A perspective on breeding and implementing durable powdery mildew resistance

Lance Cadle-Davidson

Lance.CadleDavidson@ars.usda.gov

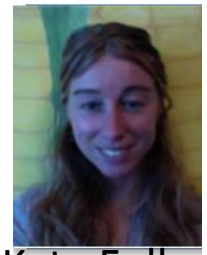
USDA United States Department Of Agriculture Agricultural Research Service

Overview

- Feed discussion: How do we ensure future generations can benefit from current efforts in PM resistance breeding?
  - Encourage: International collaboration to address this
- Share: experience of R-genes in Cornell breeding program
  - *RUN1/REN2* and *REN2/REN3*
- Update: *Vitis*Gen2 genotyping methods for gene stacking

Value of PM resistance
(California 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

Wine Economics and Policy 3:90-107

<u>Economic incentive to accelerate and increase adoption</u>

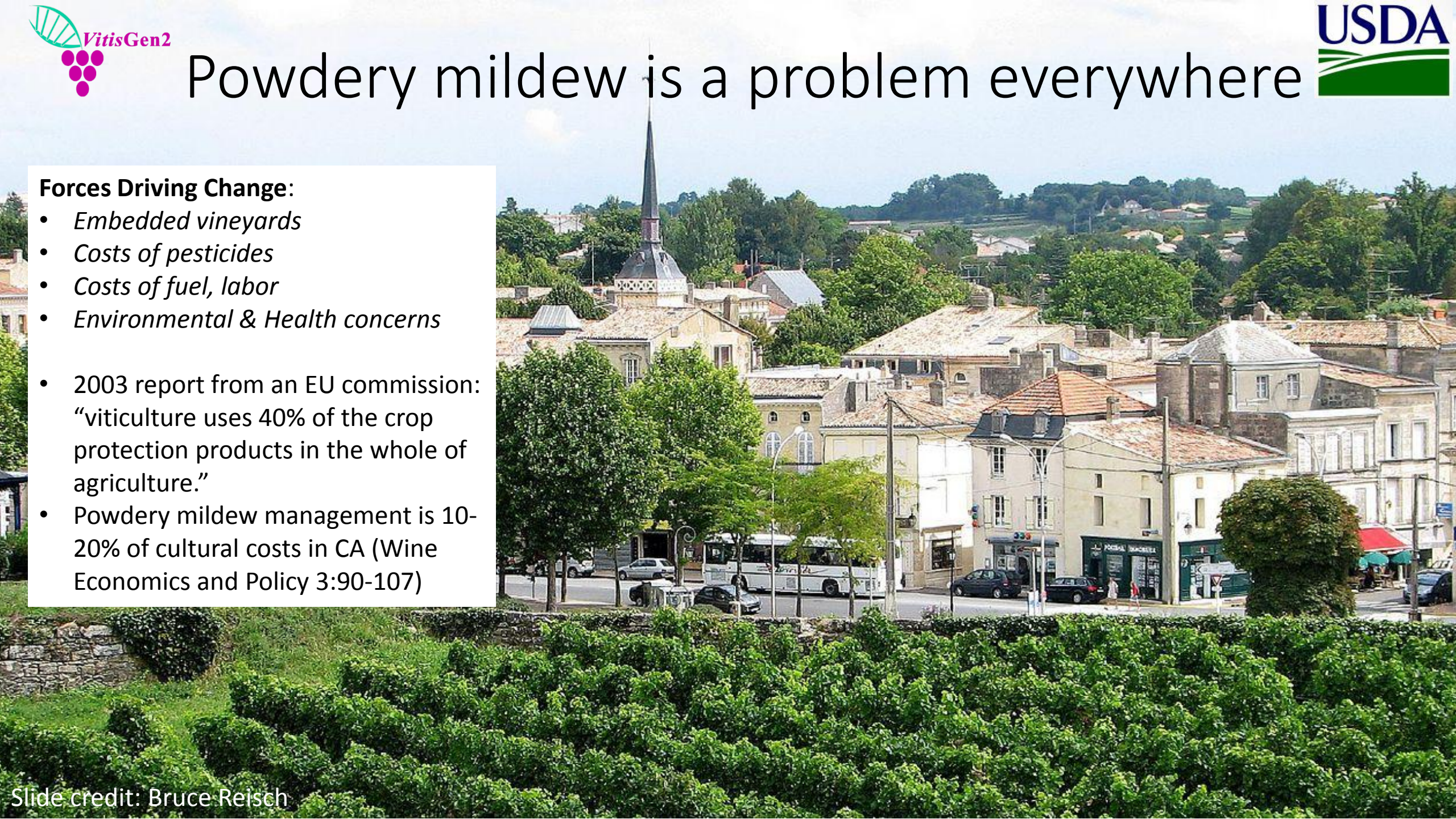
(Addresses trade-off between time and quality)

Powdery mildew is a problem everywhere

Forces Driving Change:

- *Embedded vineyards*
- *Costs of pesticides*
- *Costs of fuel, labor*
- *Environmental & Health concerns*

- 2003 report from an EU commission: “viticulture uses 40% of the crop protection products in the whole of agriculture.”
- Powdery mildew management is 10-20% of cultural costs in CA (Wine Economics and Policy 3:90-107)



Slide credit: Bruce Reisch

The rise of low-input grape cultivars

Decanter.com

Home | News | Wine | People & Places | Learning | Even

Wine News | Wine Blogs | Wine Videos | Wine Pictures

You are here: Decanter › News › Wine News

Bordeaux's Vignobles Ducourt plants 'disease resistant' vineyard

Tuesday 12 August 2014 | by Jane Anson

Like 49 Tweet 43 +1 7 Share 103

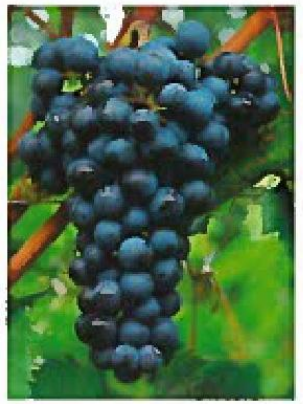
Vignobles Ducourt, which owns 440 ha in the Bordeaux region, has planted its first 'no spray vineyard' consisting of grapes crossed with disease resistant varieties.

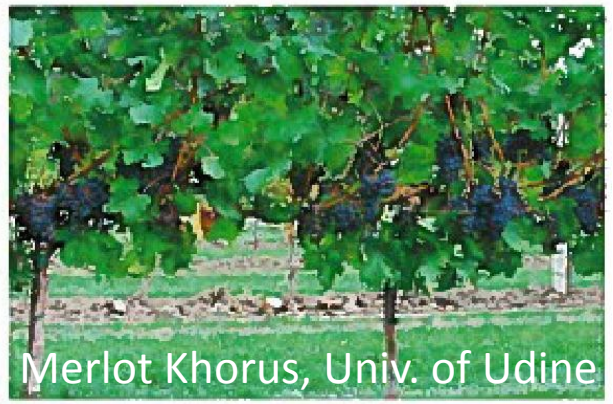
Borsmenta, University of Pécs

Vidoc, INRA

Calardis blanc, JKI

More details in Bruce Reisch's webinar at:
www.*Vitis*Gen2.org/webinars





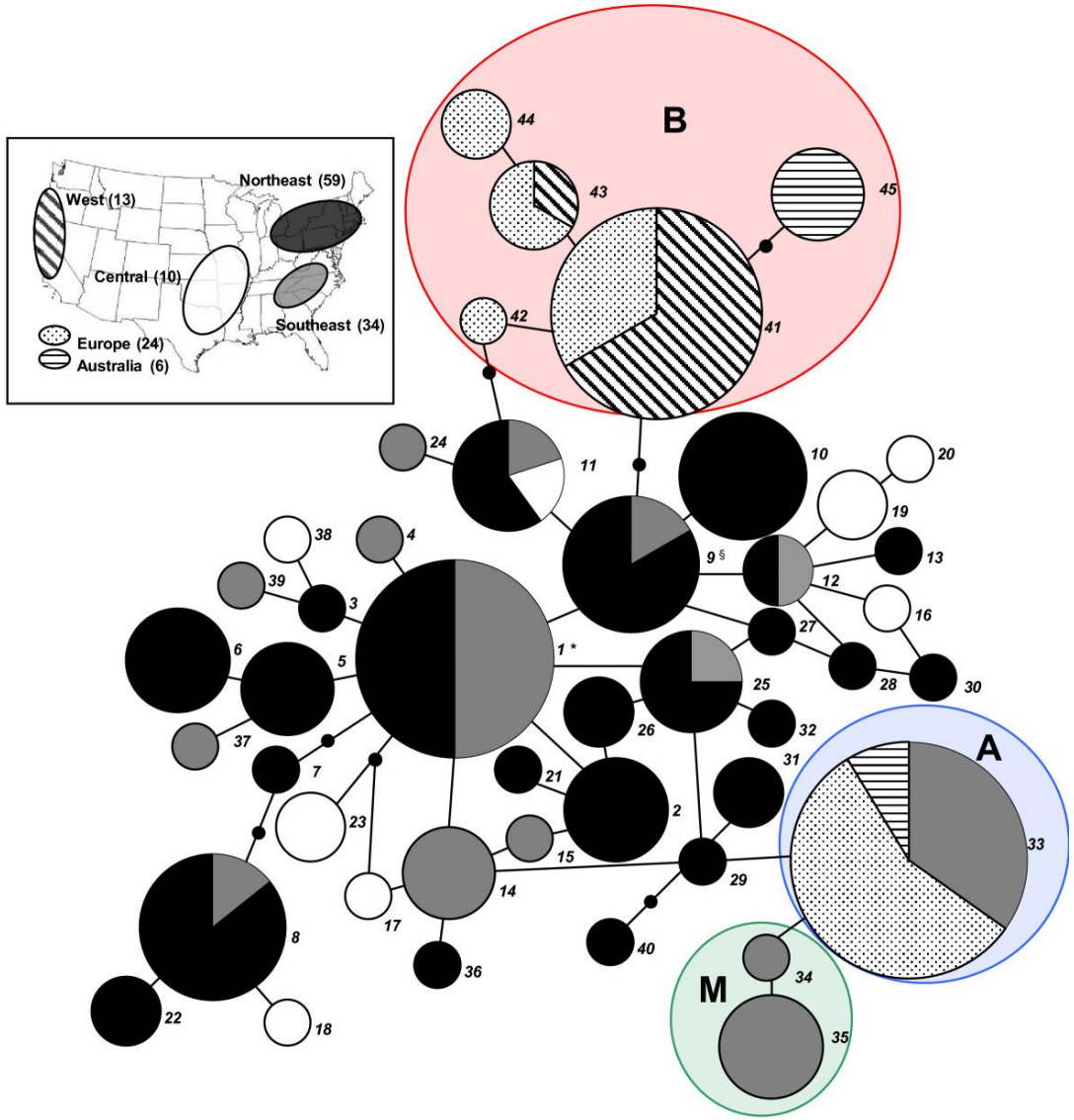
Merlot Khorus, Univ. of Udine

The Crystal Ball

How do we protect these genes for future generations?
How do we predict the future?

…Data-driven strategies

Pathogen genetics informs host genetics

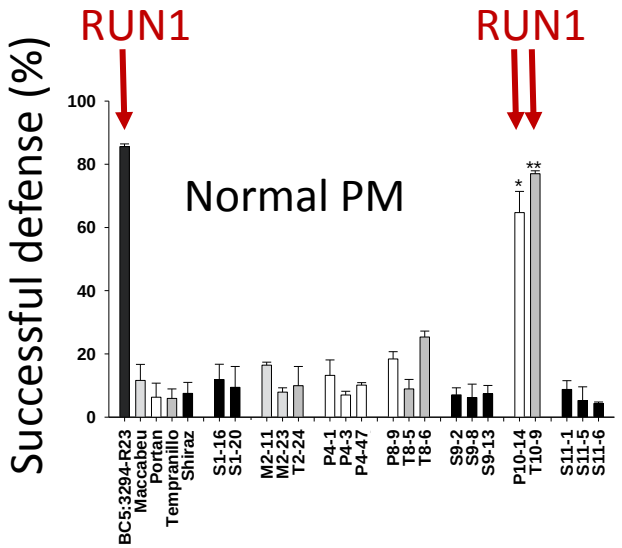


- Eastern U.S.: A center of diversity for *Erysiphe necator*
- Coevolution of pathogen with many mapped PM resistance genes

➢A crystal ball?

Milgroom, Brewer, Frenkel, Cadle-Davidson, et al.

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



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

The Plant Journal 76:661–674

Race specificity: Qualitative resistance from *RUN1*

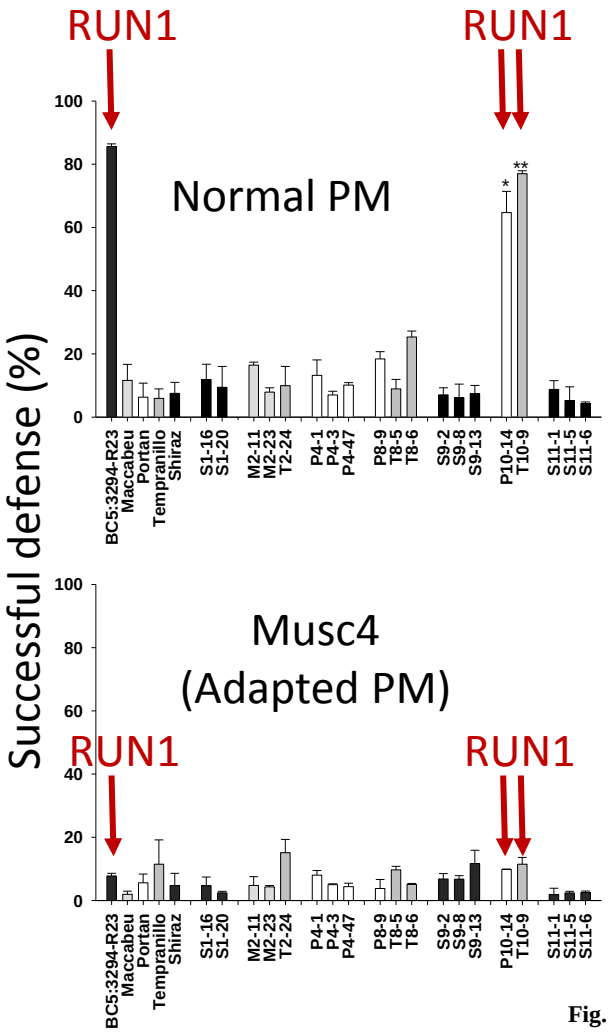


Fig. S3. Feechan *et al.*

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

The Plant Journal 76:661–674

Race specificity: Qualitative resistance from *RUN1*

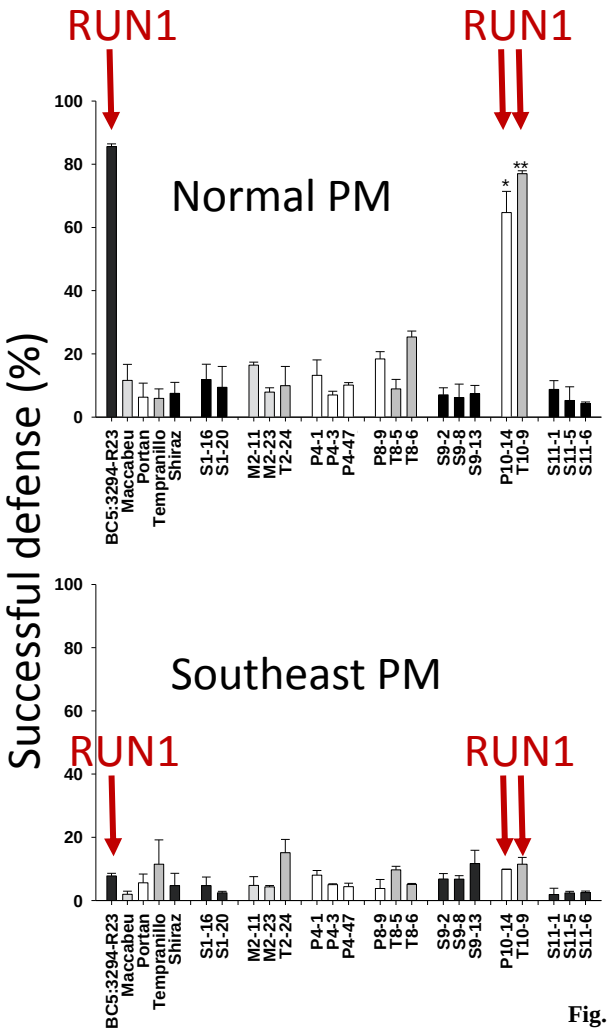


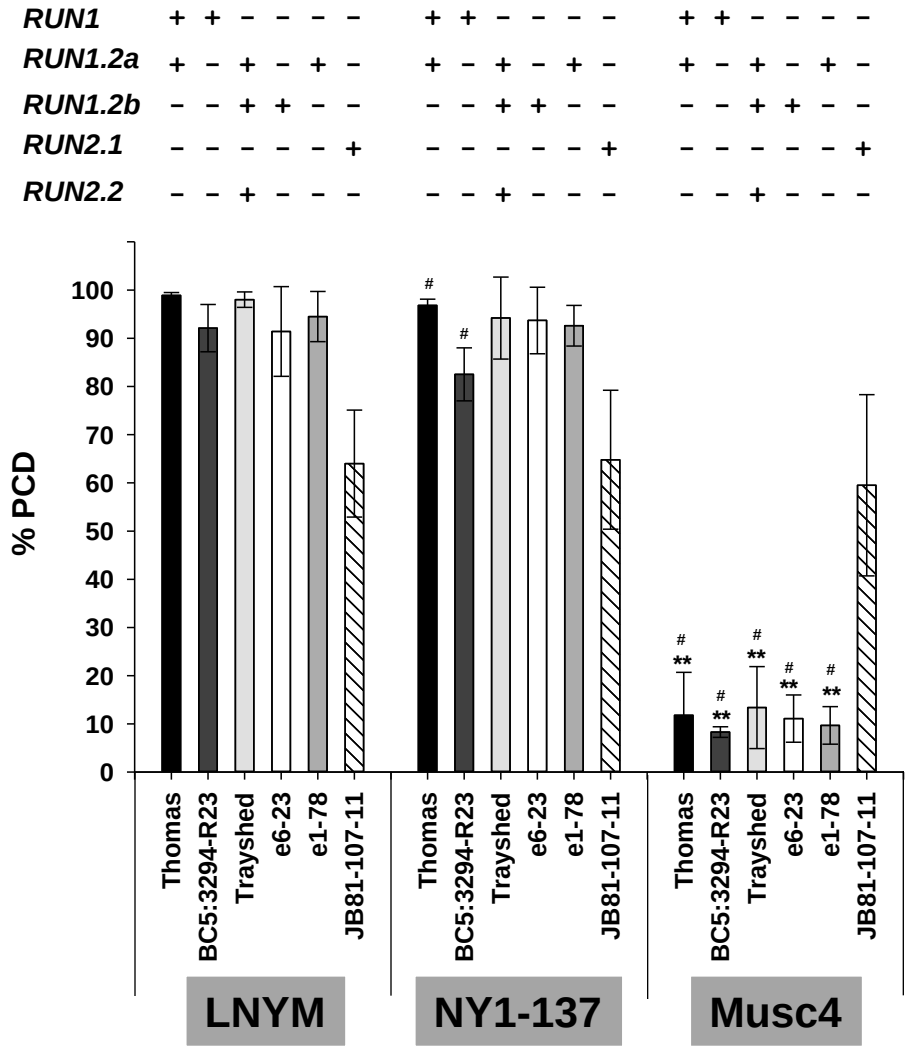
Fig. S3. Feechan *et al.*

The Plant Journal 76:661–674

- *RUN1* in vinifera
  - ➢PM Resistant…Eureka!
- Challenge with PM from Southeast U.S.
  - ➢PM Susceptible…sigh.
  - ➢Similarly, within 3 years of introduction, PM in New York could reproduce on *RUN1* vines

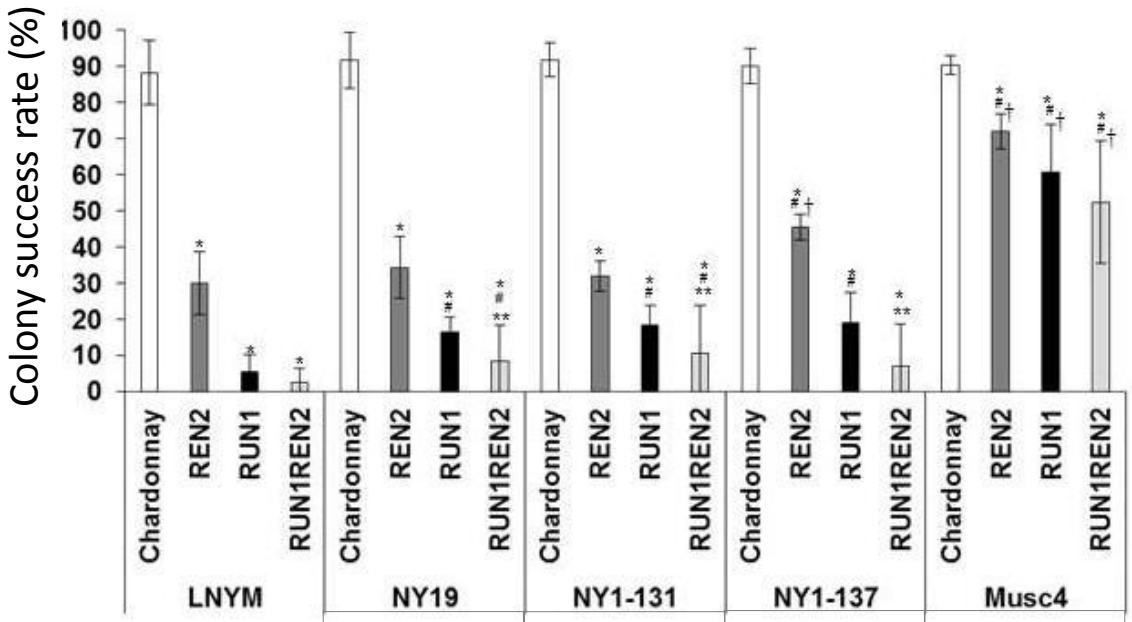
Vitis (2011) 50:173–175

RUN1.2a, RUN1.2b, and *RUN2.2:* Redundant to *RUN1?*



Phytopathology 105:1104-1113

RUN1 and REN2 = additive effects?



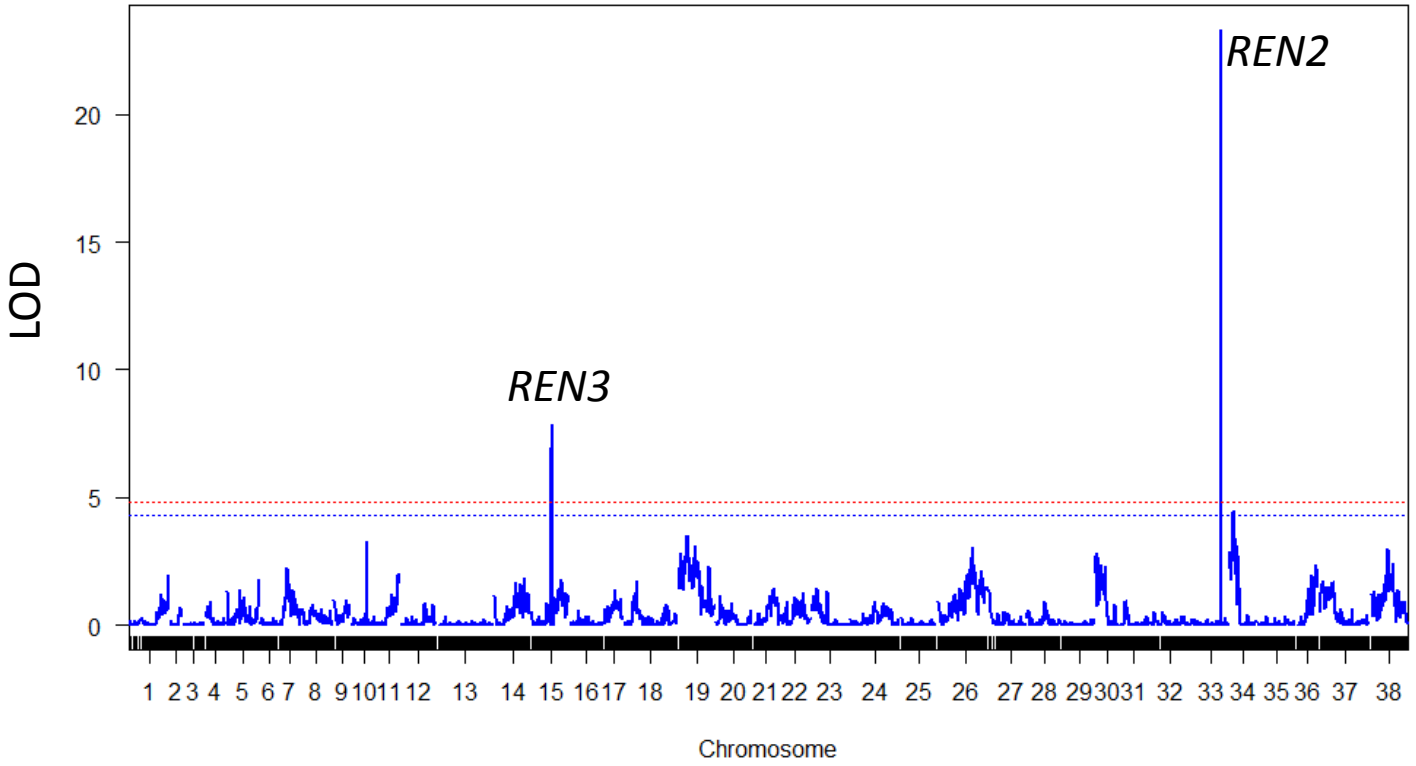
	LNYM				NY19				NY1-131				NY1-137				Musc4			
	Chardonnay	REN2	RUN1	RUN1REN2	Chardonnay	REN2	RUN1	RUN1REN2	Chardonnay	REN2	RUN1	RUN1REN2	Chardonnay	REN2	RUN1	RUN1REN2	Chardonnay	REN2	RUN1	RUN1REN2
10dpi growth:	9				9		3	3	9		5	3	9		5	3	9		5	5
10dpi conidiation:	9				9		0	0	9		0	0	9		0	0	9		5	5

9=a lot; 5=some; 0=none

Phytopathology 105:1104-1113

Race specificity: Moderate-minor resistance QTL

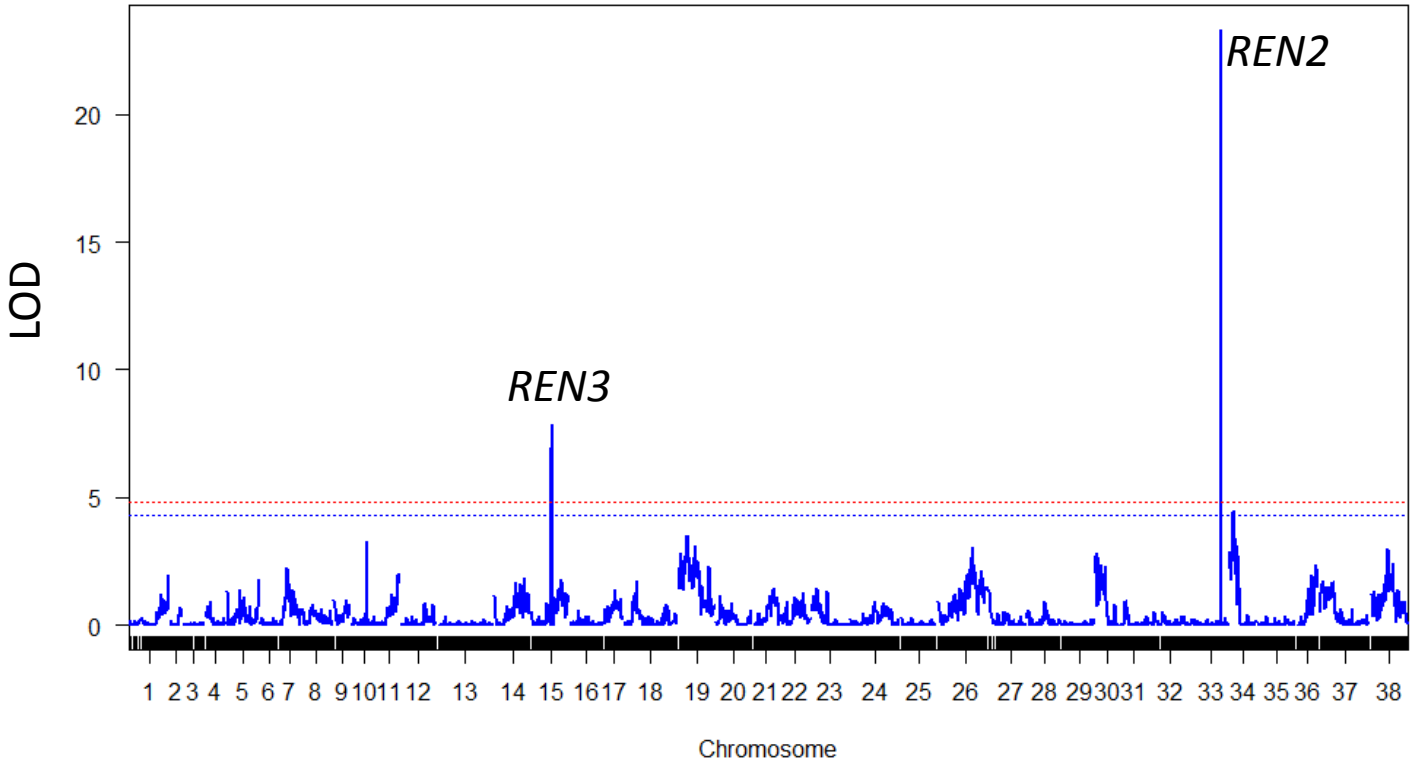
Vineyard data 1991-2008
Horizon x Illinois 547-1 (V. rupestris B38 x V. cinerea B9)



Reisch, Karn, Cadle-Davidson, et al., unpublished data

Race specificity: Moderate-minor resistance QTL

Vineyard data 1991-2008
Horizon x Illinois 547-1 (V. rupestris B38 x V. cinerea B9)



Reisch, Karn, Cadle-Davidson, et al., unpublished data
Molecular Breeding 37:1
Phytopathology 106:1159-1169

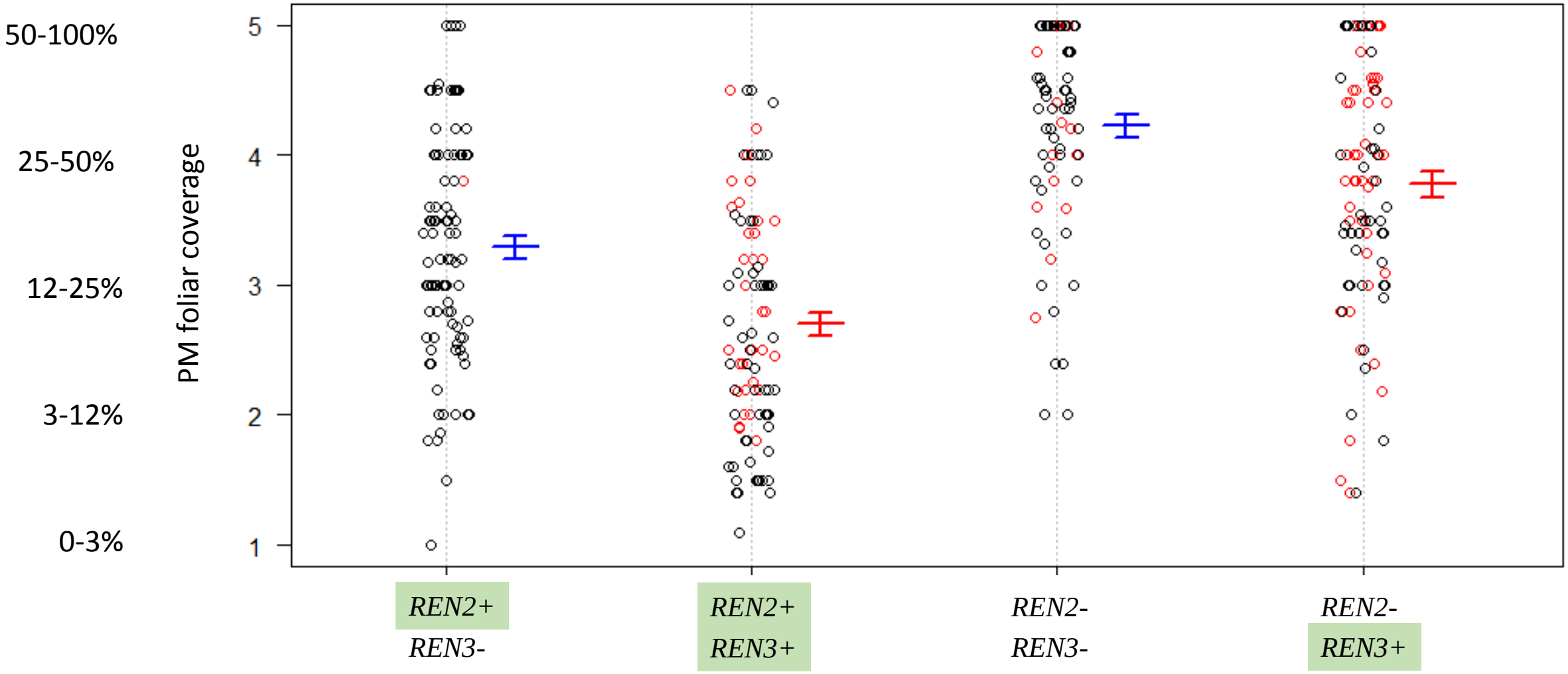
Vineyard data 2011-2014
'Horizon' × *V. cinerea* B9

Date	Chr	LOD	LOD Threshold	R^2	
08-25-11	NA	NA	NA	NA	
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	*REN3*
08-27-14	22	3.97	3.34	11.6	
09-03-14	NA	NA	NA	NA	

- *REN2* no longer detectable in vineyard
- *REN3* race-specific
  - Confirmed in lab

➢For mapping, phenotype early!

REN2 and *REN3* = additive effects



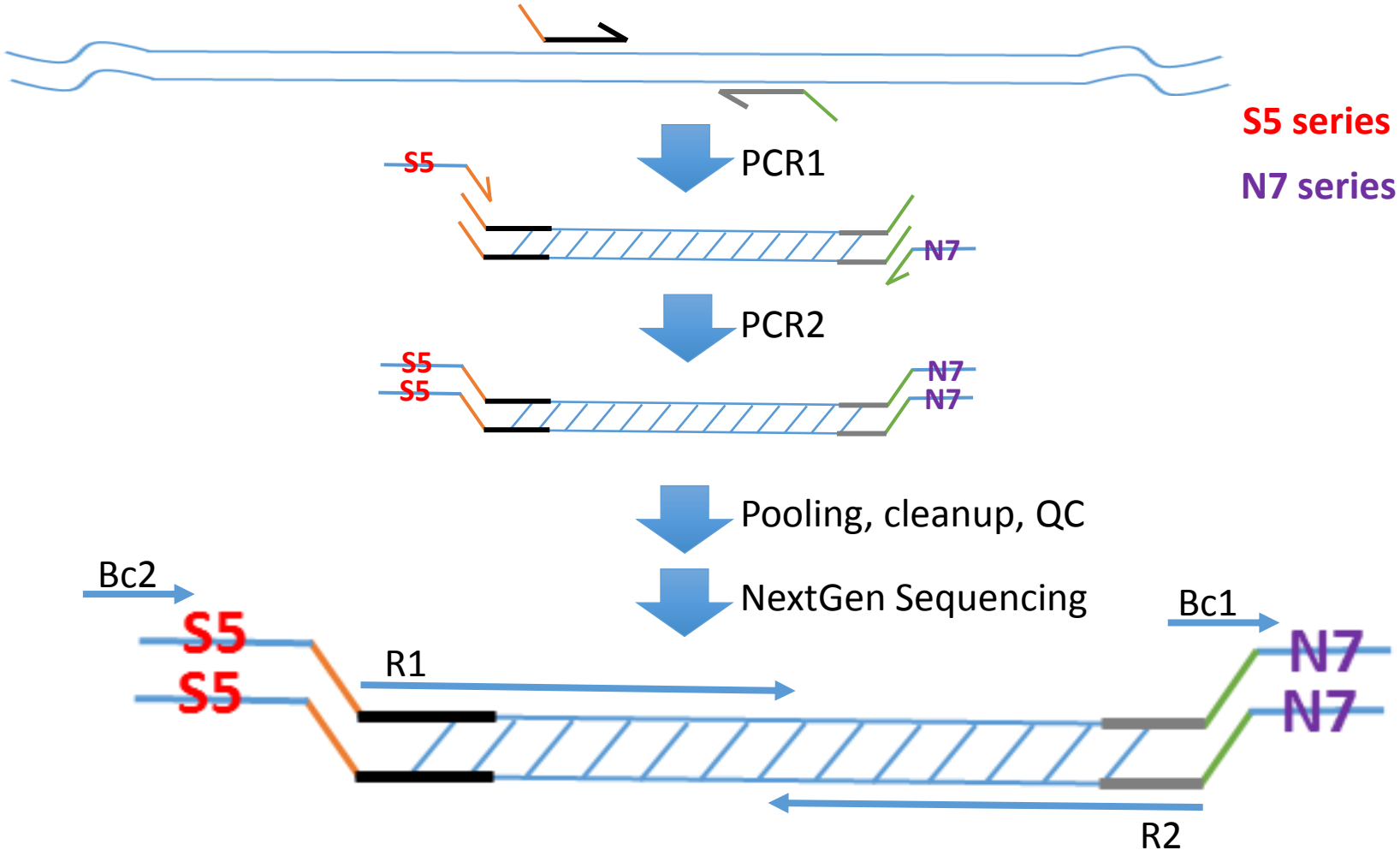
Reisch, Karn, Cadle-Davidson, et al., unpublished data

PM resistance in U.S. bunch grape breeding programs

	Locus	Chr	*Vitis* source	Race-specific	Reference	MAS?
	REN1	13	*vinifera*	Yes	Hoffman et al. 2008	Yes
	REN2	14	*cinerea*	Yes	Cadle-Davidson et al. 2016	Yes
	REN3	15	*complex*	Yes	Welter et al. 2007	Yes
Strong	***REN4***	**18**	***romanetii***	**?**	**Ramming et al. 2011**	**Yes**
Strong	***REN6***	**9**	***piazeskii***		**Pap et al. 2016**	**Yes**
	REN7	19	*piazeskii*		Pap et al. 2016	Yes
	REN9	15	*complex*	Yes	Zendler et al. 2017	Yes
	REN10	2	*complex*		Teh et al. 2017	Yes
Strong	***RUN1***	**12**	***rotundifolia***	**Yes**	**Feechan et al. 2013**	**Yes**
	RUN2.1	18	*rotundifolia*	Yes	Ramming et al. 2012	
	new	?	*aestivalis*	Yes	Ramming et al. 2012	
	new	7?	*rupestris*	Yes	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, Surya Sapkota, Avi Karn, and others

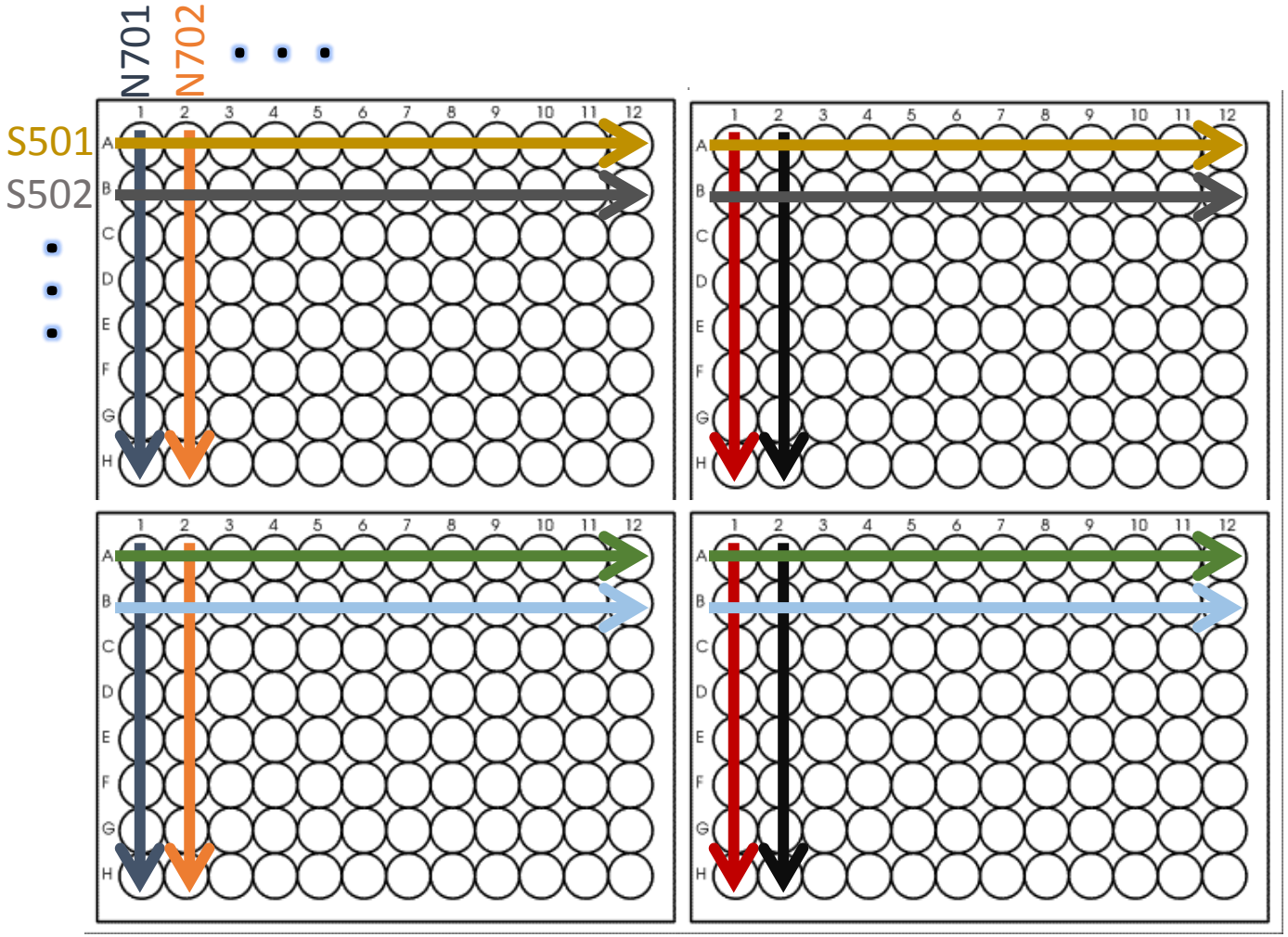
What is AmpSeq? In pictures…for one amplicon

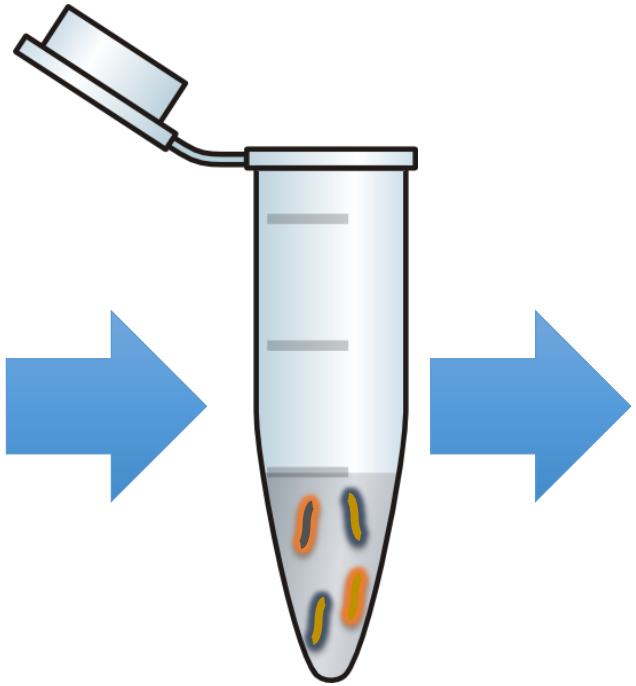


S5 series barcodes identify the sample row

N7 series barcodes identify the sample column

Imagine this across 384 samples x hundreds of amplicons per sample

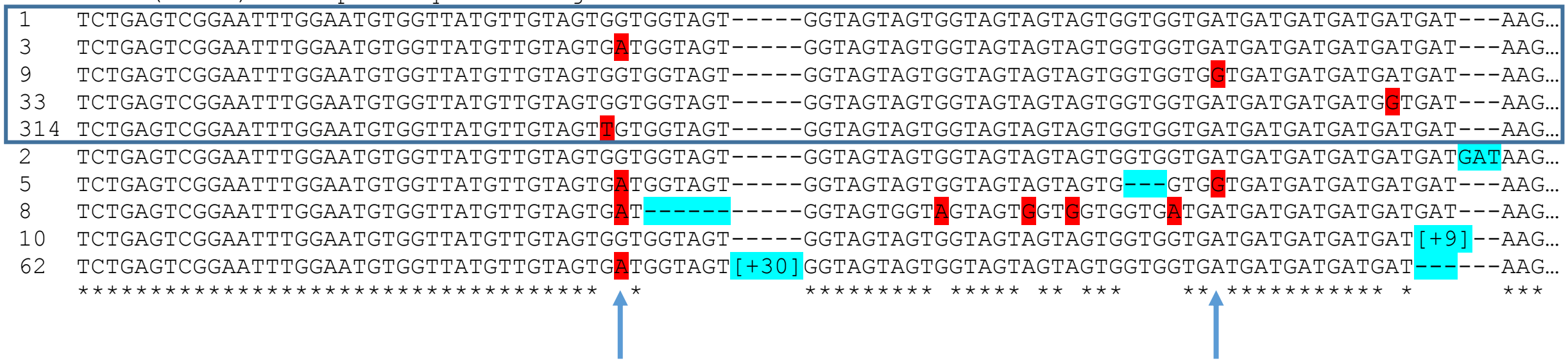




Pool all samples and amplicons in one tube

QC and sequence

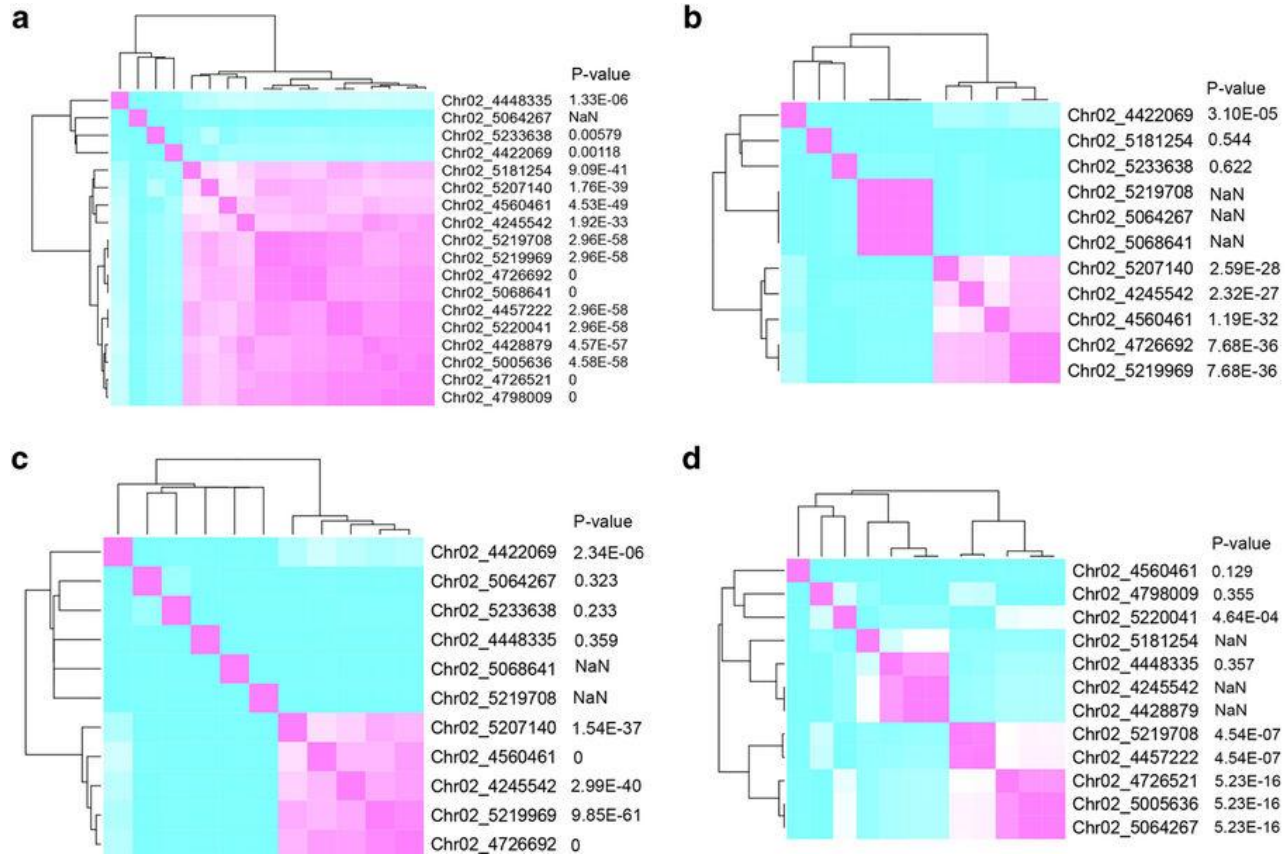
Any marker with a sequence basis can be converted to AmpSeq (eg here, simple sequence repeat, SSR)



- By electrophoresis, the top 5 alleles would all appear to be the same SSR allele.
- Two of the SNPs shown here occur with multiple SSR allele sizes.
- Excellent quality data from undetectable quantities of dirty DNA.
  - Analysis of same DNA by SSR capillary electrophoresis was a mess.

Side note on marker transferability

In *Vitis*Gen1, GBS markers developed from one breeding program did not work in other breeding programs



Horticulture Research 3:16002

Jonathan Fresnedo

Shanshan Yang

A core genome to improve transferability: Reference genomes examined

		Total bases (Mbp)	N50 (kbp)
wild	**V. cinerea B9 (pacbio)**	702	226
	V. cinerea B9 (10X)	427	374
	V. x doaniana (10X)	472	1767
	V. riparia	415	702
	V. rupestris B38 (10X)	524	545
interspecific	**Chambourcin (10X)**	523	538
	Jaeger 70 (10X)	503	292
	Concord (10x)	491	324
	Norton (454/Illumina)	459	12
vinifera	**Sultanina (Illumina)**	544	278
	CabSav_v1 (pacbio)	591	2173
	Flame (10X)	543	1980

Lance Cadle-Davidson

Jason Londo

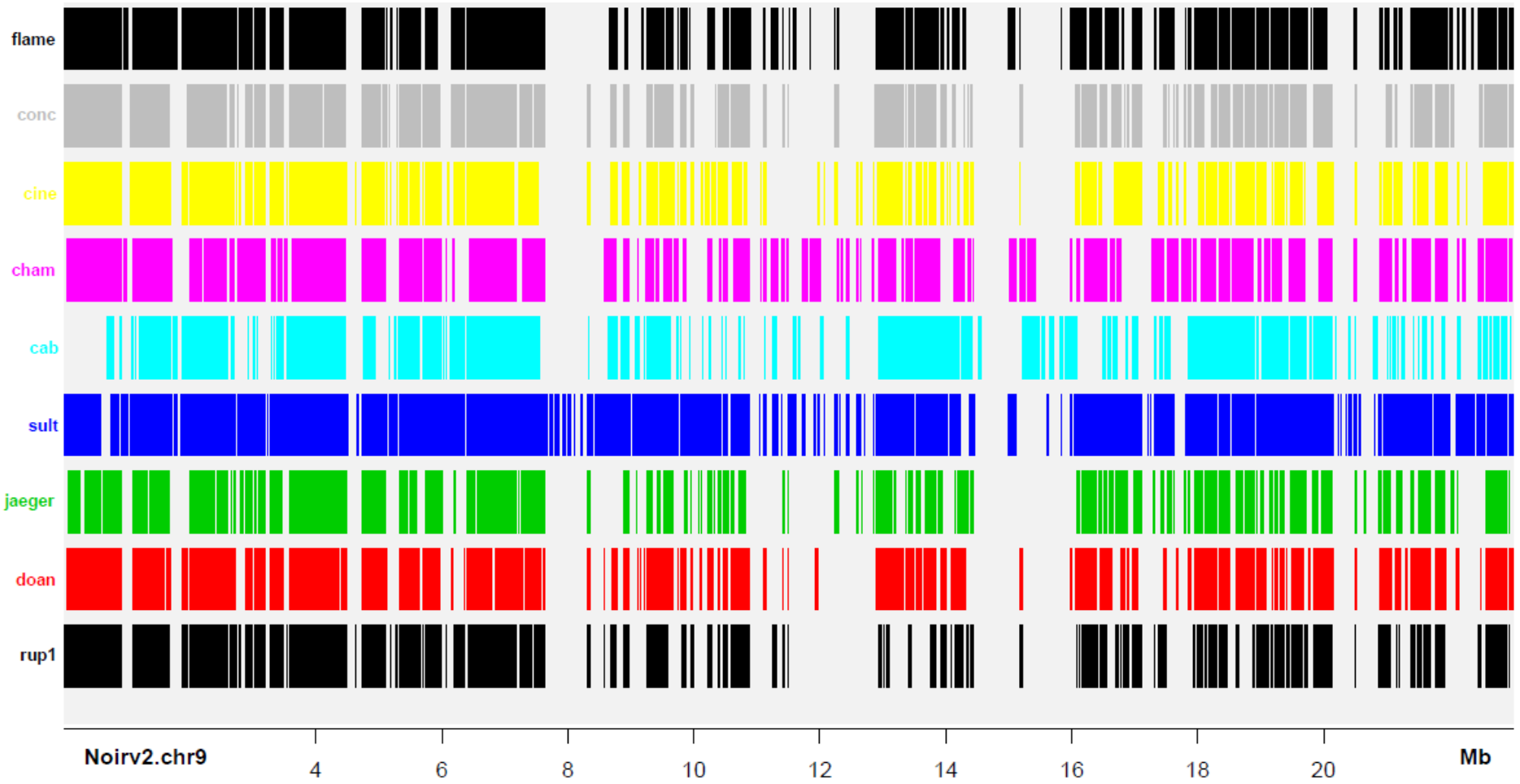
Michael Campbell

Dario Cantu

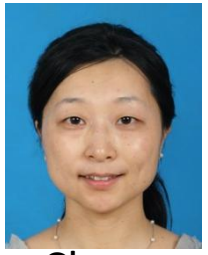
Anne Fennell

Doreen Ware

Core genome was built by aligning each genome to PN40024

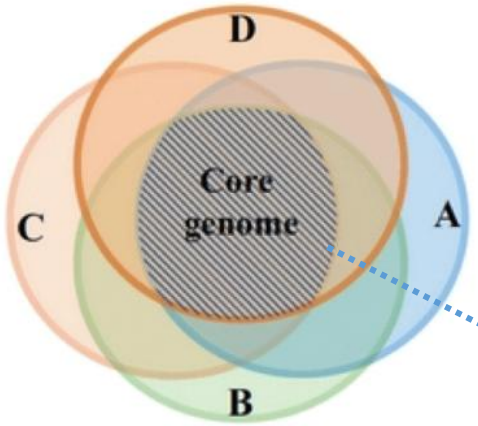


PN40024 chr9

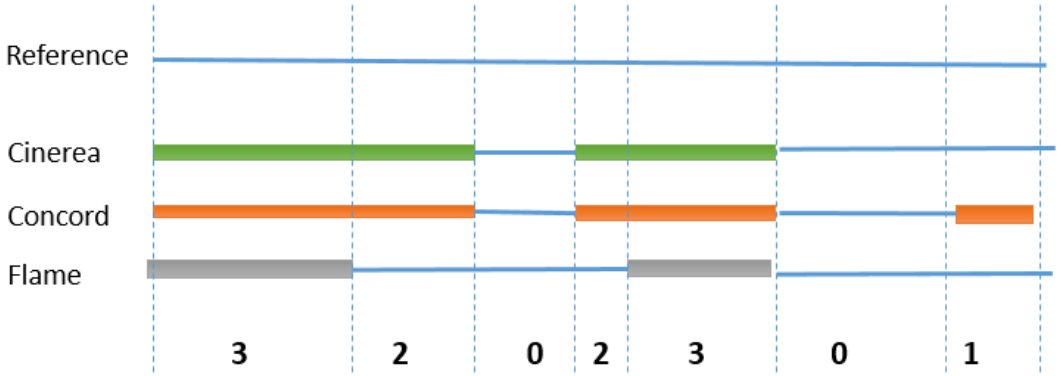


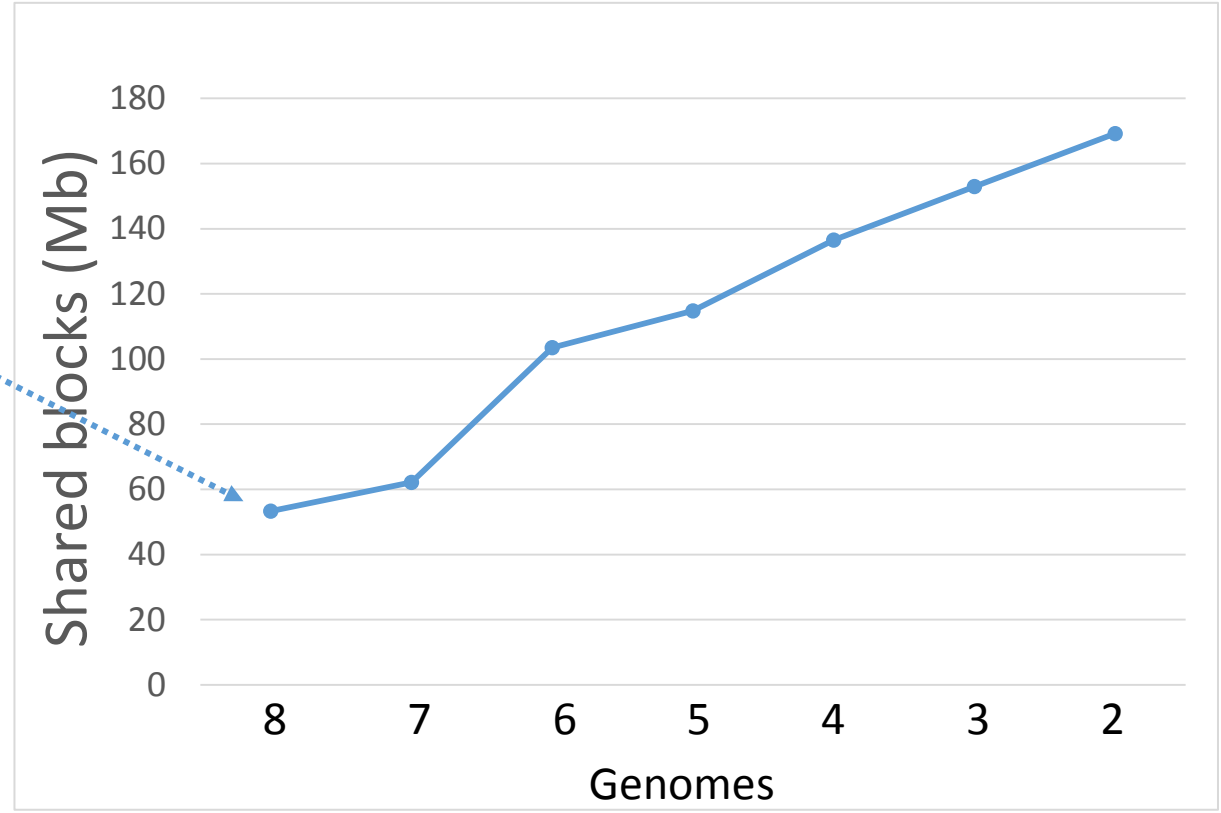
Cheng Zou

Grapevine core genome

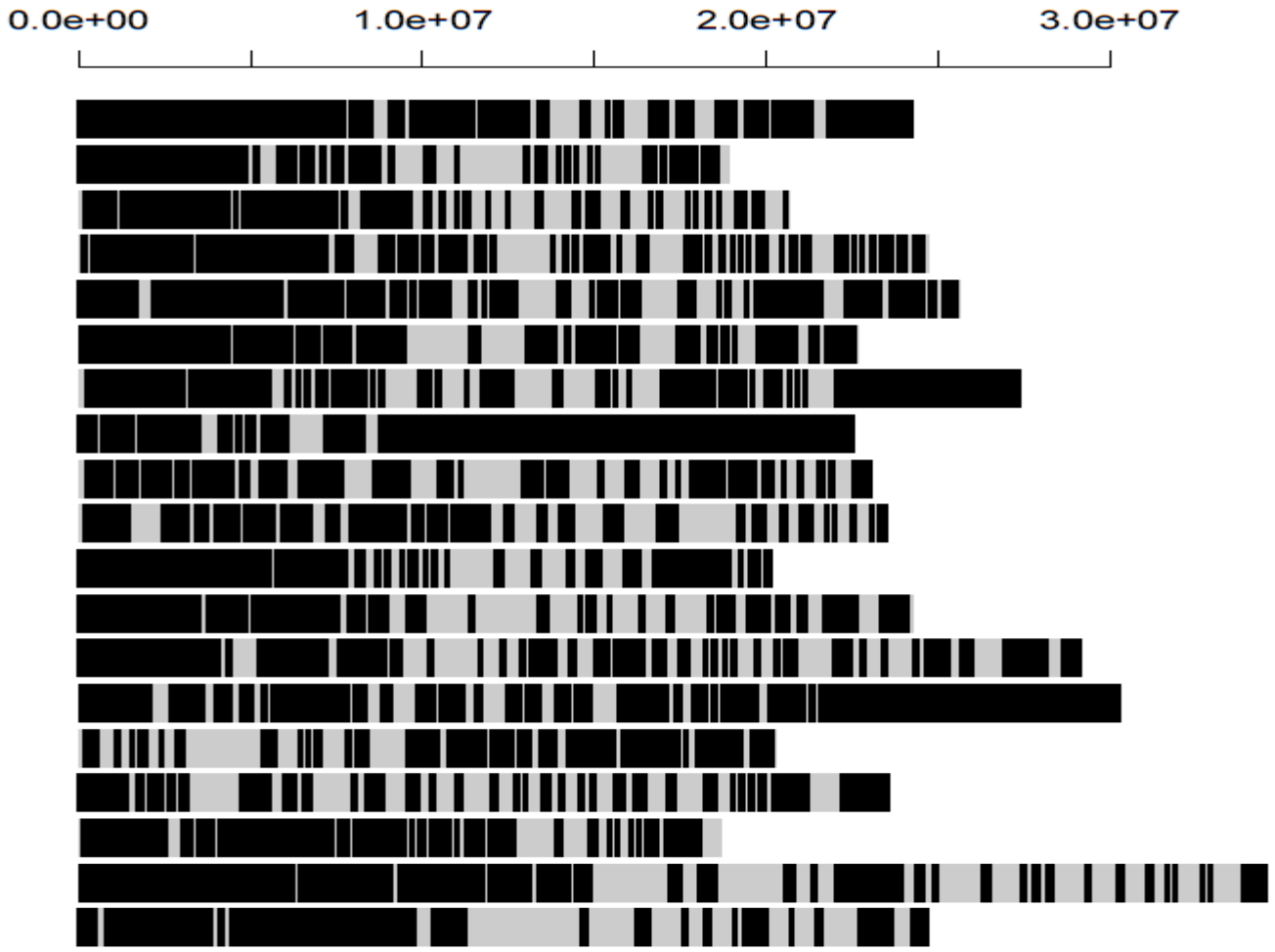


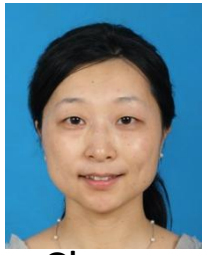
- 10% of the genome is shared among the (imperfect) genomes examined
- 70% of core genome overlaps with genes





2000 rhAmpSeq markers from core genome



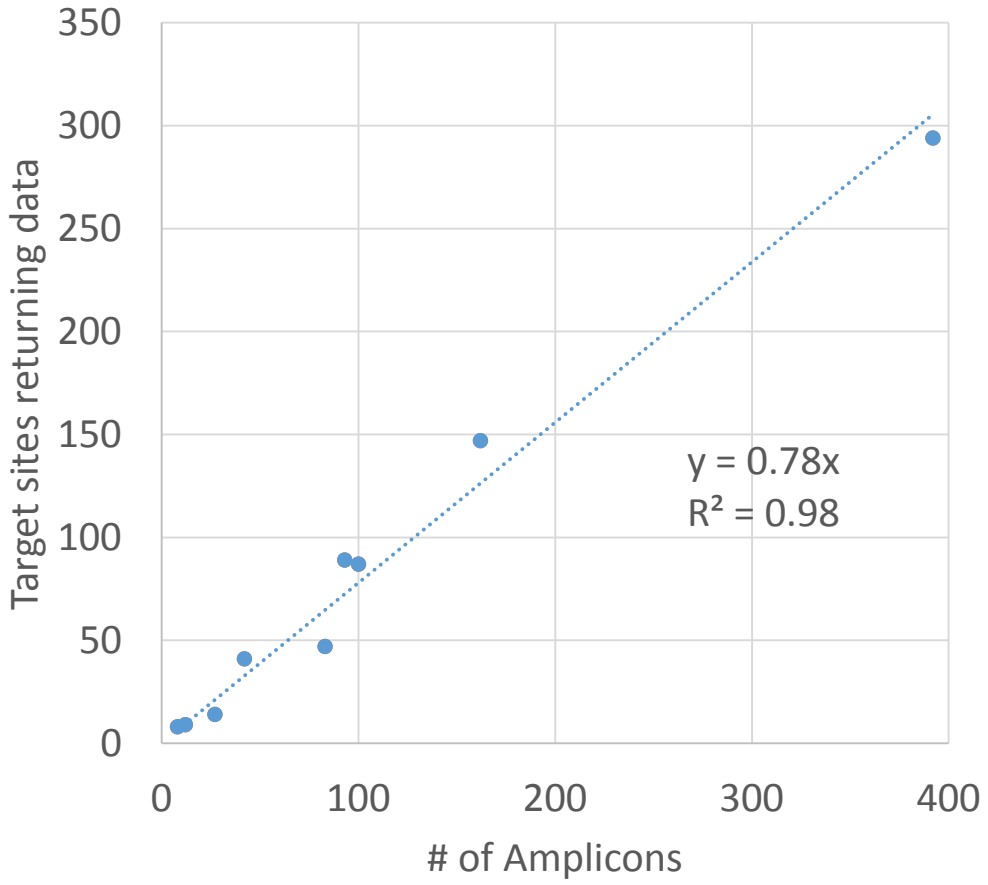


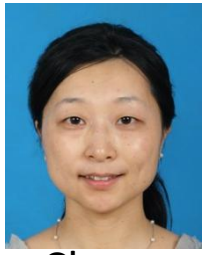
Cheng Zou

Avi Karn

rhAmpSeq compatible with thousands of multiplexed markers

Data return rates for newly designed amplicons without troubleshooting





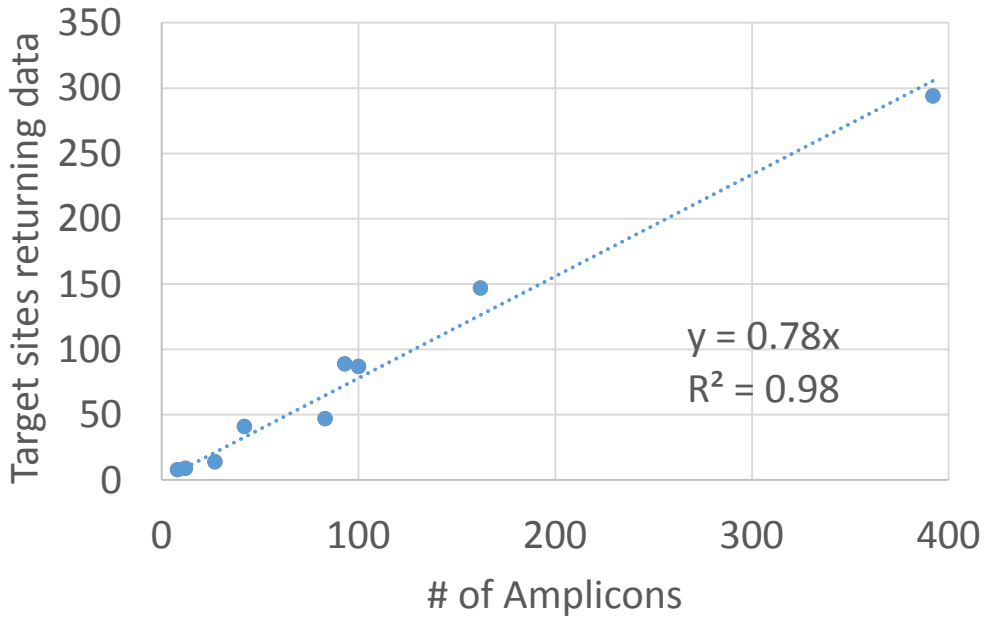
Cheng Zou

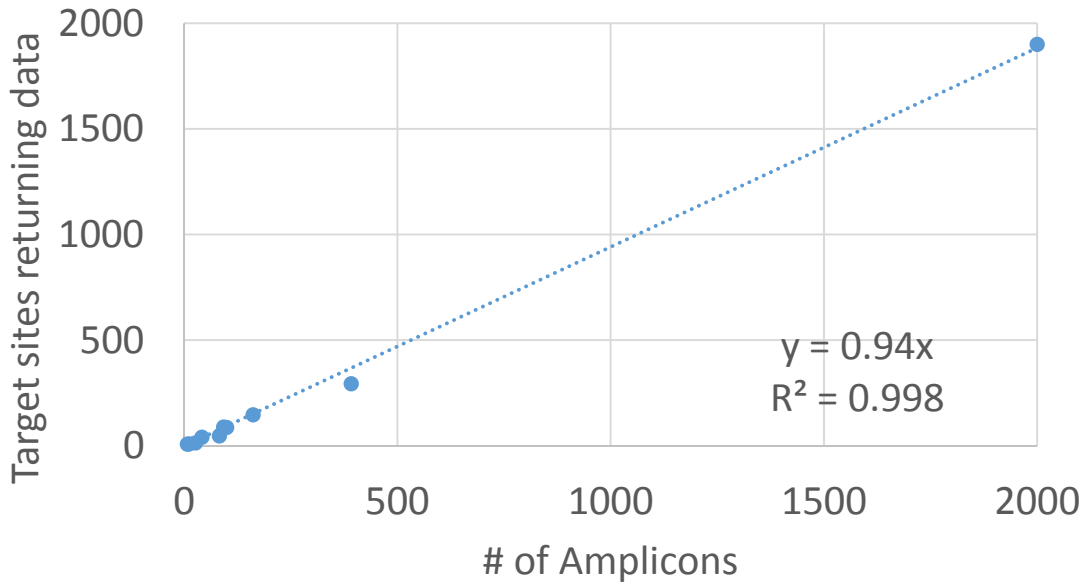
Avi Karn

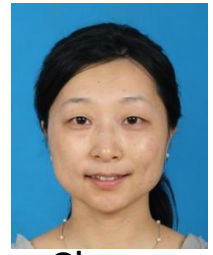
Jonathan Fresnedo

rhAmpSeq compatible with thousands of multiplexed markers

Data return rates for newly designed amplicons without troubleshooting



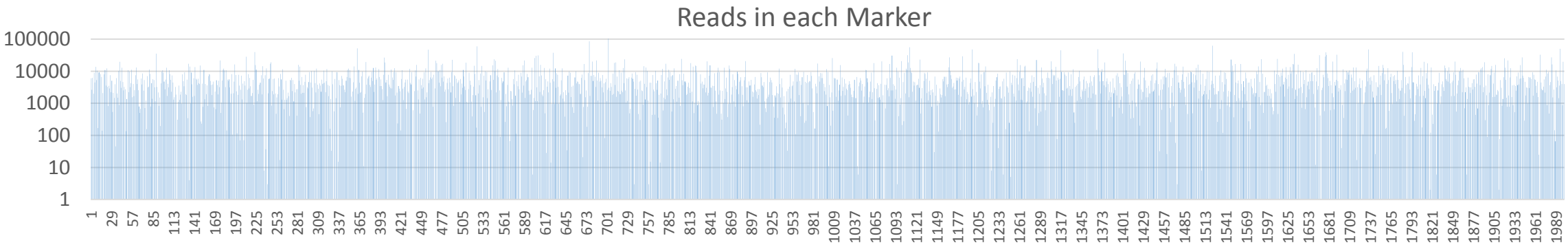




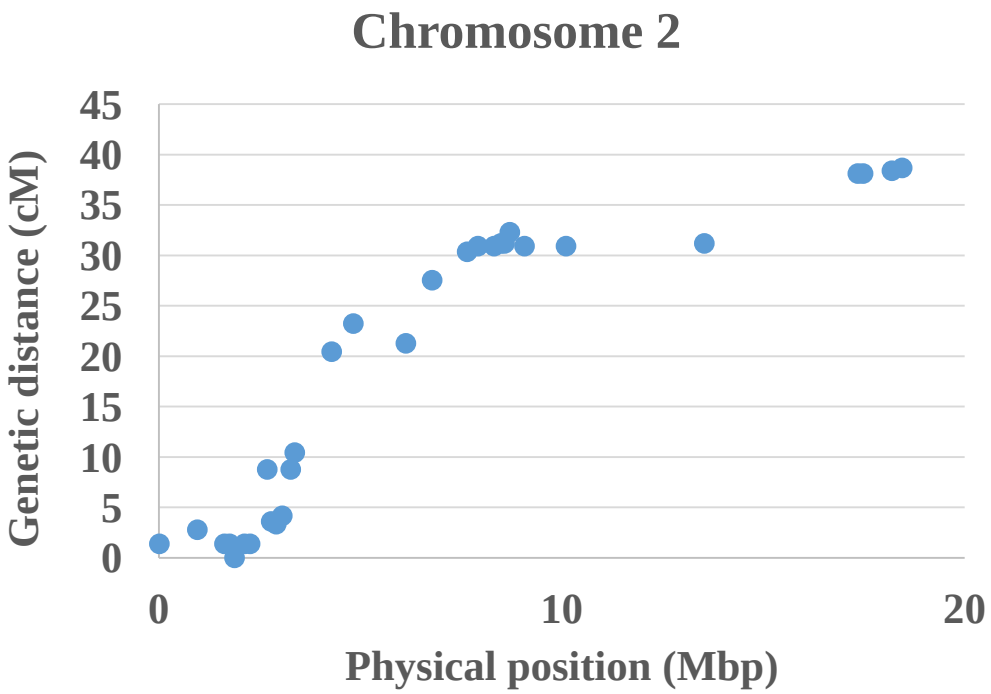
Cheng Zou

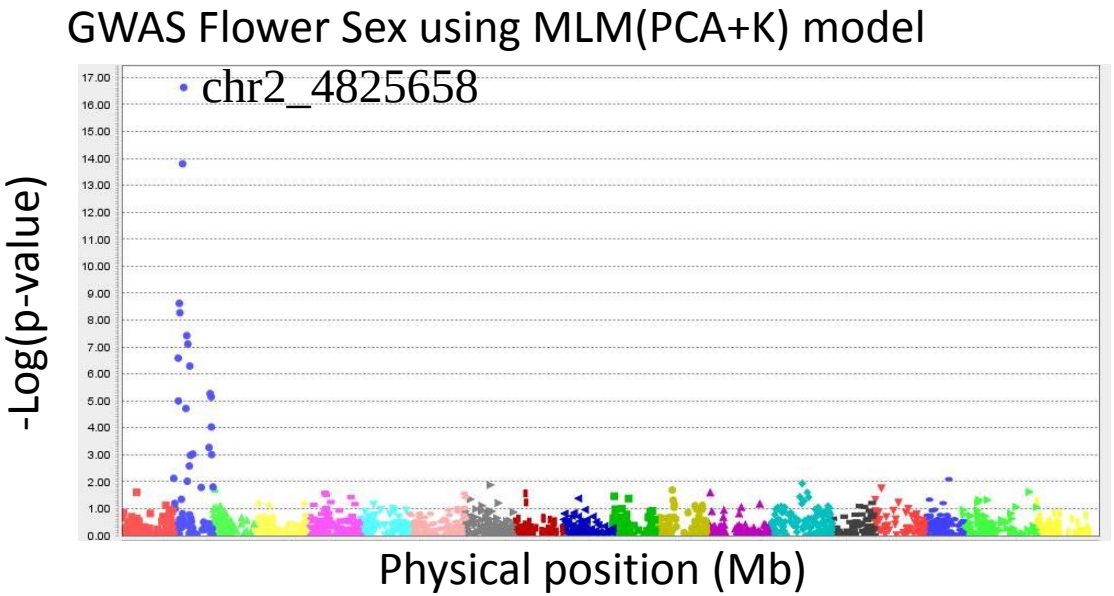
Avi Karn

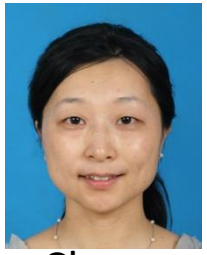
Jonathan Fresnedo



rhAmpSeq compatible with thousands of multiplexed markers







Cheng Zou

Avi Karn

Outreach: AmpSeq workshops

ICPP BOSTON Plant Health in a Global Economy JULY 29 - AUGUST 3 2018

Avi Karn

Jonathan Fresnedo

ICPP Workshop Sunday, July 29, 2018:

Hands-On Analysis of AmpSeq Data

Getting started with AmpSeq

- Make data analysis plan
- Order PCR1 primers with linkers
- Obtain DNA from samples of interest
- Decision point:
  - In-house: Order PCR2 barcode primers, multiplex PCR mix, multichannel pipet or robot
  - Outsource: Simply send primers and DNA to genotyping facility

Strongest R-genes already discovered? New alleles possibly redundant?

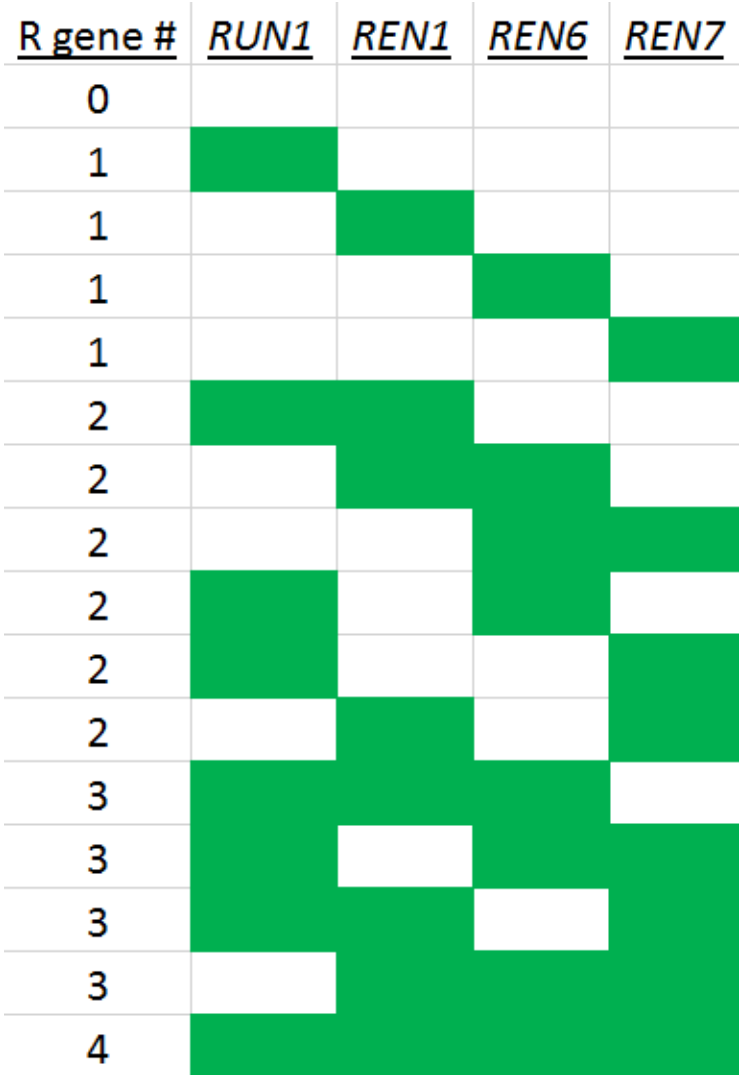
- ~16 R-gene QTL mapped in grapevine
  - Are the strongest effect loci already known?
  - How many more will we find?
    - Barley: 27 R-gene loci
    - Wheat: 41 R-gene loci, 60 alleles
- Some QTL have additive effects
- All are likely race-specific
  - ➢Justification for stacking = additivity and spectrum
- Some are redundant
  - ➢Need knowledge of which combinations are useful

Locus	Chr	*Vitis* source	Race-specific	Reference	MAS?
REN1	13	*vinifera*	Yes	Hoffman et al. 2008	Yes
REN2	14	*cinerea*	Yes	Cadle-Davidson et al. 2016	Yes
REN3	15	*complex*	Yes	Welter et al. 2007	Yes
REN4	**18**	***romanetii***	**?**	**Ramming et al. 2011**	**Yes**
REN6	**9**	***piazeskii***	**nd**	**Pap et al. 2016**	**Yes**
REN7	19	*piazeskii*	nd	Pap et al. 2016	Yes
REN9	15	*complex*	Yes	Zendler et al. 2017	Yes
REN10	2	*complex*	nd	Teh et al. 2017	Yes
RUN1	**12**	***rotundifolia***	**Yes**	**Feechan et al. 2013**	**Yes**
RUN2.1	18	*rotundifolia*	Yes	Ramming et al. 2012	
new	?	*aestivalis*	Yes	Ramming et al. 2012	
new	7?	*rupestris*	Yes	Barba et al. 2015	

Plant Pathology 64: 1396–1406
Crop and Pasture Science 63:997-1006

Which combinations are useful?

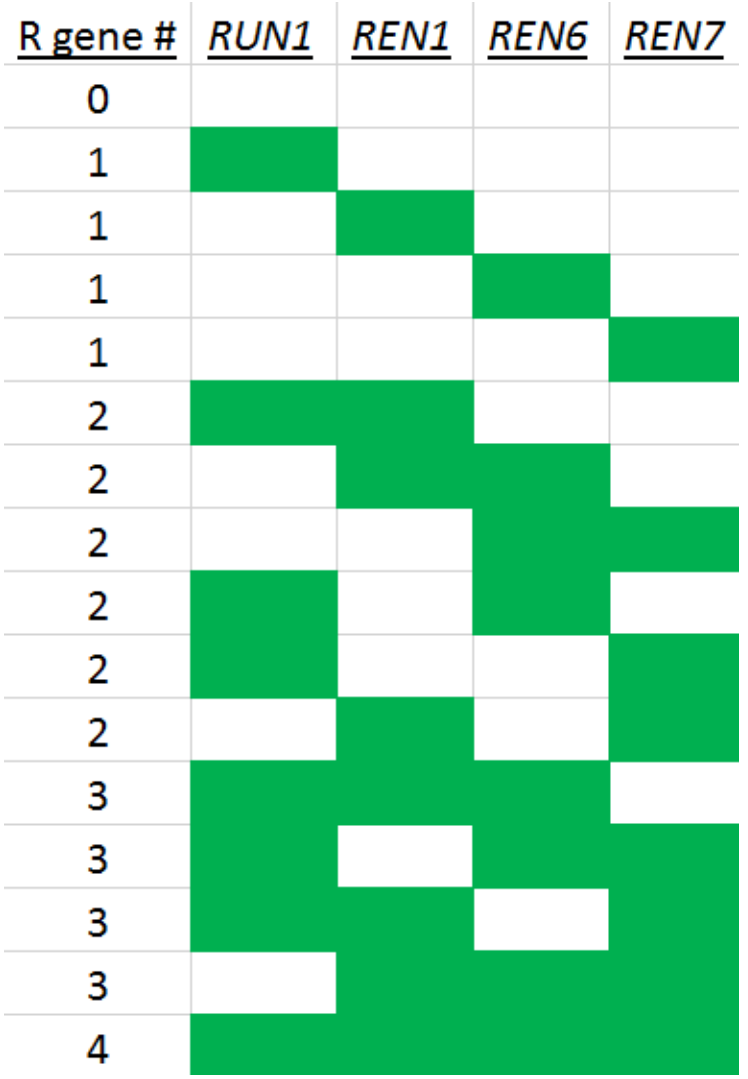
- Inform strategies: Test combinations of *RUN1*, *REN1*, *REN2*, *REN3/9*, *REN4*, *REN6*, *REN7* and *REN10.*
  - Example: *RUN1*, *REN1*, *REN6*, *REN7* = 16 combinations



Which combinations are useful?

- Inform strategies: Test combinations of *RUN1*, *REN1*, *REN2*, *REN3/9*, *REN4*, *REN6*, *REN7* and *REN10*.
  - Example: *RUN1*, *REN1*, *REN6*, *REN7* = 16 combinations
  - Crosses made for 18 of 28 pairs by Clark, Ledbetter, and Reisch

	RUN1	REN1	REN2	REN3/9	REN4	REN6	REN7	REN10
REN10	RED	RED		RED	RED			
REN7								
REN6								
REN4	RED	RED		RED				
REN3/9	RED	RED						
REN2								
REN1	RED							
RUN1								

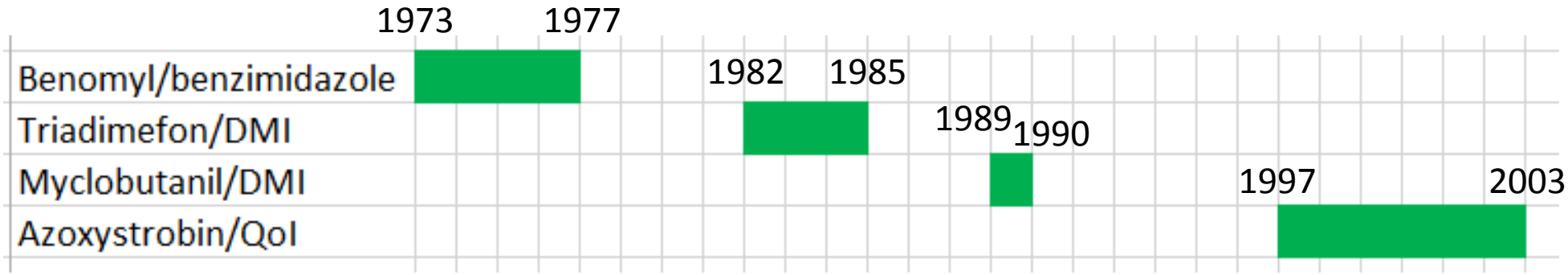


The lesson of the glass wall

Lessons from fungicide resistance

- To learn about how the pathogen will overcome host resistance, plenty of lessons from Fungicide Resistance.

Timeline from release to first report of Fungicide Resistance



"Those who cannot remember the past are condemned to repeat it."

- George Santayana

Lessons from fungicide resistance

High risk: Strobilurins (Group 11); Ridomil products (Group 4); benzimidazoles (defunct Benlate and current Topsin-M, Group 1).

Medium-to-high risk: SDHIs (Group 7); Rovral (Group 2); ametoctradin (one component of Zampro, Group 45); Ranman (Group 21)

Medium risk: DMIs (Group 3); the APs (Group 9; Vangard, Scala, one component of Switch); Quintec (Group 13); Vivando (Group U 08)

Low-to-medium risk: Group 40 fungicides (dimethomorph, one component of Zampro); Elevate (Group 17); fludioxonil (Group 12).

Low risk: Mancozeb, captan, ziram, sulfur, copper, oils, salts

Lessons from fungicide resistance

High risk: Strobilurins (Group 11); Ridomil products (Group 4); benzimidazoles (defunct Benlate and current Topsin-M, Group 1).

Medium-to-high risk: SDHIs (Group 7); Rovral (Group 2); **ametoctradin (one component of Zampro, Group 45)**; Ranman (Group 21)

Medium risk: DMIs (Group 3); the APs (Group 9; Vangard, Scala, one component of Switch); Quintec (Group 13); Vivando (Group U 08)

Low-to-medium risk: **Group 40 fungicides (dimethomorph, one component of Zampro);** Elevate (Group 17); fludioxonil (Group 12).

Low risk: Mancozeb, captan, ziram, sulfur, copper, oils, salts

What can we learn from fungicide strategies?

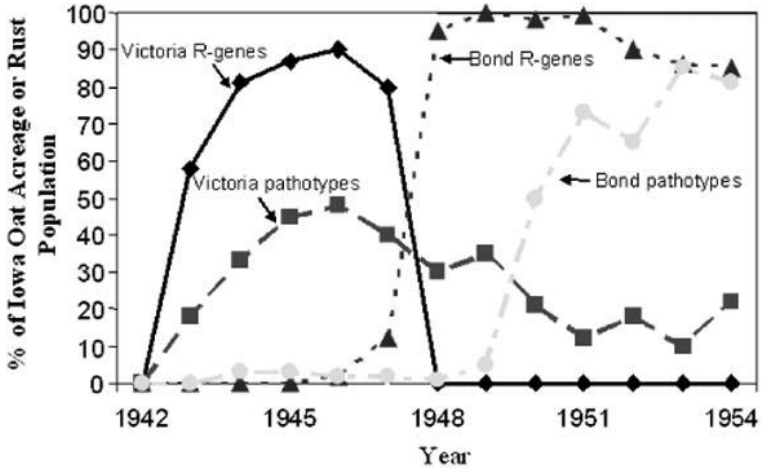
Be honest: the pathogen will win battles; our job is to delay or prevent it from winning the war.

For fungicides	For host resistance
Combine different modes of action • Often a higher risk with lower risk	Combine different mechanisms • But all are high risk
Rotate to effective, unrelated chemicals	Cannot rotate
Limit size of pathogen population • Don't use at-risk to rescue	Keep nearby vines disease-free • Don't use at-risk to rescue
Limit reproduction of resistant individuals	Limit reproduction of virulent
For quantitative resistance, increase dose or activity	Cannot increase dose after planted
Apply best chemicals when disease control is most important.	Apply chemicals after flowering, before pathogen's sexual phase? Engage pathologists!

Lessons from Wayne Wilcox

What can we learn from other crops?

Oat rust



Annu Rev Phytopathol. 14:355–382

- Barley PM: Resistance typically lasts 3 years for individual genes, 5 years for stacked genes.
  - Exception: *mlo* effective since 1940s.
- Oat rust (left): Virulence within 1-2 years.
- Quantitative resistance (QR) typically involves at least 4-5 QTL and may be more durable than qualitative resistance (but also exceptions).
- Gene pyramids: redundancy, additive effects, or synergistic mechanisms?
  - HR + reduce sporulation or ontogenic resistance
  - Need more info
- Variety mixtures

Infect Genet Evol. 2014:446–455
Mol Ecol. 10:1–16

Legal/educational assets to protect R-genes

➢Human nature: Ensuring that growers cooperate (apply the requested pesticides, timing, and rates) could be challenging.

- Education: Growers are familiar with fungicide stewardship
  - ➢Develop best practices for managing genetics (built into vine) and chemistry (applied by grower).
- Patented variety, not otherwise managed
  - Adding cultural practice requirement is possible, but mechanism usually not exercised.
- Managed varieties (eg SweeTango apple): dictate who and how club varieties are grown
  - Contractually require growers to conduct certain cultural practices
  - Lease the vines, with contractual obligations

Thank you to Peter Cousins, Matt Clark, Bruce Reisch, and Craig Ledbetter for helpful comments!

My recommendation from this

Limited supply of useful, non-redundant resistance alleles available->need durability.

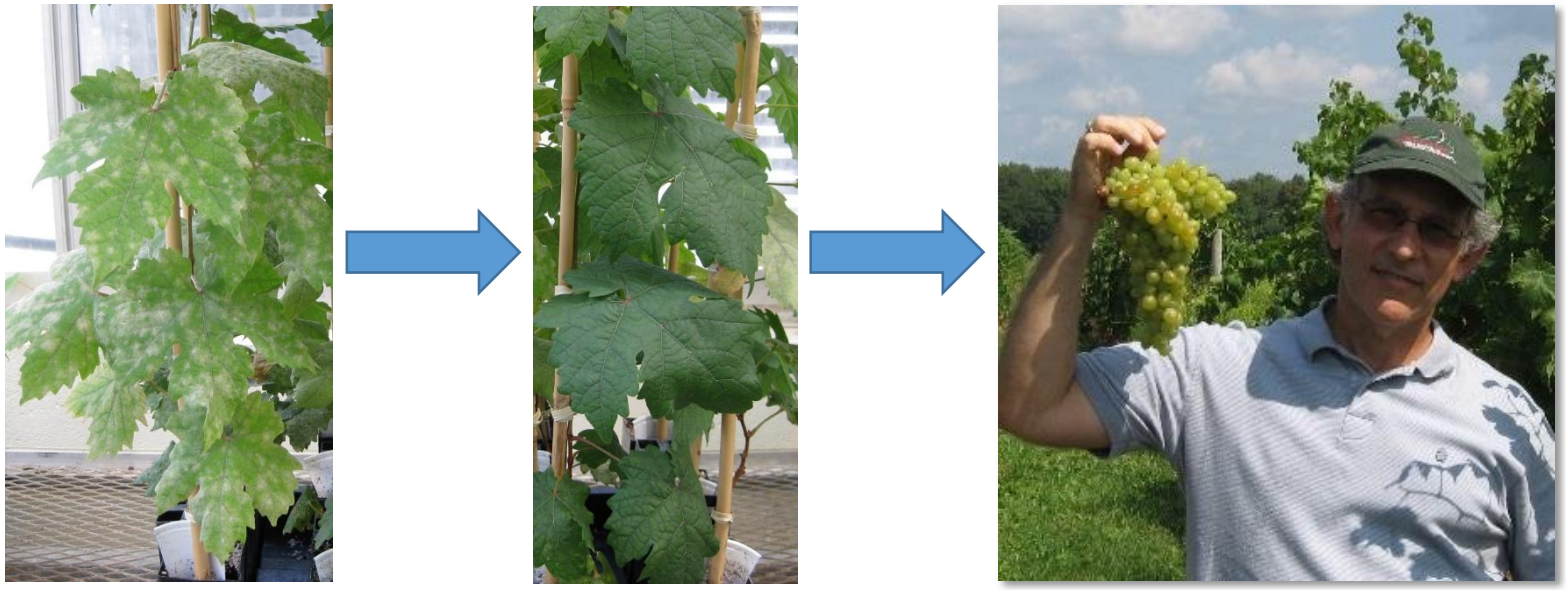
- Stacking with knowledge of complementarity should be helpful.
  - Minor resistance effects of genetic background should help as well.
- Annual fungicide application(s) will be critical.
  - Remove ‘No-spray’ from lexicon.
  - (Biologicals are a viable option.)
  - Use education to encourage Annual spray(s).
  - Manage varieties to mandate Annual spray(s) contractually.
  - Spray when fruit is susceptible; other sprays will depend on local epidemiology
- Powdery mildew management in nearby blocks will be critical.
- Variety mixtures (different R-gene stacks in adjacent rows) may be helpful.

At Cornell/USDA:

- We can receive other R-genes for standardized phenotyping.
- Need an effort like OsCaR in center of origin, as a crystal ball of best management practices.

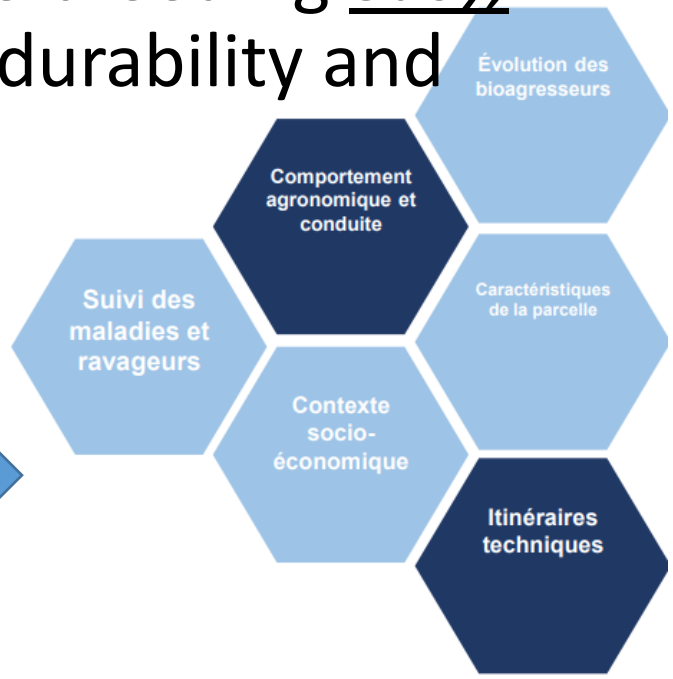
*Vitis*Gen2 Genotyping and PM Goal

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



*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*, and integrated strategies for durability and increased adoption.



Strategies to increase adoption

Acknowledgments

Executive Committee:
Bruce Reisch, Lead
Lance Cadle-Davidson
Jason Londo
Gavin Sacks
Tim Martinson
Julian Alston

Project Manager:
Fred Gouker

Genetics Team:
Jason Londo, Lead
Lance Cadle-Davidson
Dario Cantu
Qi Sun
Peter Schweitzer
Anne Fennell

Powdery Mildew Team:
Lance Cadle-Davidson, Lead
David Gadoury
Mark Rea

Fruit Quality Team:
Gavin Sacks, Lead
Anna-Katharine Mansfield
Rachel Naegele, TG Lead

Funding:
USDA-NIFA Specialty Crops Research Initiative
Project Number 2017-51181-26829

Helpful discussions
Peter Cousins, Ian Dry, Ed Buckler

Extension and Outreach Team:
Tim Martinson, Lead
Matthew Clark
Michelle Moyer
Keith Striegler

Breeding Team:
Bruce Reisch, Lead
Lance Cadle-Davidson
Matt Clark
Anne Fennell
Harlene Hatterman-Valenti
Chin-Feng Hwang
Craig Ledbetter
Jason Londo
Rachel Naegele
Andy Walker

Trait Economics Team:
Julian Alston, Lead
Olena Sambucci
Karina Gallardo
Bradley Rickard