



Mapping and Detection of Downy Mildew and Botrytis bunch rot Resistance Loci in Norton-based Population

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***Vitis aestivalis*-derived
'Norton'
State Grape of Missouri**

Norton has been grown in Missouri for over 160 years, but little is known about the genetics of its disease resistance, cold hardiness and berry quality.



Genetics of Norton (Missouri State) Grapes

Norton has naturally evolved resistance to

- 1. Powdery mildew**
- 2. Downy mildew**
- 3. Berry rot complex including *Botrytis*
Bunch rot, Bitter rot and Black rot**
- 4. Insect Phylloxera**
- 5. Cold hardiness**



Norton vs Cabernet Sauvignon

***Vitis aestivalis*-derived ‘Norton’**

Cold hardy and Resistant to most fungal pathogens
Good wine quality

***Vitis vinifera* ‘Cabernet Sauvignon’**

Cold sensitive and Susceptible to most fungal pathogens
Great wine quality

A need exists to breed for grapevines that would combine the superior wine quality of *V. vinifera* with the disease resistance and cold hardiness of Norton.

Interspecific Hybrid Identification

Genetic profiles (allele sizes in bp) of grape varieties at various SSR loci

Primers	VVMD5	VVMD7	VVMD27	VVS2	VrZAG62	VrZAG79
Norton	233/247	237/246	184/186	135/137	181/205	250/254
Cabernet Sauvignon	231/240	239/239	173/187	141/154	189/195	246/246

Crosses tested for interspecific hybrid production

Crosses	# Plants evaluated	# True hybrids	% True hybrids
Norton (♀) x Cabernet Sauvignon (♂)	286	252	88.1
Norton (♂) x Cabernet Sauvignon (♀)	24	21	87.5



Genotyping



Norton Linkage Map Construction

- 1. 1,157 SSR markers were test on the parents and 4 progenies for polymorphism**
- 2. 414 polymorphic markers were identified and screened through a-182 genotype population**
- 3. 411 markers clustered in 19 linkage groups**

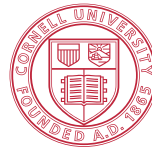
Missouri State
UNIVERSITY



VitisGen I Project (2011-2016)



- A 5-year project funded by the USDA-National Institute of Food and Agriculture (NIFA) Specialty Crops Research Initiative
- Combines the expertise of breeders, geneticists, pathologists, physiologists, chemists, enologists, computational biologists, sociologists, economists, and the grape industry
- 12 research institutions



Dr. Bruce Reisch
Cornell University



Cornell University
Cooperative Extension





VitisGen Project

Genotyping

Genotyping-by-Sequencing (GBS)

Single Nucleotide Polymorphism (SNP)

Ultimate Goal: 50,000 SNPs/Population

43,971 SNPs have been identified.

A consensus map of 3,825 SNPs has also been developed.



Genotyping:

Genotyping-by-Sequencing (GBS)

Single Nucleotide Polymorphism (SNP)

Simple Sequence Repeat (SSR)

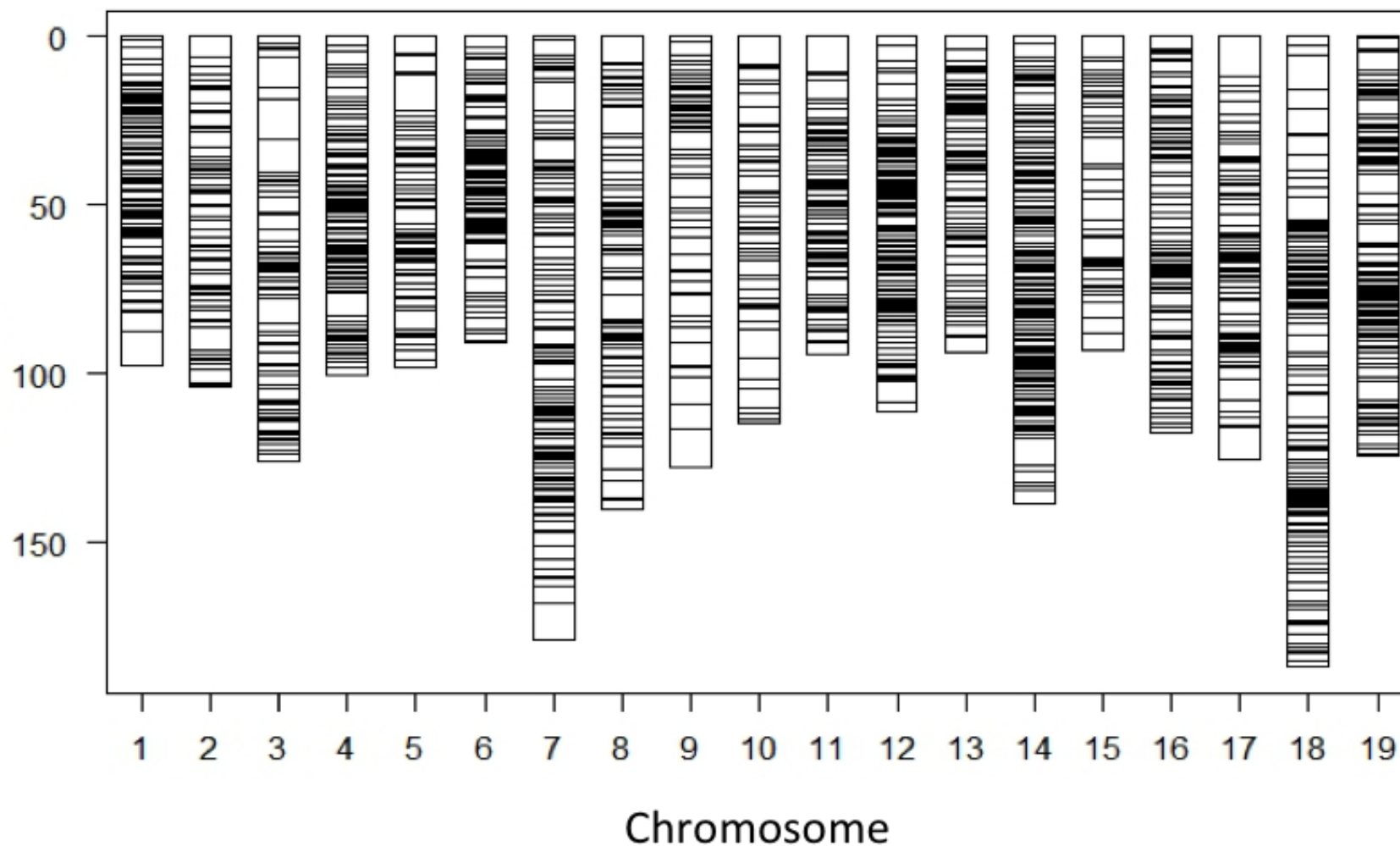
43,971 SNPs have been identified

A consensus map of 3,825 SNPs

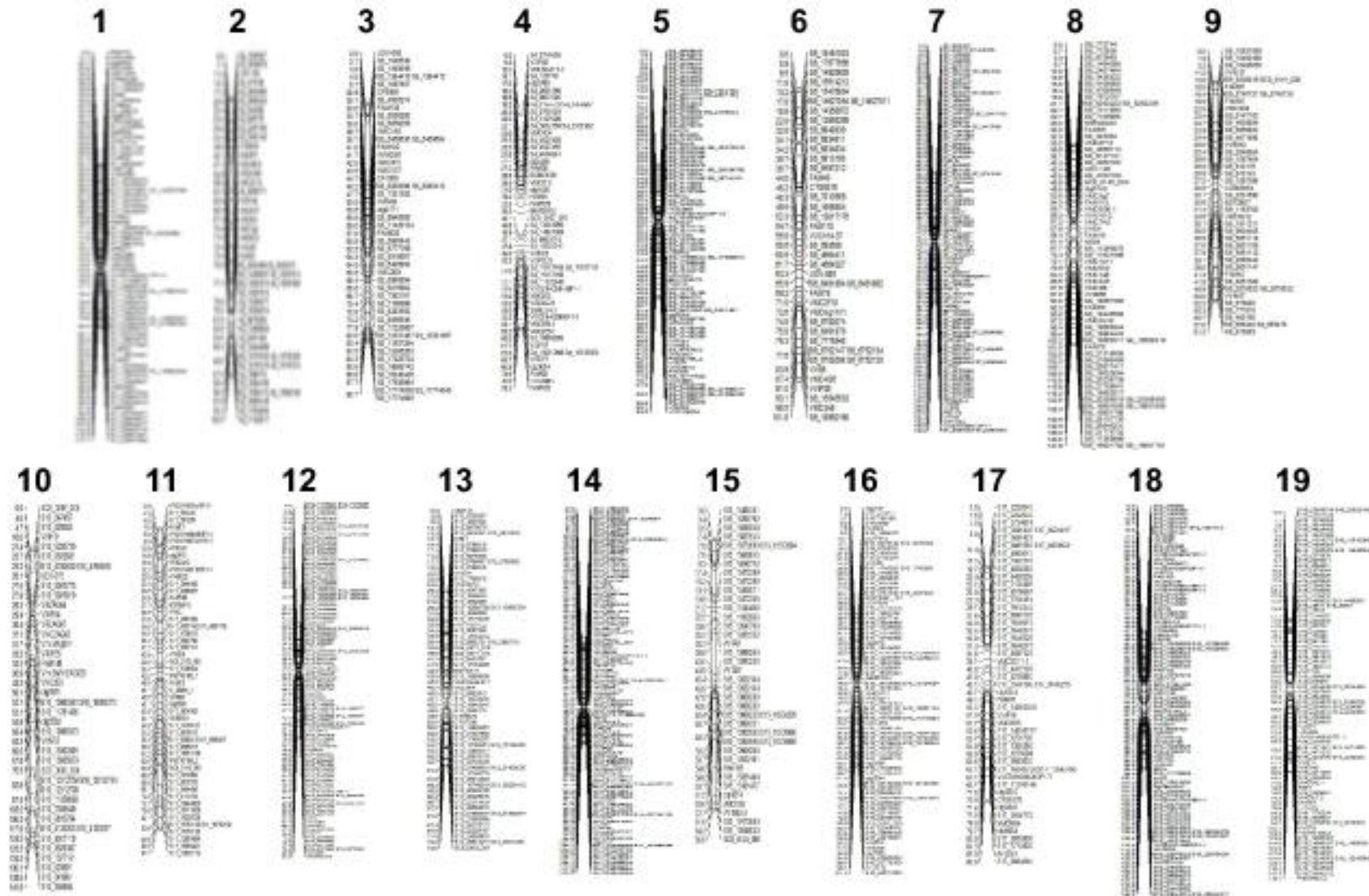
A consensus map of 411 SSRs

**Integration of Genetic Maps to construct
a high-resolution map with both SSR and
SNP markers using JoinMap 4.1 software**

Consensus Map



Norton Haploid Map



Downy Mildew

Norton

**Cabernet
Sauvignon**



Adaxial Side

Norton

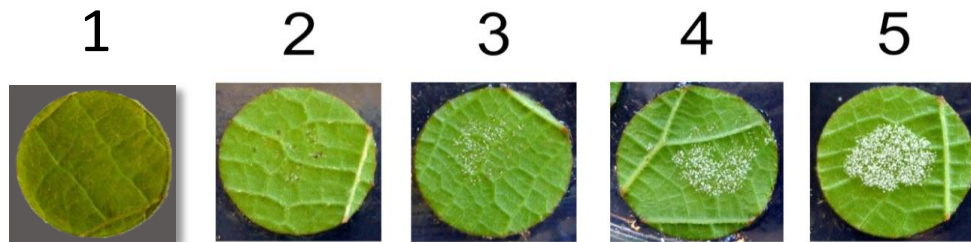
**Cabernet
Sauvignon**



Abaxial Side

Laboratory Assay

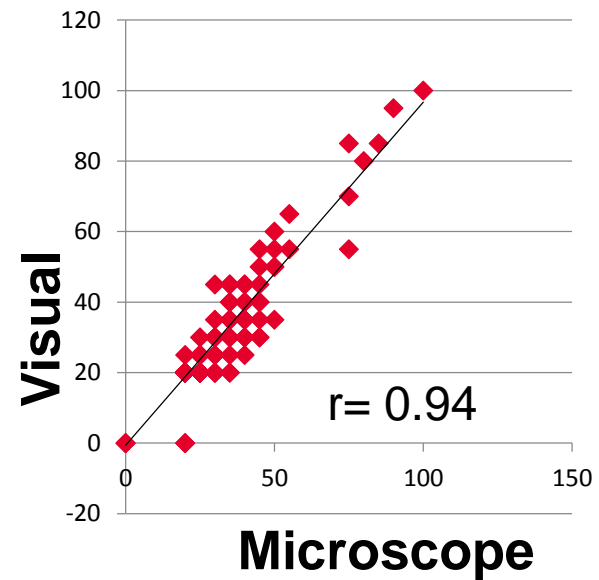
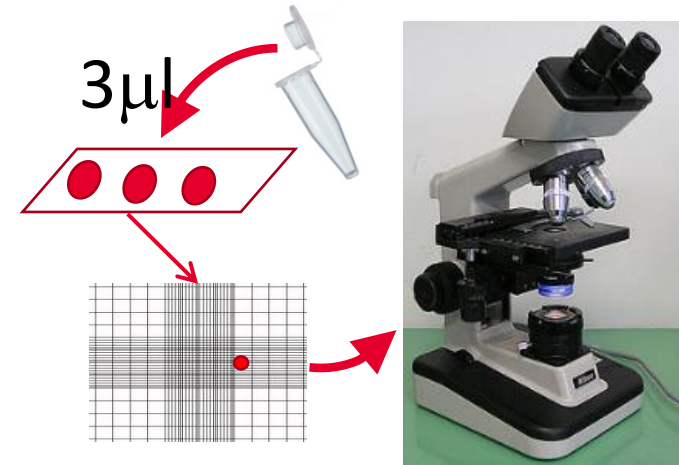
Visual rating



Norton

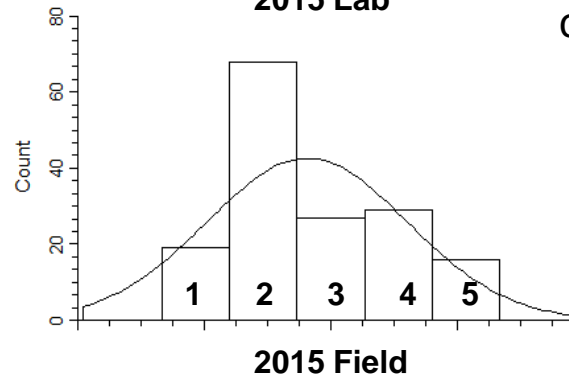
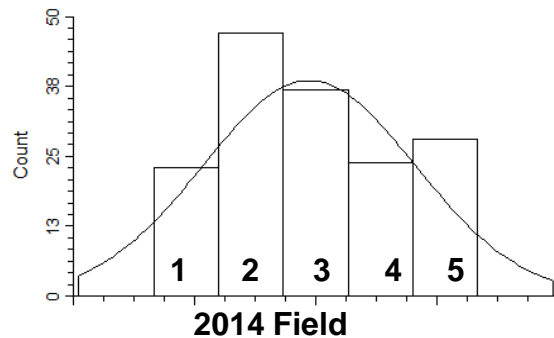
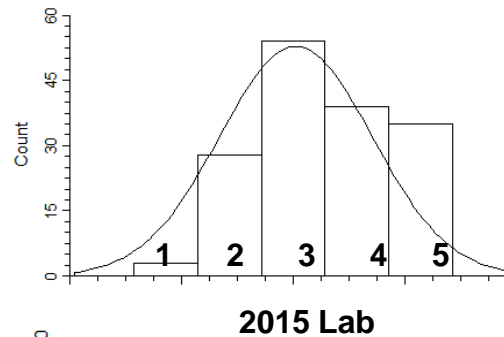
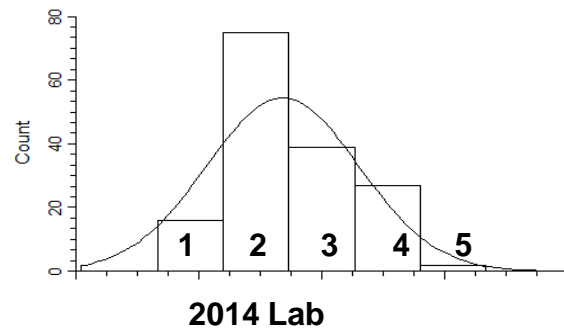
Cabernet
Sauvignon

Field Assay



Downy Mildew

Population Size	159
Repetitions	6
Duration	9 DAI



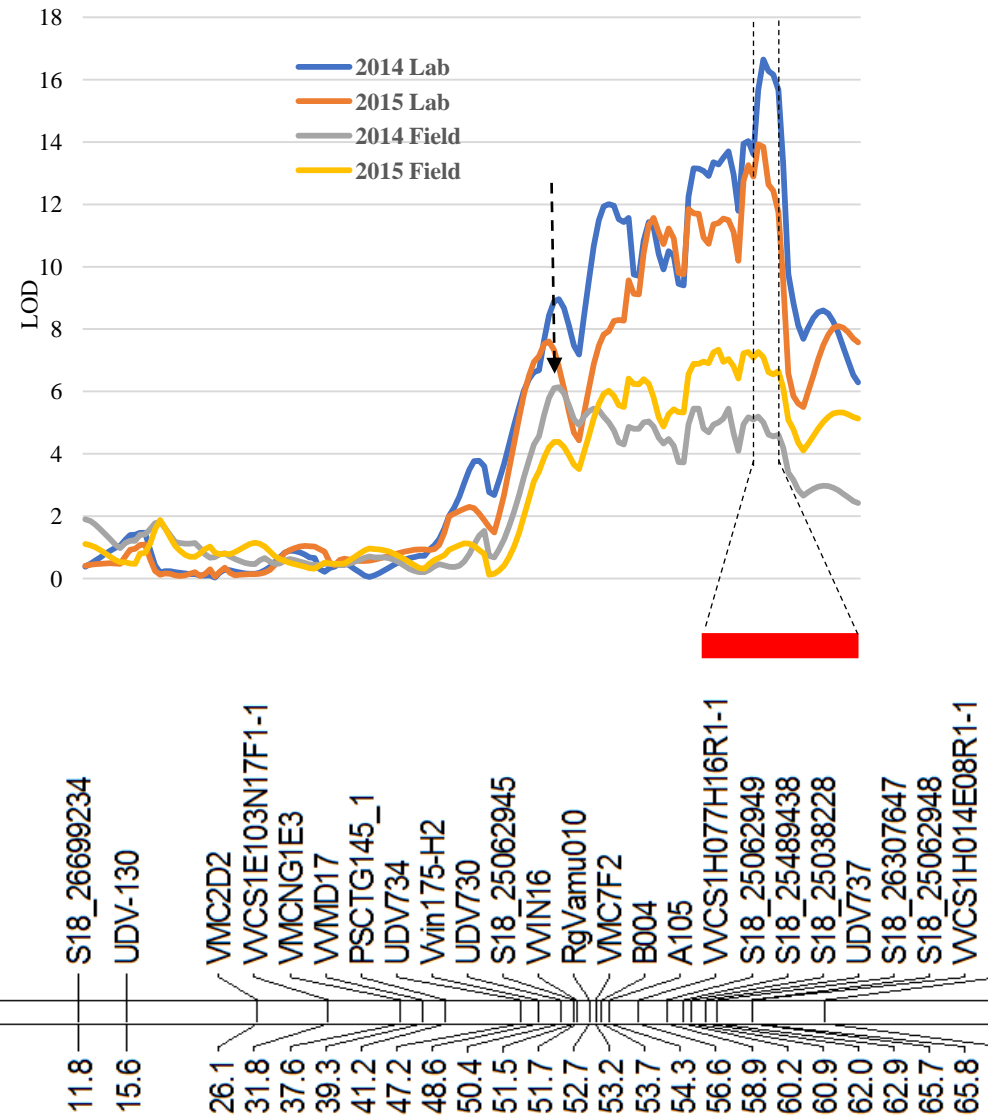
	2014 lab	2015 lab	2014 Field	2015 Field
2014 lab	1.00	0.69	0.66	0.63
2015 lab		1.00	0.57	0.59
2014 Field			1.00	0.79
2015 Field				1.00

Strong correlations were observed among data sets (Spearman correlation coefficient = 0.57 to 0.79)

Downy Mildew Resistance (*Rpv 25*)

*LOD value of 16.4 explaining 33.8% of the total phenotypic variation flanked by markers VVCS1H077H166R1-1 (56.6 cM) and UDV737 (60.9 cM).

*Three SNP-trait association were detected between the two flanking SSR markers, further reducing the interval distance to 0.7-2.3 cM.



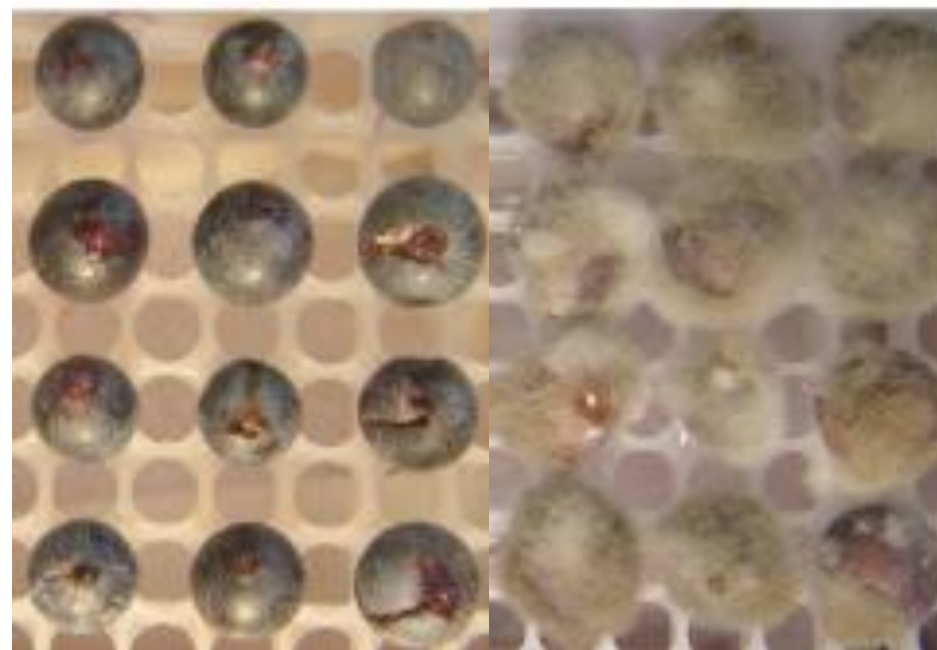
Linkage Group 18

Botrytis Bunch Rot

Botrytis cinerea

Cabernet
Sauvignon

Norton



Botrytis Bunch Rot

Botrytis cinerea

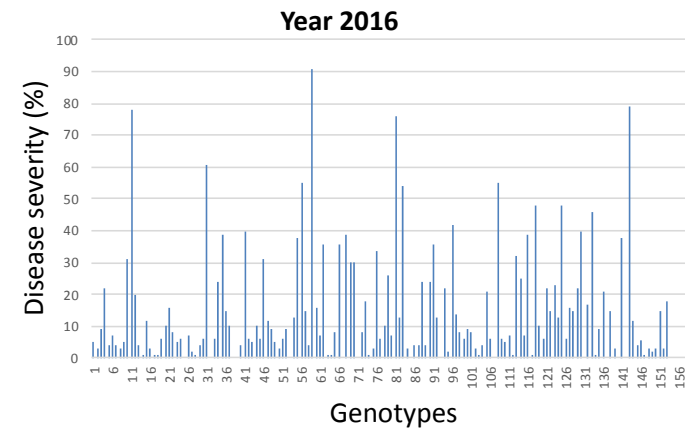
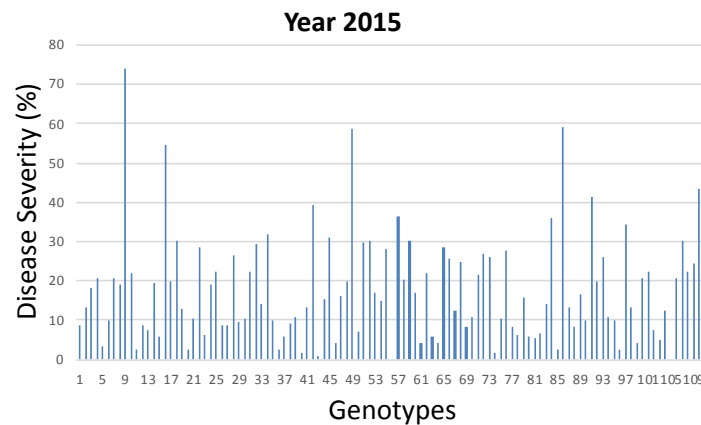
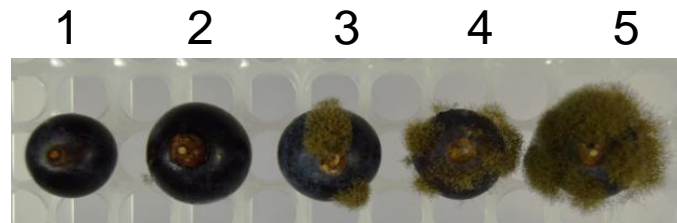
Table 1. Average disease incidence and severity between two grape cultivars; Norton and Cabernet Sauvignon (CS) under different stages.

Harvesting Stage	Disease Incidence				Disease Severity			
	2012		2013		2012		2013	
	Norton	CS	Norton	CS	Norton	CS	Norton	CS
E-L Stage 36	4.2	100.0	8.3	100.0	1.7	96.7	3.3	96.7
E-L Stage 37	0.0	83.3	12.5	95.8	0.0	80.8	5.8	86.7
E-L Stage 38	0.0	91.7	16.7	100.0	0.0	91.7	6.7	96.7
E-L Stage 39	4.1	100.0	16.7	100.0	1.6	96.7	10.8	100.0
E-L Stage 40	0.0	100.0	10.0	100.0	0.0	100.0	6.7	98.3

Values are mean for five replicates for each stage between two cultivars for both years.
Significant differences (two-sampled t test) are designed at $P > 0.05$.

Botrytis Bunch Rot

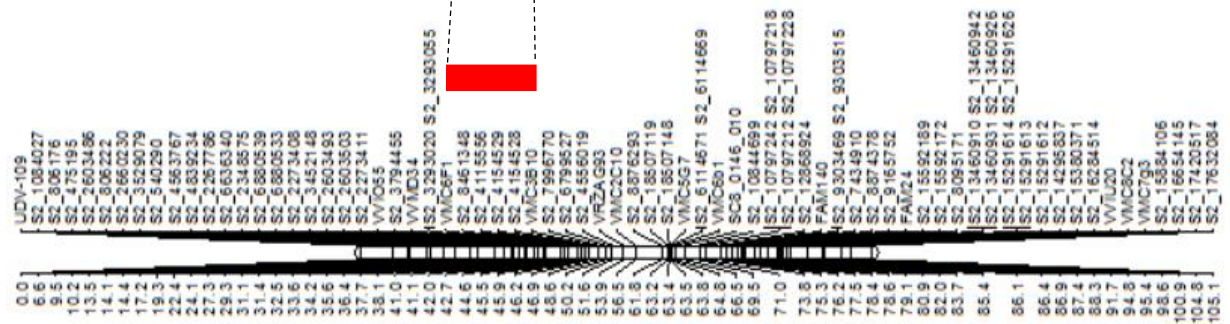
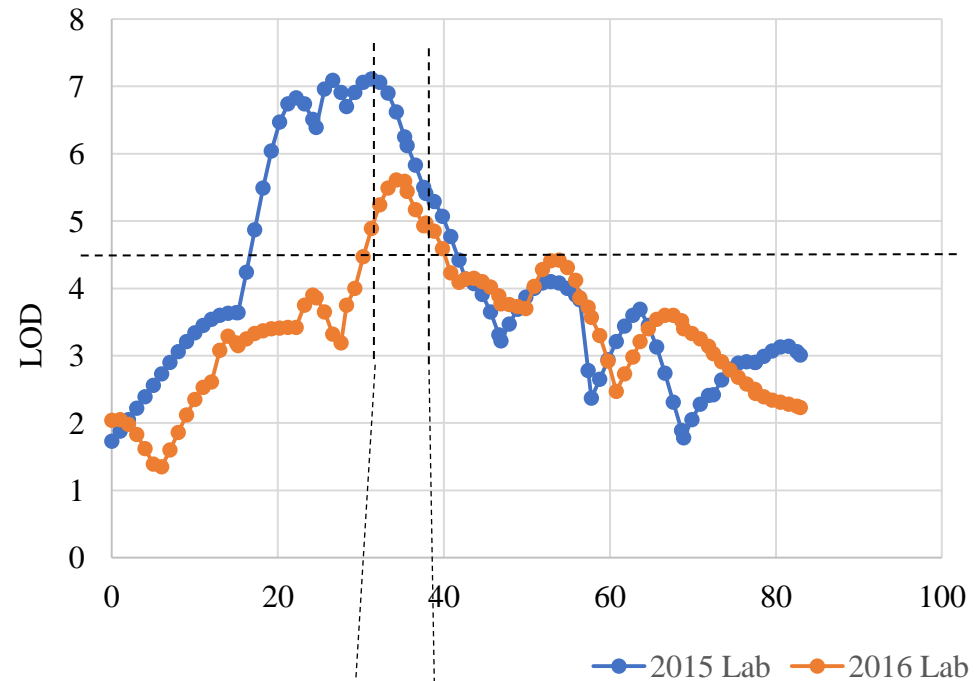
Population Size	158
Repetitions	8
Duration	10 DAI



Botrytis Bunch Rot Resistance

*LOD value of 7.1 explaining 18.4% of the total phenotypic variation flanked by markers VMC6F1 (42.7 cM) and VMC3B10 (46.9 cM).

*Four SNP-trait associations were detected between the two flanking SSR markers, further reducing the interval distance to 0.3-1.9 cM.



Linkage Group 2

Norton x Vignoles



Summary

1. GBS data can be used to saturate the grape genome with SNPs in a pseudo-testcross population.
2. SNPs and SSRs can have complementary roles: first, to identify genome regions associated with traits of interest using SNPs, and second, to perform marker-assisted selection using SSRs.
3. **The overall goal of this program is to provide molecular genetic support to expedite a Norton grape breeding effort with the ultimate goal of developing improved cultivars well adapted to Missouri conditions.**



North American Grape Breeders Conference

Arkansas, California, Florida, Georgia, Minnesota and New York

2015 Cornell University; 2017 UC Davis

2019 Missouri State University

August 15-16, 2019

Field Day – Thursday

Missouri State Fruit Experiment Station, Mtn. Grove. MO

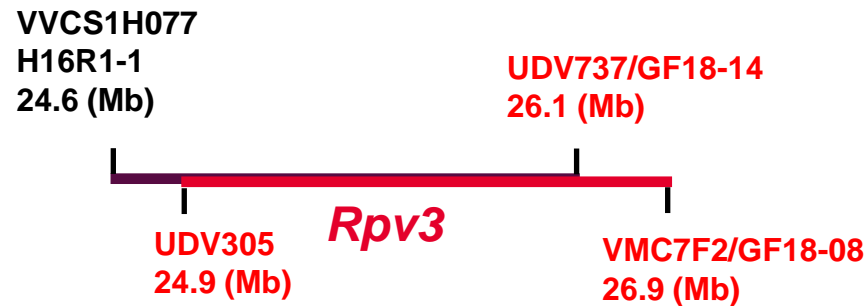
MSU breeding vineyard (*VitisGen II*) & winery; Virus cleaning network

St. James Winery, St. James, MO

Research Presentation – Friday

Bond Learning Center, Springfield, MO

Linkage Group 18



1. Di Gaspero et al. (2012) studied the selective sweep in *Rpv3* using its flanking SSR loci UDV305 and UDV737. Seven different haplotypes were generated; however, none of the haplotypes were present in 'Norton'/'Cynthiana'.
2. The *Rpv3* related SSR markers including UDV305, UDV108, UDV112 and VMC7F2 didn't show polymorphism in Norton.
3. Downy mildew resistance in 'Norton' is most likely due to the presence of a new locus within the shared region of *Rpv3* (UDV305 and UDV737), or in the unique region between markers of VVCS1H077H16R1-1 and UDV305.

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