*Vitis*Gen2 Approaches to Powdery Mildew Resistance

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USDA United States Department Of Agriculture Agricultural Research Service

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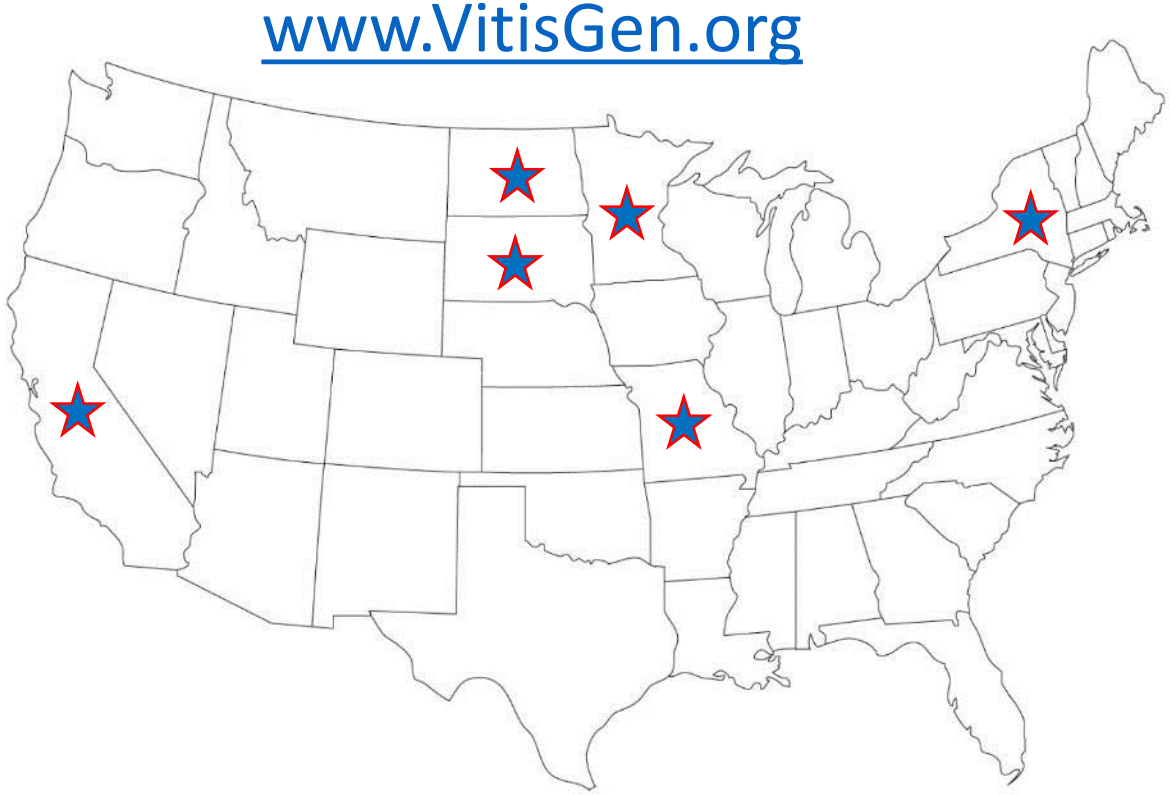
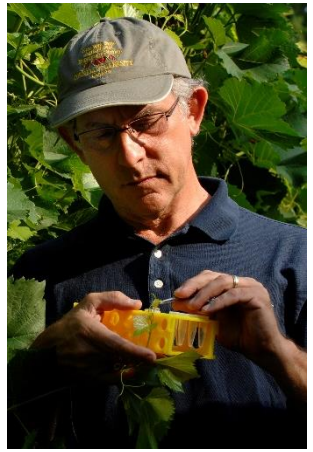
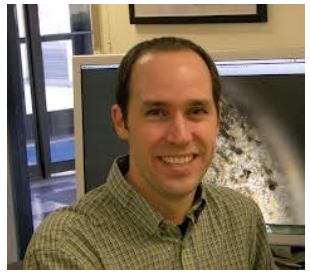
VitisGen unites breeding programs nationwide

www.VitisGen.org







Key *Vitis*Gen components

Provide tools for traditional breeding:

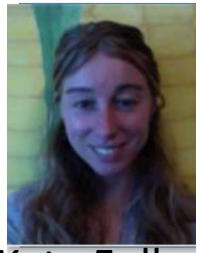
- Funding to maintain mapping populations
- Centralized genetic mapping and marker-assisted selection
- Centralized phenotyping
  - Powdery mildew resistance
  - Cold tolerance (*Vitis*Gen1)
  - Fruit chemistry
- Local phenotyping
  - Regionally important traits and unplanned opportunities

Key *Vitis*Gen2 changes

- From markers to genes
- Multi-location plantings
- Translate research tools to inform management decisions
- Automated phenotyping, computer vision
- Release grapevines with powdery mildew resistance
- Recurring column in Wines and Vines
- Every team member involved in Extension
  - Webinar series
- Willingness-to-pay Economic analyses

Value of PM resistance
(CA Raisin grape example, in $millions)


Julian Alston


Kate Fuller


Olena Sambucci

	Adoption Lag (Years)			
Adoption Rate	**10**	**20**	**30**	**40**
20%	$124M	92	69	51
60%	372	277	206	153
100%	620	461	343	255

Incentive to accelerate and increase adoption

PM Phenotyping

Vineyard approach

1. Allow natural infection of unreplicated vines
2. Record disease severity on 4- or 5-point scale (typically)

<u>Some uncontrolled variables:</u>

- Powdery mildew genetics
- Non-random inoculum
- Year-to-year environment
- Spatial variability across vineyard
- Spatial variability within vine
- Lighting conditions to see PM
- Timepoint during epidemic
- Subjectivity and bias in rating

REN2 (Chr14) with IPGRI ratings (9-point)

Date	Linkage Group	LOD	LOD Threshold	R^2
09-17-12	NA	NA	NA	NA
09-25-12	NA	NA	NA	NA
09-17-13	4	3.69	3.40	9.6
	15	3.60	3.40	9.4
08-27-14	22	3.97	3.34	11.6
09-03-14	NA	NA	NA	NA

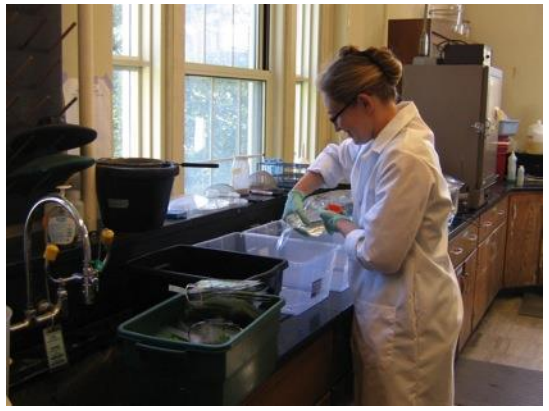
Observation: Difficult to detect or confirm minor and moderate QTL.

PM Precision Phenotyping

Laboratory approach

Hypothesis: Field evaluation works for qualitative resistance, but need tighter control for minor and moderate quantitative resistance analysis.

Solution: Breeders ship leaves to a phenotyping center for processing surface sterilized 1-cm leaf discs.



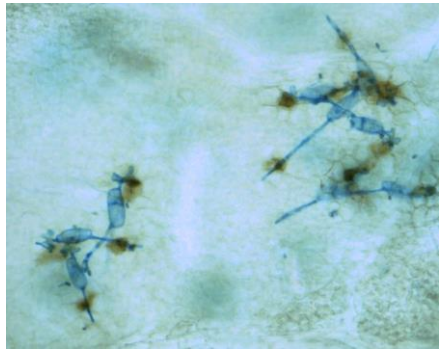
Up to 260 leaf disks on agar are inoculated with a single isolate of powdery mildew.

PM Precision Phenotyping

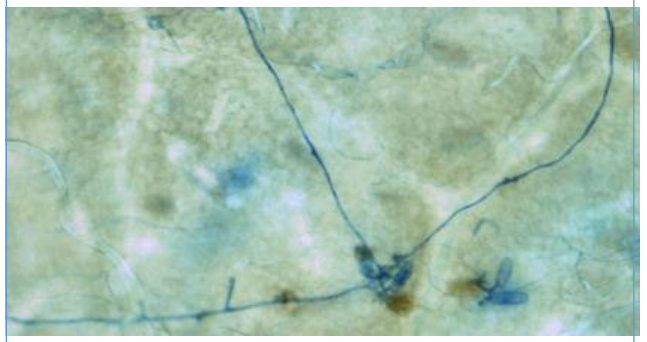
Laboratory approach

Plan A: Over 230,000 spores in Years 1 and 2 were rated at 48 hpi for:

Appressorium only = Penetration Resistance



Single hypha = Colony Resistance

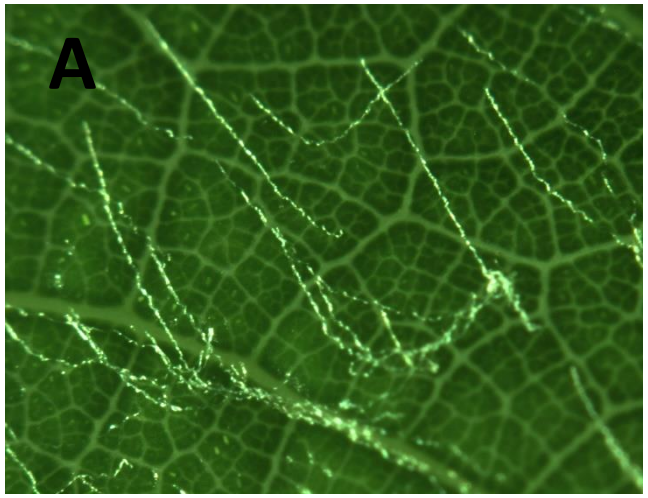


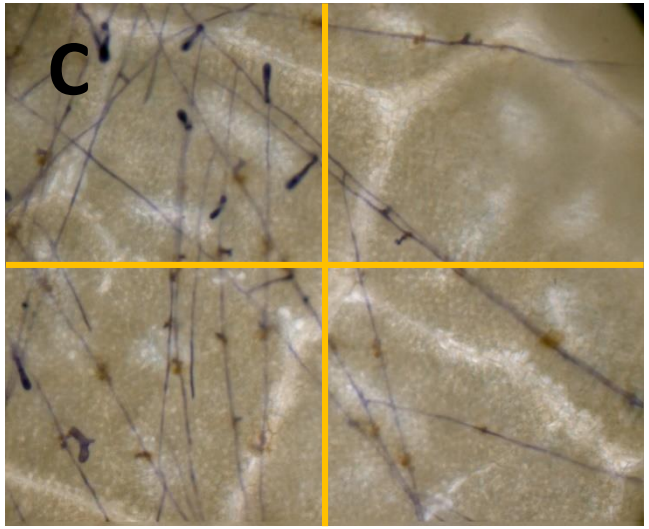
Colony = No Resistance

- Widely accepted approach
- Great quantitative distribution
- Poor heritability
- Poor reproducibility
- Time and resources wasted

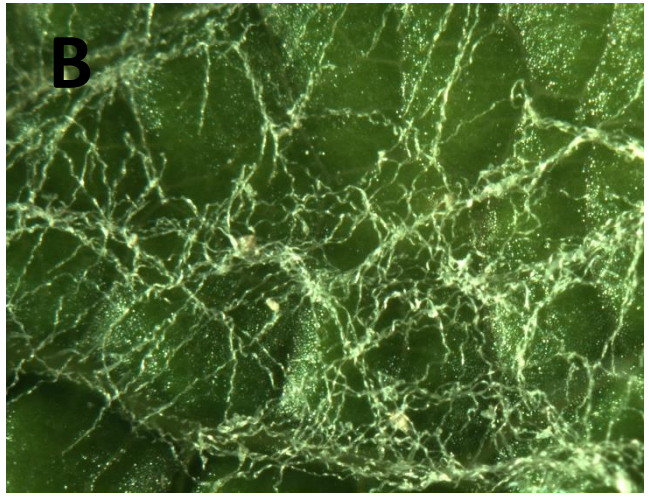
Hyphal transect quantification

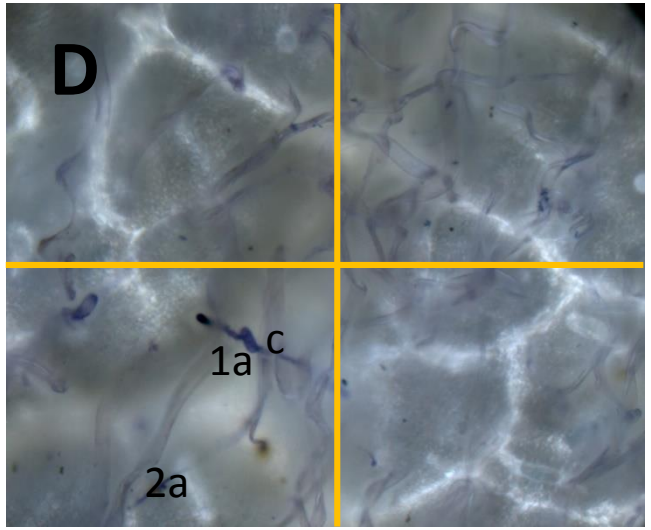
Susceptible

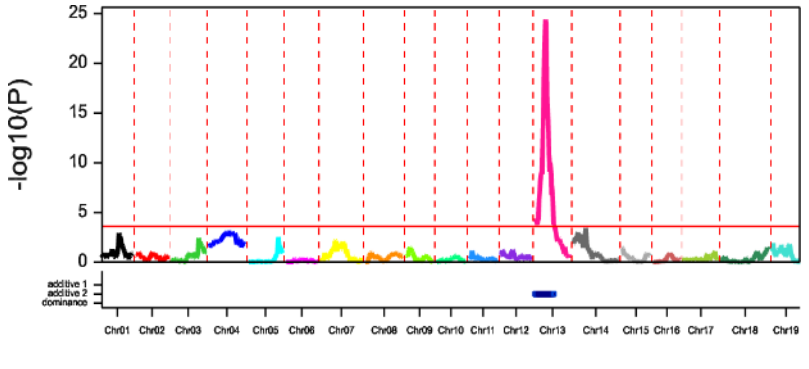




Resistant (but many trichomes)







David Gadoury

Jonathan Fresnedo

Phytopathology 106:1159-1169.

PM Center vs. Field ratings

REN2 locus (Chr14)

Data	LOD	R^2
2012 Transects	n.s.	NA
2014 Transects1	4.2	11.7
2014 Transects2	3.5	12.7
08-25-11 Field	n.s.	NA
09-17-12 Field	n.s.	NA
09-25-12 Field	n.s.	NA
09-17-13 Field	n.s.	NA
08-27-14 Field	n.s.	NA
09-03-14 Field	n.s.	NA

REN1 locus (Chr13)

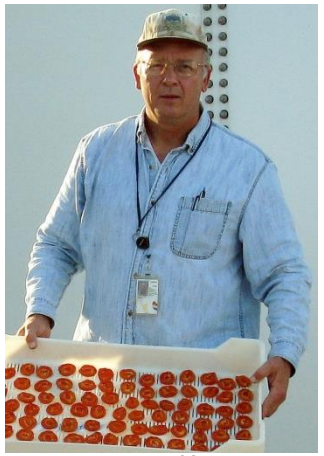
Data	LOD	R^2
2012 Transects	8.2	27.1
2013 Transects	15.3	46.2
2014 Transects1	22.0	58.0
2014 Transects2	17.2	54.9
2014 Early Field	7.4	18.4
2015 Early Field	6.4	18.9
2015 Late Field	n.s.	NA

Phytopathology 106:1159-1169.

David Gadoury

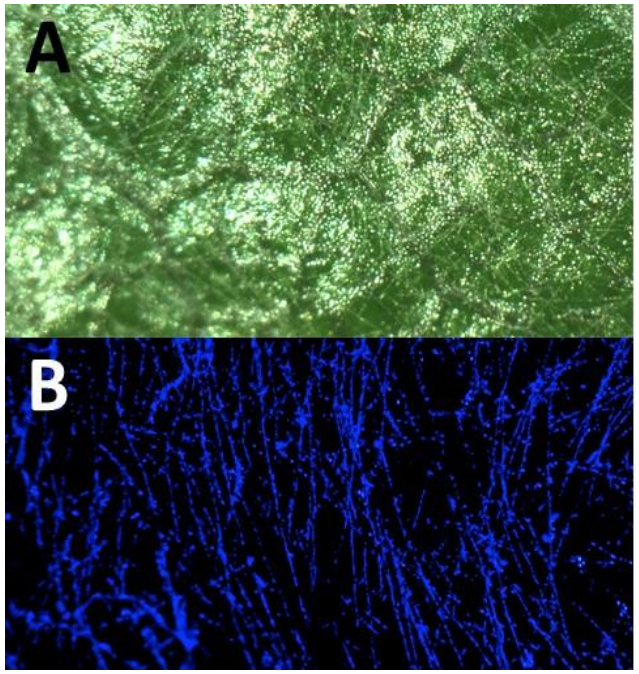
Paola Barba

Bruce Reisch



Craig Ledbetter

*Vitis*Gen2: Automated disease quantification

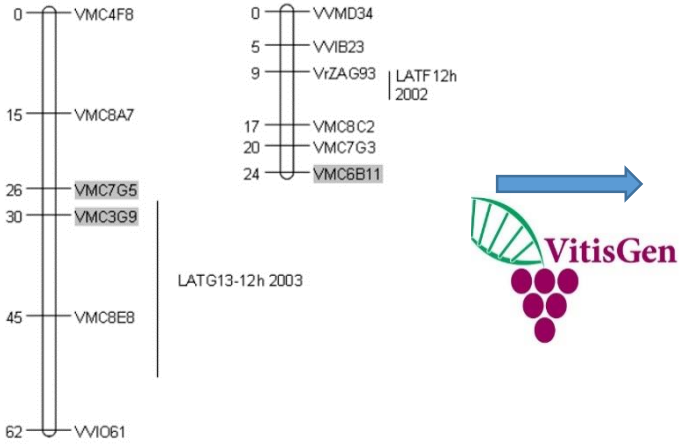


Tim Plummer

Andrew Bierman

Breanne Kisselstein

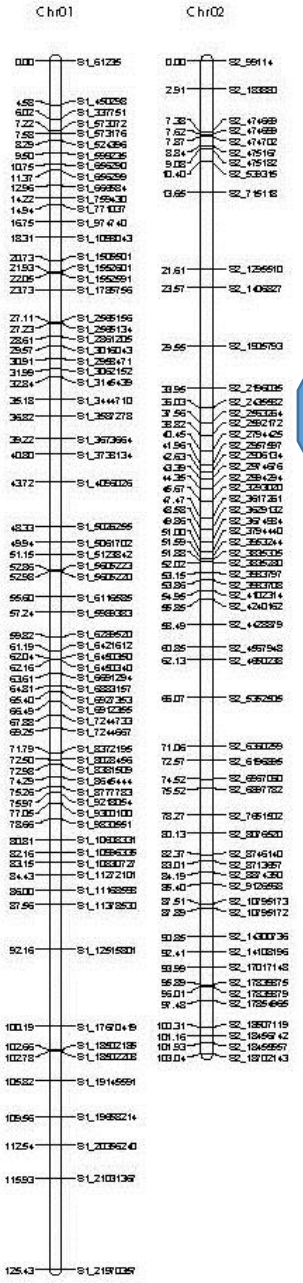
High resolution maps to genomes and genes



SSR map (2009)

	SSR map	SNP map
Progeny	119	424
Markers	120	1449
Linkage groups	21	19
Distance between markers (cM)	14.87	1.67
Genome coverage (%)	83.7	95.1

PlosONE 11(3): e0149560 and 10(8): e0134880



SNP map (2014)

Trehalose-6-phosphate phosphatase	4897621..4903784	VIT_202s0154g00110
Unknown	4914168..4914984	VIT_202s0154g00120
Exostosin (Xyloglucan galactosyltransferase KATAMARI 1)	4921780..4923581	VIT_202s0154g00130
3-oxoacyl-[acyl-carrier-protein] synthase 3 A, KASIII	4921664..4935576	VIT_202s0154g00140
PLATZ transcription factor	4949210..4950534	VIT_202s0154g00150
Ethylene overproducer like 1 (ETO1) *	-	-
Flavin-containing monooxygenase family	4951841..4956070	VIT_202s0154g00160
Flavin-containing monooxygenase 3	4957715..4960913	VIT_202s0154g00170
Flavin-containing monooxygenase 3	4962382..4965728	VIT_202s0154g00180
Flavin-containing monooxygenase 3	4974431..4978234	VIT_202s0154g00190
Unknown protein (*VviFSEX**)	4981728..4986851	VIT_202s0154g00200
WRKY DNA-binding protein 21	4989461..4990306	VIT_202s0154g00210
Adenine phosphoribosyltransferase APRT3 / APRT1 *	-	-
Unknown	5025223..5026178	VIT_202s0154g00220
Phosphatidic acid phosphatase / PAP2 *	-	-

GGRU efforts on gene editing

New hybrid grapes
~$800 / ton

Old variety grapes
~$1600 / ton

Non-transgenic clones of existing varieties.

- Gene knock-out
- Allele swapping

See GGRU poster for more details

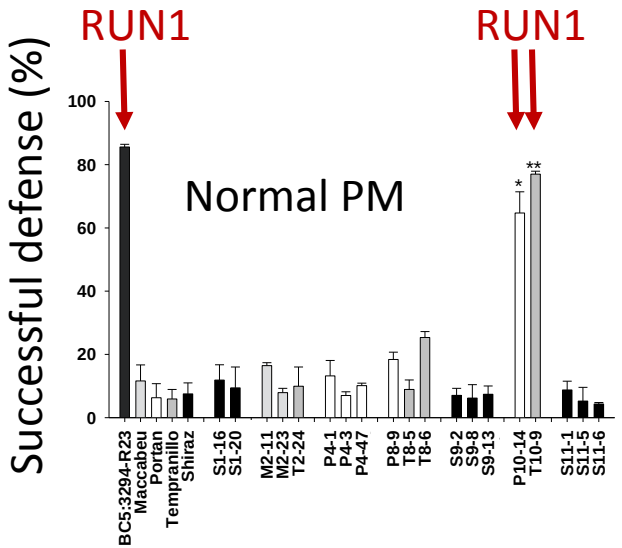
PM resistance in U.S. bunch grape breeding programs

	Locus	Chr	*Vitis* source	Reference
	REN1	13	*vinifera*	Hoffman et al. 2008
	REN2	14	*cinerea*	Cadle-Davidson et al. 2016
	REN3	15	*complex*	Welter et al. 2007
Strong	***REN4***	**18**	***romanetii***	**Ramming et al. 2011**
Strong	***REN6***	**9**	***piazeskii***	**Pap et al. 2016**
	REN7	19	*piazeskii*	Pap et al. 2016
	REN10	2	*complex*	Teh et al. 2017
Strong	***RUN1***	**12**	***rotundifolia***	**Feechan et al. 2013**
	RUN2.1	18	*rotundifolia*	Ramming et al. 2012
	new	?	*aestivalis*	Ramming et al. 2012
	new	7?	*rupestris*	Barba et al. 2015

Acknowledgments: Bruce Reisch, David Ramming, Craig Ledbetter, Andy Walker, Summaira Riaz, Pal Kozma, Matt Clark, Jim Luby, David Gadoury, Bob Seem, Ian Dry, Angela Feechan, Michael Milgroom, Joe Smilanick, Molly Cadle-Davidson, Marianna Kocsis, Paola Barba, Omer Frenkel, Marin Brewer, Raj Majumdar, Siraprapa Mahanil, Sara Lagerholm, Michelle Schaub, Anna Nowogrodzki, Hema Kasinathan, Mary Jean Welser, Paige Appleton, Wei Zhang, Nancy Consolie, Jackie Lillis, Erin Galarneau, Franka Gabler, and others

Now, simply crank out PM resistant cultivars

“Race specificity” *Vitis rotundifolia* example (*RUN1*)



- *RUN1* in vinifera
  - PM Resistant…Eureka!

“Race specificity”
Vitis rotundifolia example (*RUN1*)

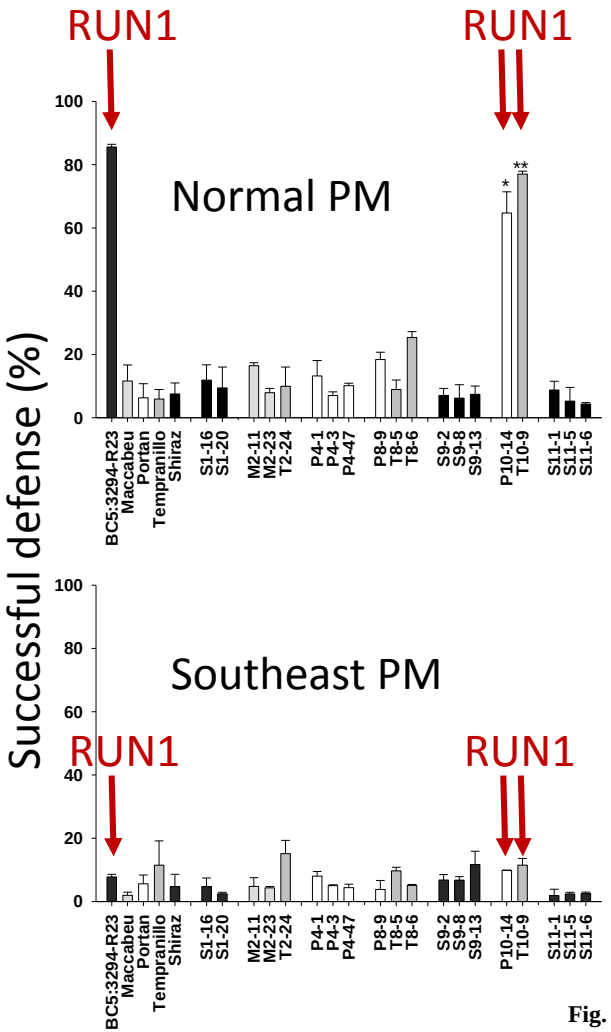


Fig. S3. Feechan *et al.*

- *RUN1* in vinifera
  - ➢PM Resistant…Eureka!
- Challenge with PM from Southeast U.S.
  - ➢PM Susceptible…sigh.

"Race specificity"
Vitis rotundifolia example (*RUN1*)

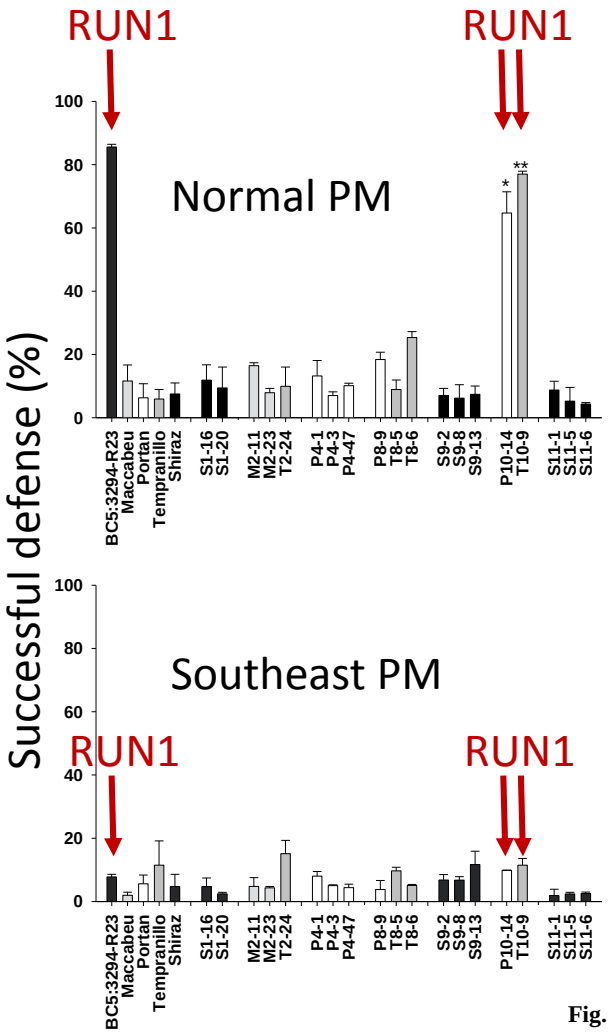


Fig. S3. Feechan *et al.*

- *RUN1* in vinifera
  - ➢PM Resistant…Eureka!
- Challenge with PM from Southeast U.S.
  - ➢PM Susceptible…sigh.
  - ➢Similarly, we find PM in NY that can reproduce on *RUN1* vines

Vitis (2011) 50:173–175

PM resistance in U.S. bunch grape breeding programs

	Locus	Chr	*Vitis* source	Race-specific	Reference
	REN1	13	*vinifera*	Yes	Hoffman et al. 2008
	REN2	14	*cinerea*	Yes	Cadle-Davidson et al. 2016
	REN3	15	*complex*	Yes	Welter et al. 2007
Strong	***REN4***	**18**	***romanetii***	**?**	**Ramming et al. 2011**
Strong	***REN6***	**9**	***piazeskii***	**nd**	**Pap et al. 2016**
	REN7	19	*piazeskii*	nd	Pap et al. 2016
	REN10	2	*complex*	nd	Teh et al. 2017
Strong	***RUN1***	**12**	***rotundifolia***	**Yes**	**Feechan et al. 2013**
	RUN2.1	18	*rotundifolia*	Yes	Ramming et al. 2012
	new	?	*aestivalis*	Yes	Ramming et al. 2012
	new	7?	*rupestris*	Yes	Barba et al. 2015

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PM resistance in U.S. bunch grape breeding programs

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Strong	***REN6***	**9**	***piazeskii***	**nd**	**Pap et al. 2016**	**Yes**
	REN7	19	*piazeskii*	nd	Pap et al. 2016	Yes
	REN10	2	*complex*	nd	Teh et al. 2017	
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Plan: PM resistance mapping

- Test combinations of resistance genes for durability prediction
- Build pipeline subsequent to *RUN1*, *REN4*, *REN6*, etc.
  - Six new wild sources of PM resistance
    - Four crossed to CA Table Grape (Ledbetter)
    - Two crossed to NY Wine Grape (Reisch)
  - Vineyard and Laboratory evaluation
  - Genetic mapping
  - AmpSeq markers developed if germplasm proves useful

*Vitis*Gen2 Genotyping and PM Goal

Provide germplasm, strategies, protocols, and marker technology (cost-effective and accessible) to make PM resistance breeding *easy, efficient, and effective*.