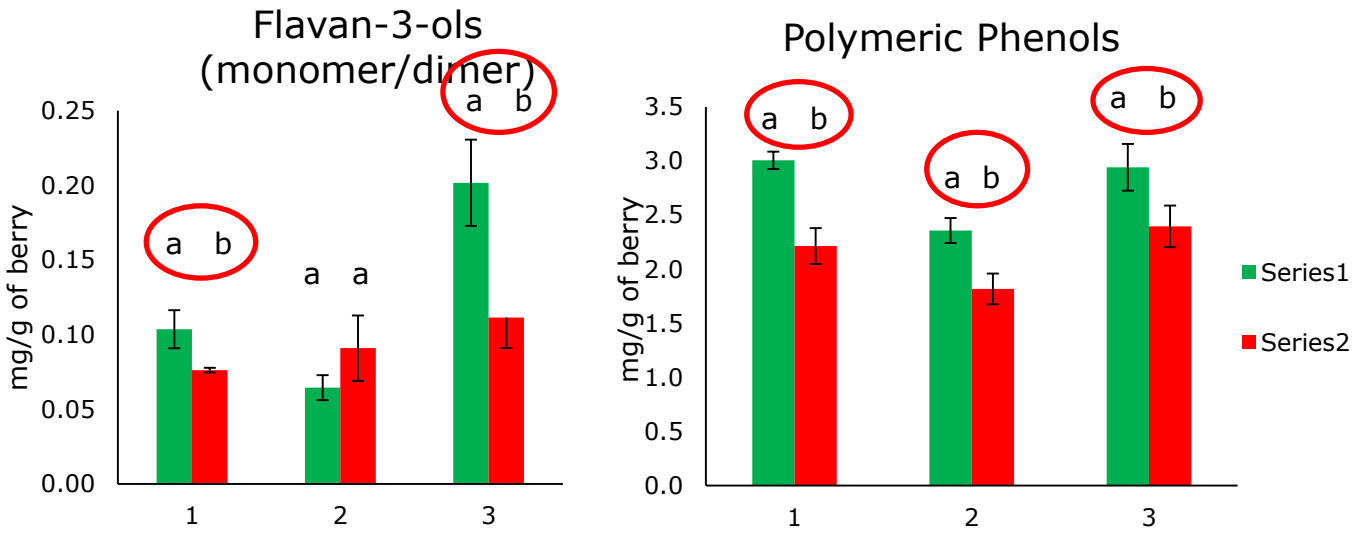


Figure: RP-HPLC phenolic profile results of RB (-) and RB (+) Chardonnay at harvest in 2014

2015 RP-HPLC Phenolic Profile

- **CH 1a RB(+):** ↓ flavan-3-ols concentration

  ↑ polymeric phenols concentration (agrees with Protein Precipitation assay)

2014 Phenolic Profile

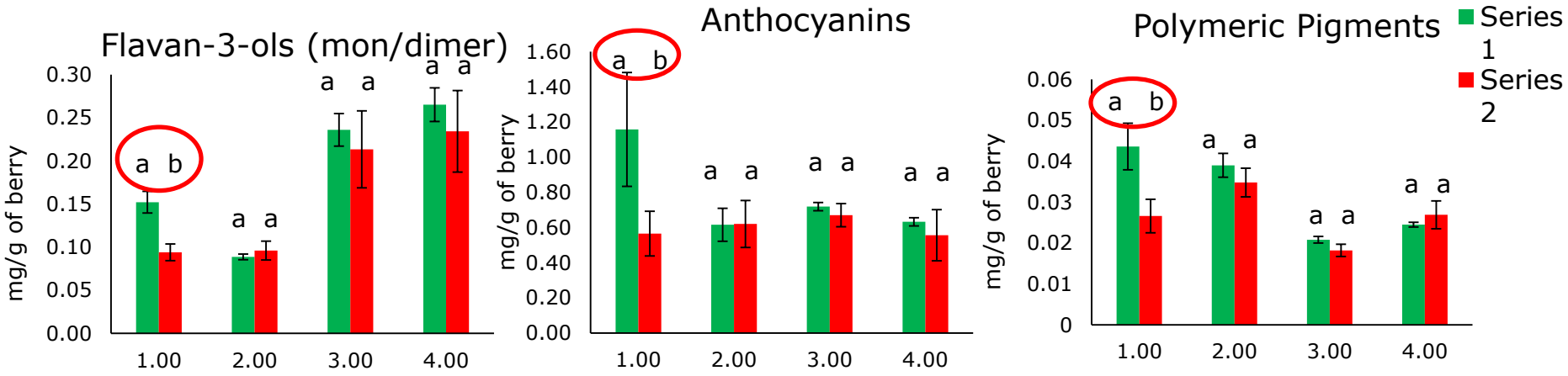
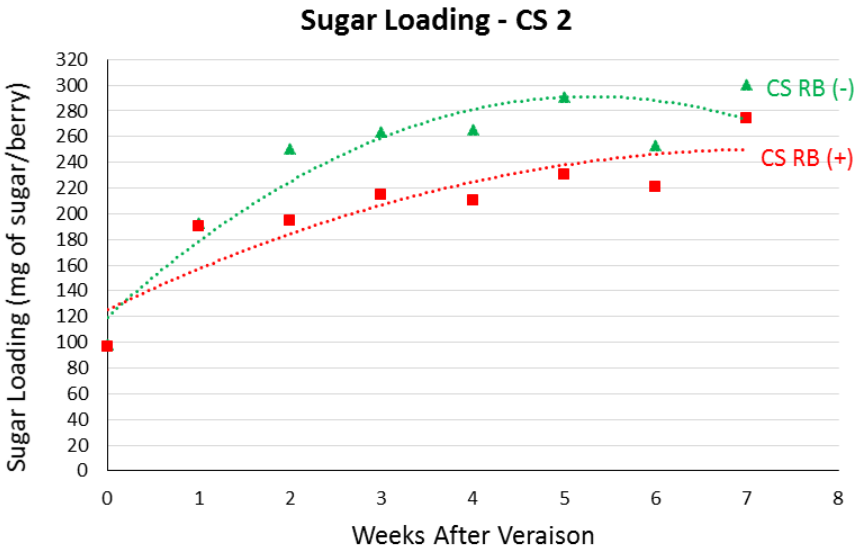


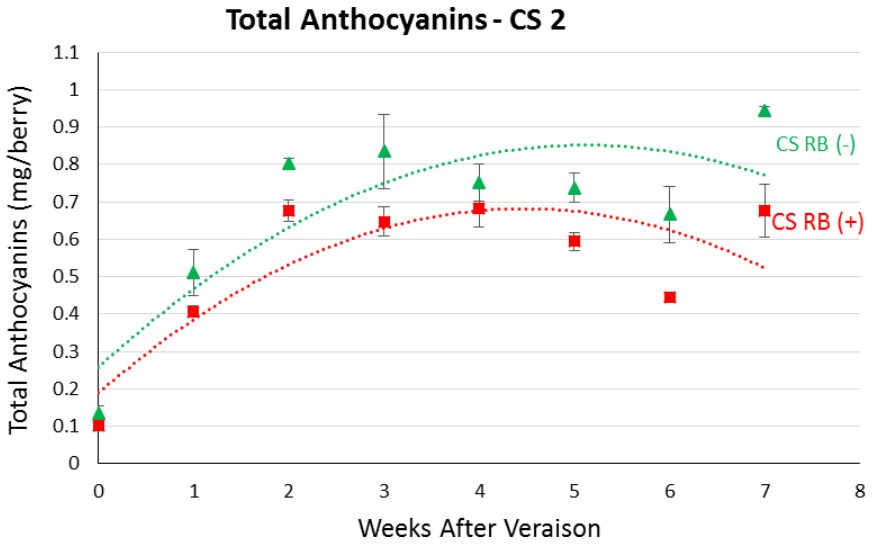
Figure: RP-HPLC phenolic profile results of RB (+) and RB (-) grapes at harvest for Cabernet Sauvignon (CS) and Merlot (ME) from 4 different sites in Napa, CA.

2015 Phenolic Profile

- **CS2 RB(+):** ↓ anthocyanins and polymeric pigments
  ↑ polymeric phenols (agrees with PP) and flavan-3-ols

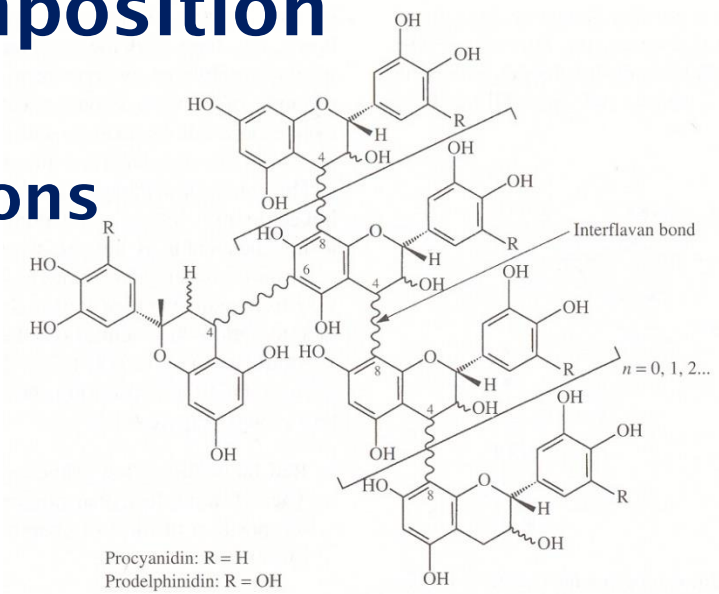
2015 CS grape ripening





Results: Tannin composition by phloroglucinolysis

- Tannin analysis showed signf differences among diffr varieties
  - No diffr due to disease status of grapes (mDP, % gallo units, % galloylation)
- It looks as if tannin composition similar
  - However method limitations



2014 – Skin and Seed Tannin Analyzed by Phloroglucinolysis

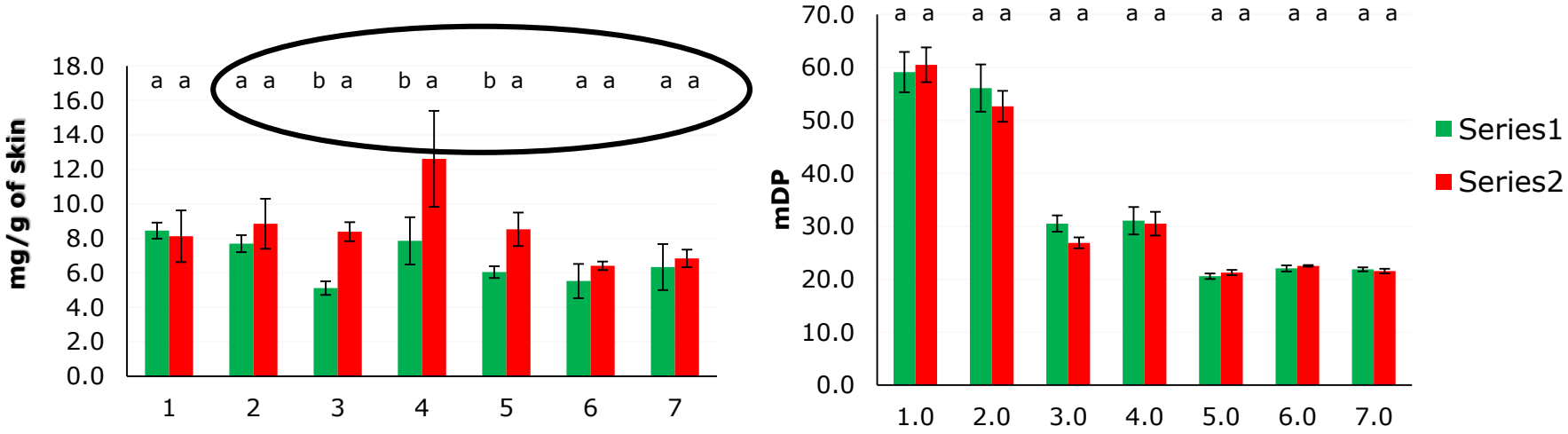


Figure: Mean Degree of Polymerization (mDP) and skins tannins on CS, CH and ME from 7 different sites in 2014 by phloroglucinolysis

2015

- RB (+) CS 2 and CH 1a had significant ↑ concentration of tannins in the skins
- Skins tannins and mDP had the same trend as observed in 2014
- RB (-) and RB (+) seed from both 2014 and 2015 did not show differences regarding tannin concentration and mDP

Results: Wine chemical composition 2014

Wine	GRBaV Status	EtOH% (v/v)	pH	TA (g/L)	RS (g/L)	AA (g/L)
CH 1a	-	16.1 ± 0.2*	3.6 ± 0.2*	5.2 ± 0.1	1.9 ± 0.2*	0.1 ± 0.0*
	+	15.4 ± 0.0*	3.8 ± 0.2*	5.6 ± 0.0	1.1 ± 0.2*	0.1 ± 0.0*
ME 2 (b)	-	15.3 ± 0.1*	3.7 ± 0.2	5.2 ± 0.1	0.2 ± 0.0	0.0 ± 0.0
	+	14.1 ± 0.1*	3.7 ± 0.2	5.3 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
CS 1 (a)	-	14.6 ± 0.3*	3.2 ± 0.2*	7.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0*
	+	13.0 ± 0.1*	3.2 ± 0.2*	7.1 ± 0.4	0.1 ± 0.0	0.1 ± 0.0*
CS 2 (b)	-	15.8 ± 0.1*	3.9 ± 0.2*	4.8 ± 0.0*	0.3 ± 0.0	0.1 ± 0.0*
	+	14.9 ± 0.0*	3.7 ± 0.2*	5.5 ± 0.5*	0.2 ± 0.0	0.1 ± 0.0*

CH = Chardonnay; CS = Cabernet Sauvignon; ME = Merlot

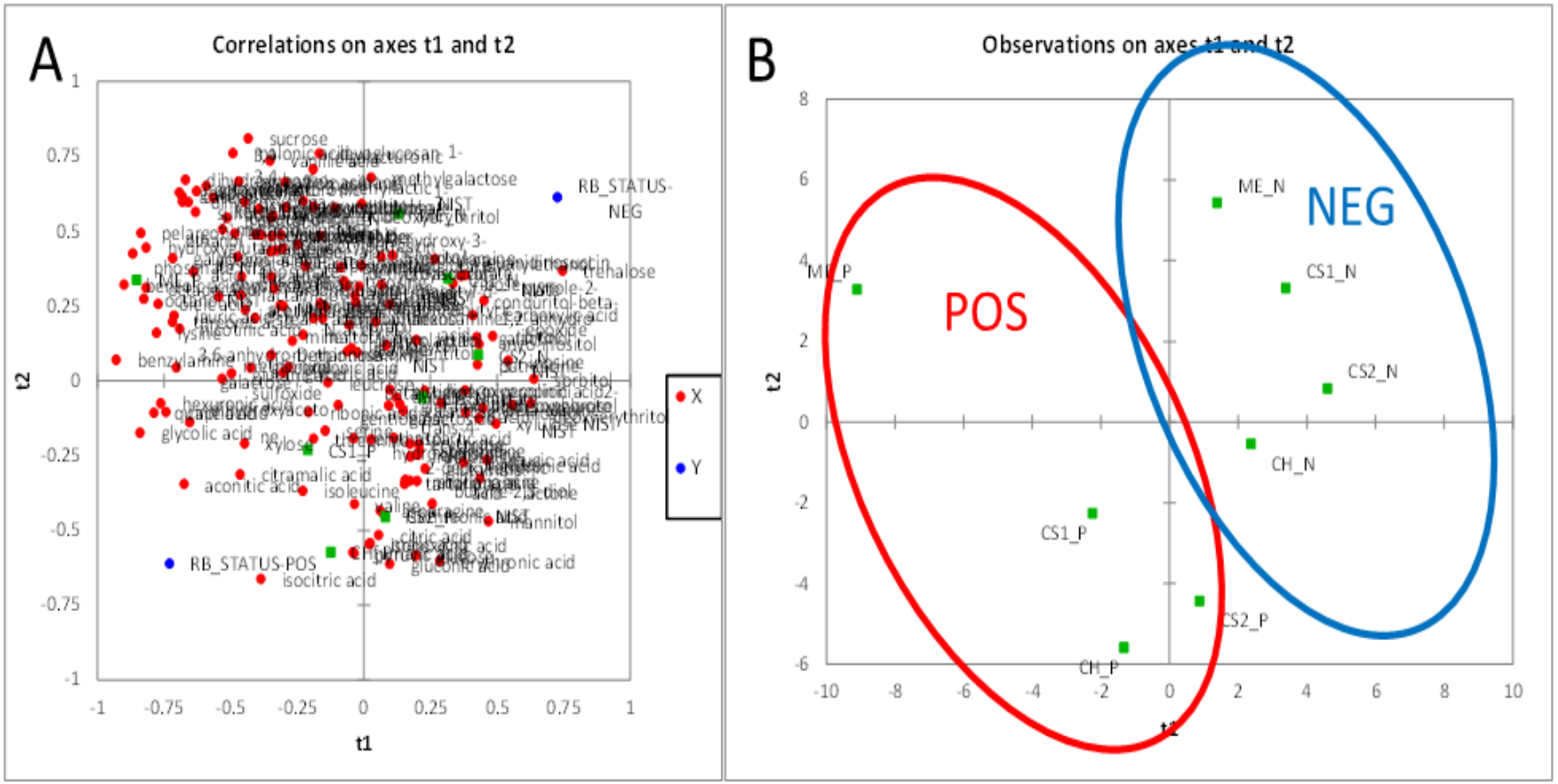
**Indicate significance at $p < 0.05$ within a site*

Results: Wine chemical composition 2015

Wine	GRBaV status	EtOH% (v/v)	pH	TA (g/L)	Free SO2 (mg/L)	VA (g/L)
CH1a	-	16.0	3.45	6.23	27.7	0.10
	+	14.8	3.75	6.26	27.0	0.10
CS2	-	15.2	3.82	5.56	32.3	0.34
	+	12.9	3.62	6.0	34.0	0.31

Different letters indicate significance at $p < 0.05$

PLS-DA of wine metabolomics data 2014



Phenolic Profile RP-HPLC and Phloroglucinolysis

Table: RP-HPLC phenolic profile results of RB (-) and positive wines (n=3 for CS Site 2 and n=2 for CS Site 1 and ME Site 2)

	Wine	Flavan-3-ols (mg/L)	Hydroxy-cinnamic acid (mg/L)	Flavonols (mg/L)	Total Anthocyanins (mg/L)	Polymeric Pigments (mg/L)	Tannins (mg/L)	mDP
2014	CS 2 RB (-)	41.14 ± 0.43	29.67 ± 8.15	62.86 ± 0.35	146.40 ± 9.59	39.72 ± 3.37 *	275.08 ± 24.14	14.51 ± 1.05
	C2 2 RB (+)	42.87 ± 1.20	37.86 ± 0.40	62.08 ± 2.46	189.48 ± 20.46	24.35 ± 2.23 *	343.15 ± 24.38	15.41 ± 0.53
	CS 1 RB (-)	66.76 ± 1.93 *	26.34 ± 0.38 *	61.61 ± 0.30 *	275.42 ± 8.32 *	23.18 ± 1.70 *	204.82 ± 5.32 *	14.61 ± 0.62
	CS 1 RB (+)	59.44 ± 3.04 *	22.41 ± 1.49 *	67.63 ± 0.36 *	243.50 ± 10.1 *	19.33 ± 0.78 *	269.76 ± 24.35 *	15.03 ± 0.72
2015	ME 2 RB (-)	81.95 ± 0.19 *	42.29 ± 0.68	86.87 ± 4.00	254.41 ± 1.24	19.89 ± 1.11	526.52 ± 42.73 *	11.50 ± 0.26
	ME 2 RB (+)	101.72 ± 0.28 *	43.95 ± 0.98	91.99 ± 3.13	250.13 ± 3.18	18.94 ± 1.13	734.82 ± 51.20 *	11.24 ± 0.47

Phenolic profile of CS 2 wines

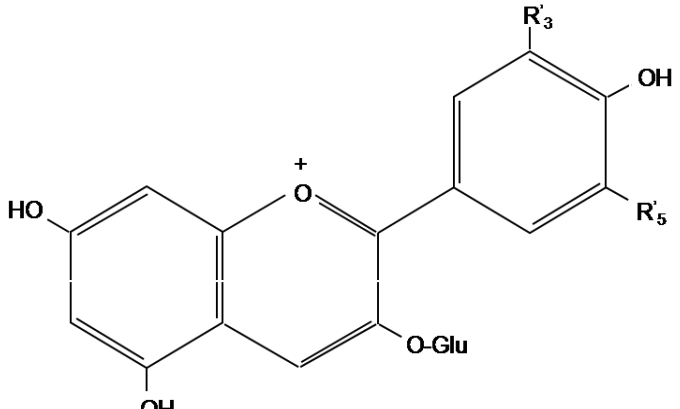
RB (+) ↑ concentration of catechin, epicatechin

↓ concentration of total anthocyanins and polymeric pigments

**Indicate significance at p < 0.05 within a site*

GRBaV Impact on Grape and Wine Phenol Composition

- Variably response to RB disease within variety and per season
- Not a direct relationship with wine composition
  - Due to matrix and extraction effects?

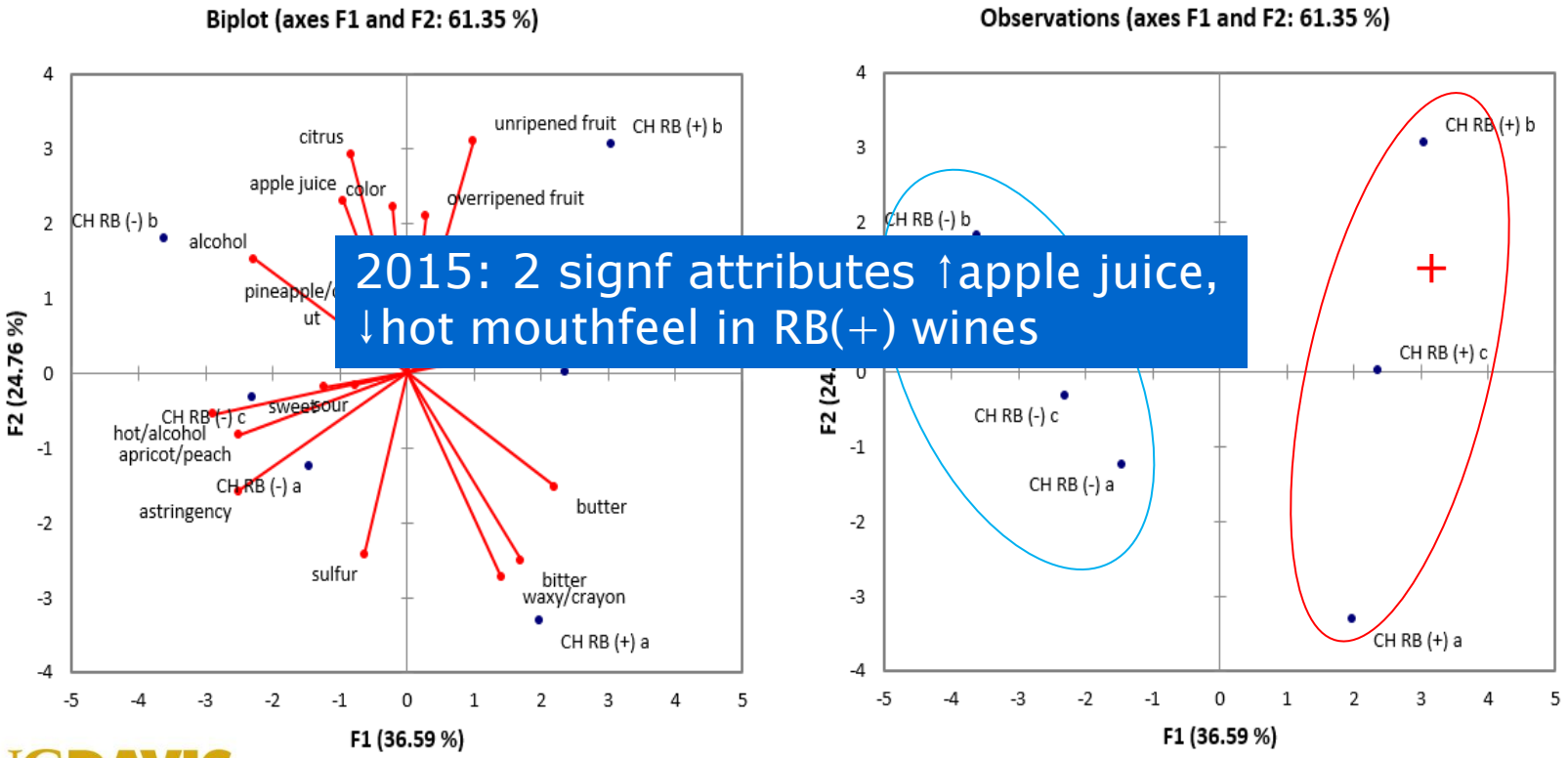


Anthocyanin

Sensory: Descriptive analysis (DA)

White wine sensory data 2014

PCA scores and loading plot



2015: 2 signf attributes ↑apple juice, ↓hot mouthfeel in RB(+) wines

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PCA separation of the wines although very little diffr

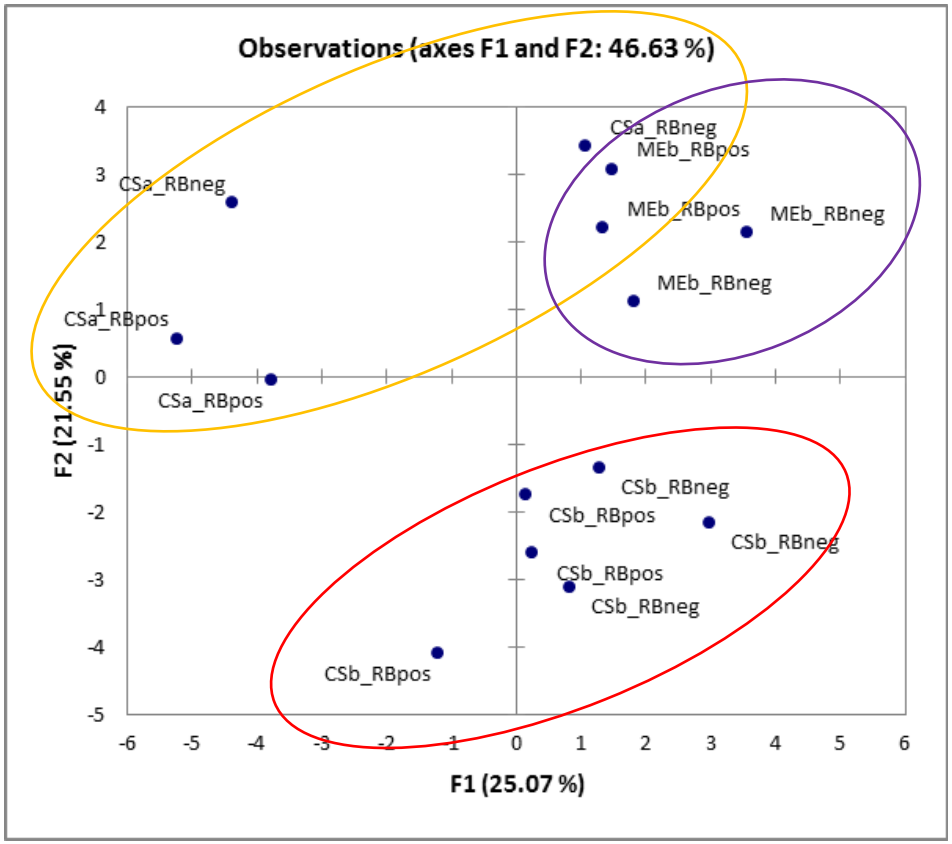
- **Only 1 out of 18 attributes sigf diffr**

Corrected F values for red DA attributes – 2014 data

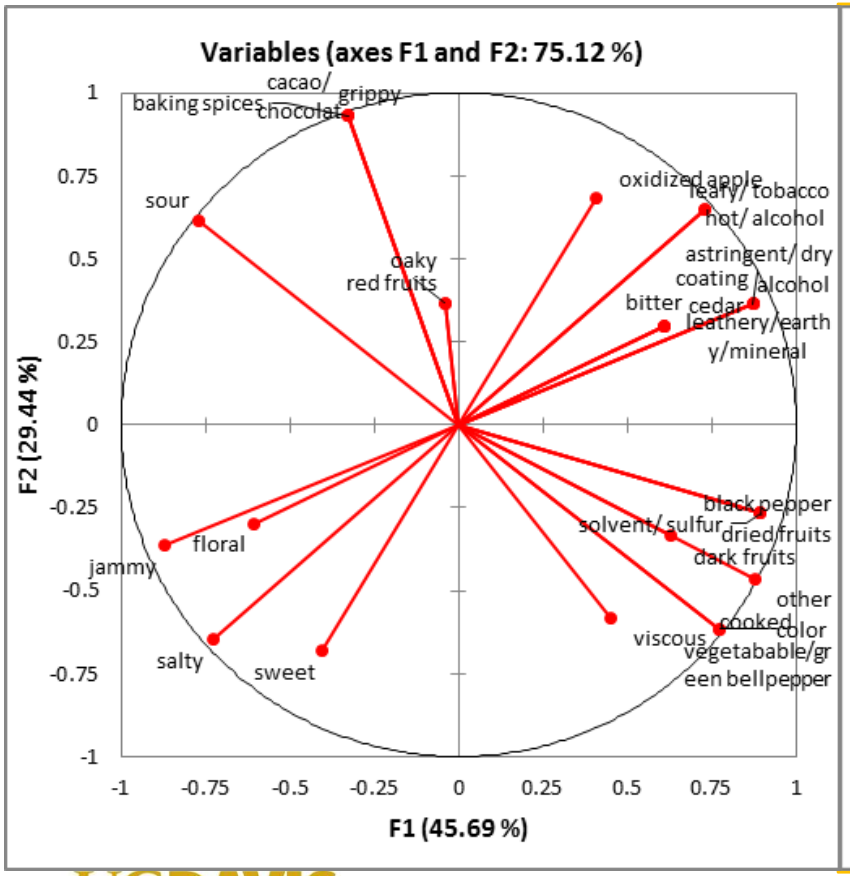
Attributes	F value wine	Significant
red fruits	1.184	no
dark fruits	1.393	no
dried fruits	2.744	yes**
oxidized apple	0.484	no
jammy	0.654	no
cooked vegetables/green bellpepper	1.551	no
leafy/tobacco	2.382	no
ceder	1.085	no
leathery/earthy/mineral	0.874	no
okay	0.970	no
alcohol	3.405	yes***
solvent/sulfur	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

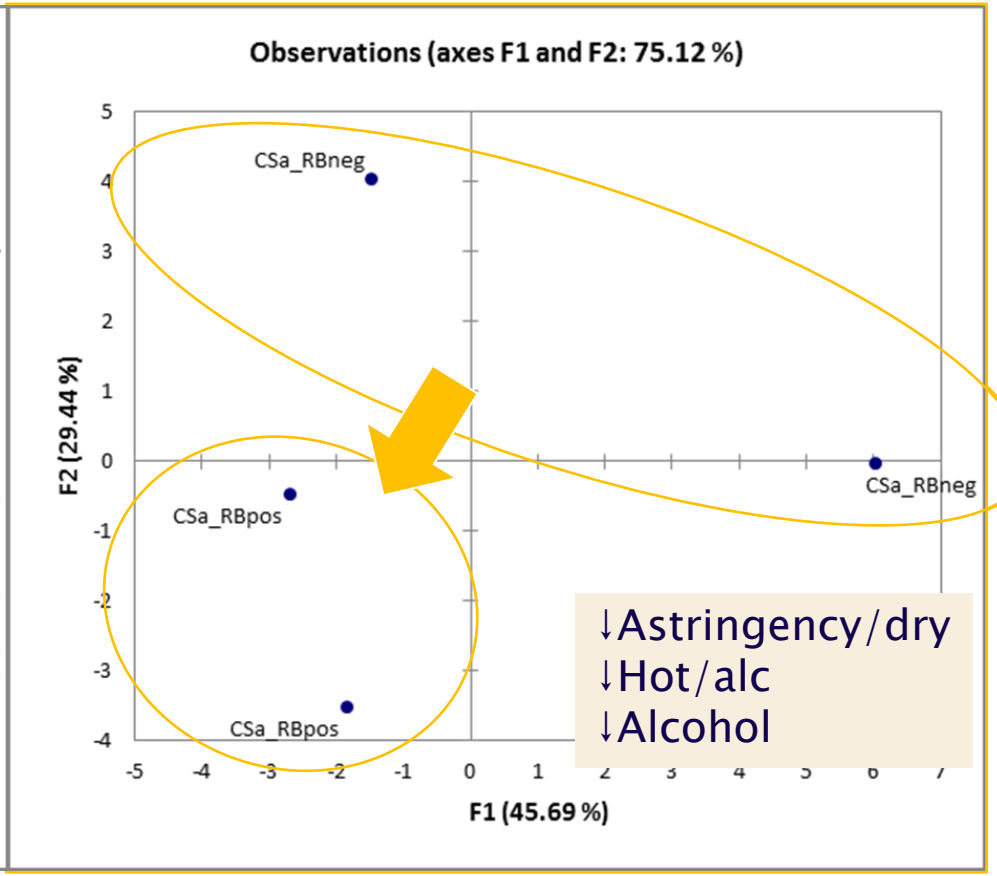
*, ** and *** indicate significance at respectively $p < 0.05$, $p < 0.01$, $p < 0.0001$

PCA score plot



PCA: Descriptive analysis of CS (1)a





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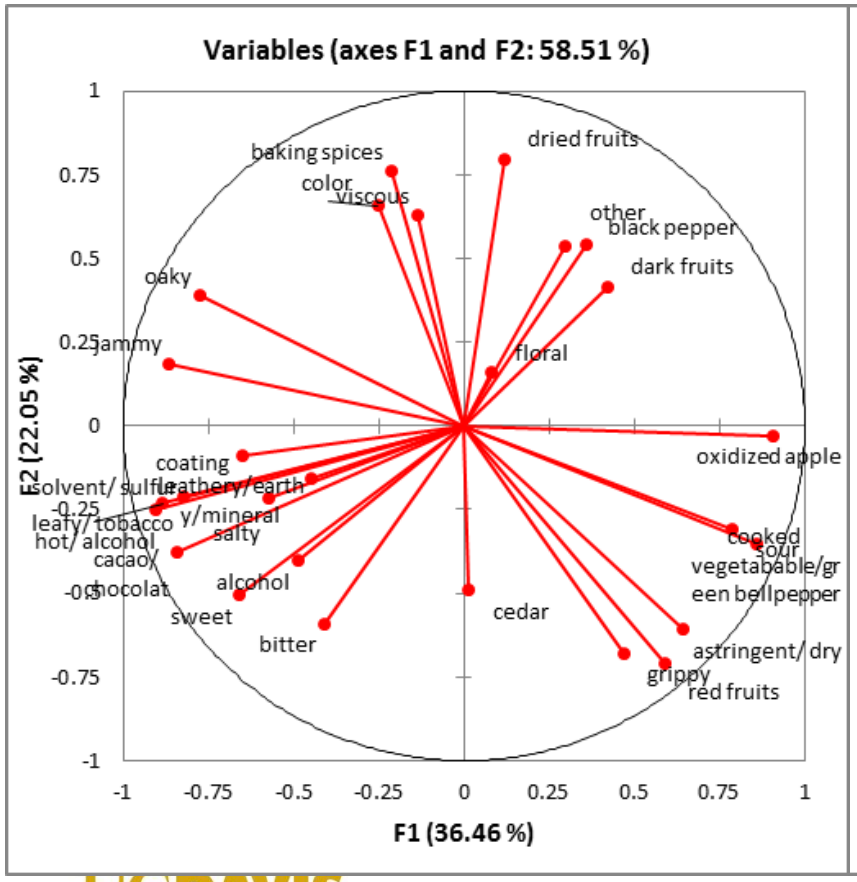
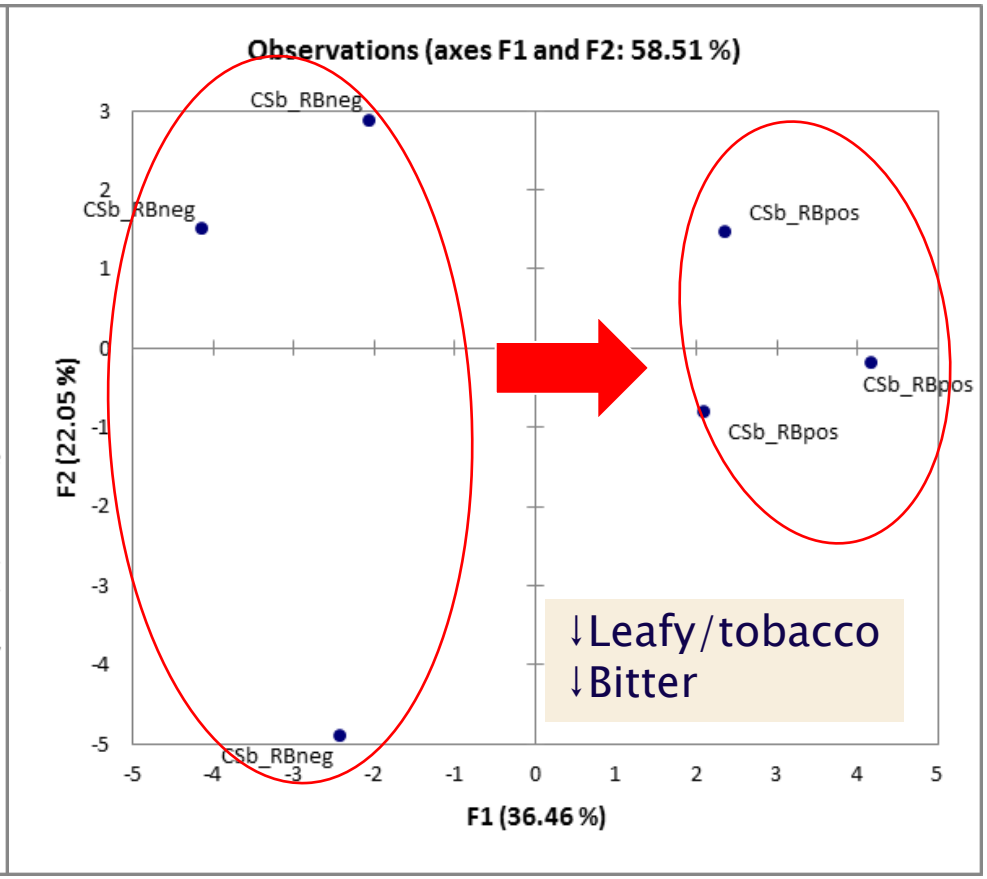
Phenolic analyses: RB (+) ↓ [anthocyanin], [pol pigments], [pol phenols] and % Alc

PCA: Descriptive analysis of CS (2)b





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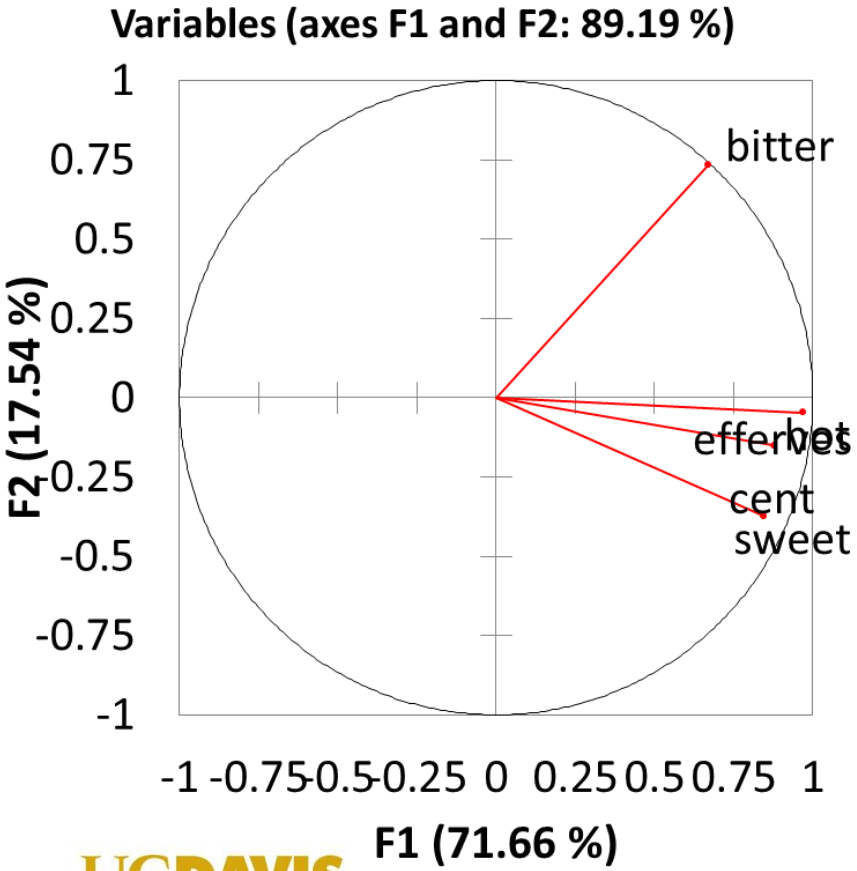
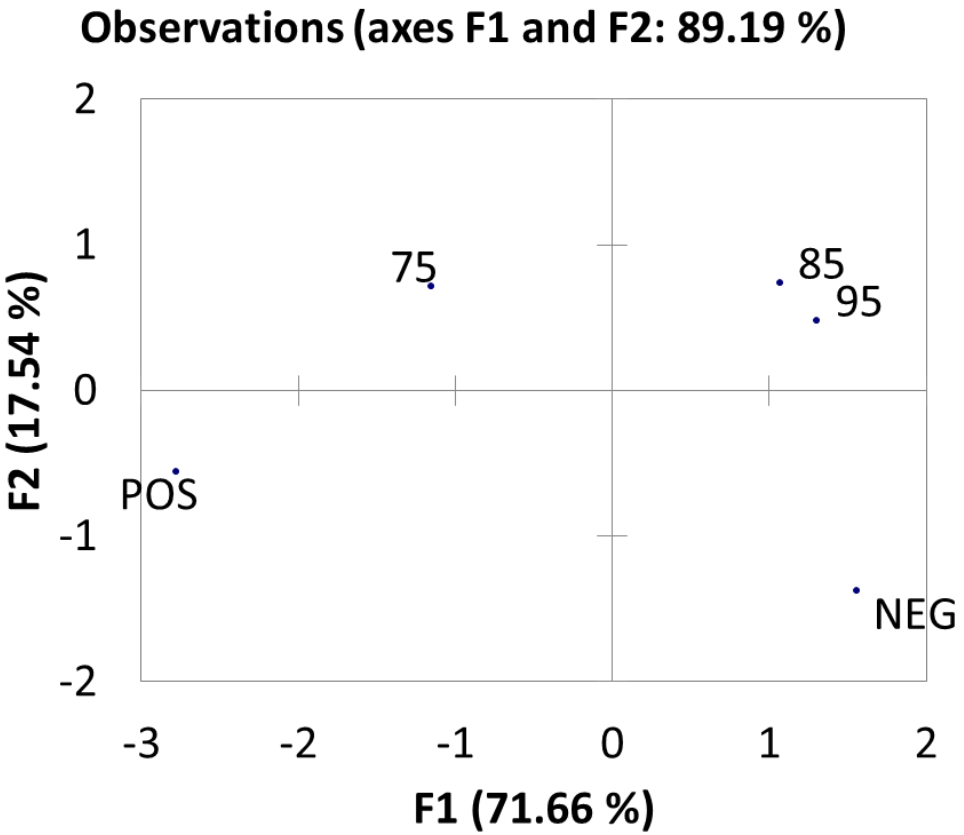
- Phenolic analyses: RB (+) only small differences
- ↓ [anth], [pol pigments], [pol phenols], % Alc

CS 2 – 2015: Averaged fermentation reps – signf attributes





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What does it mean?

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

In Summary

- Results indicate RB impact is not variety but site specific
  - Seasonal impact
- Untargeted metabolomics indicated large impact on primary metabolites
  - Organic acids
  - Sugars
  - Amino acids
  - Polysaccharides
- Some volatile and non-volatile secondary metabolites (phenols, aroma precursors) also impacted

Next Steps

- Make wines from RB (+) and (-) grapes with the same sugar content
  - Sequential harvesting - 2016
- Continue to explore impact of site on variety
  - Find correlation with soil, nutrients…..
- Targeted analysis combined with transcriptomics to identify metabolic pathways altered by RB disease resulting in changes in biochemical composition
- Use impact on gene expression to develop potential counter measures

THANK YOU