

A LONG AND WINDING ROAD....

The Discovery of the Red Leaf Viruses, the Leafrolls and Red Blotch

Deborah Golino UC
Davis





Foundation
Plant
Services

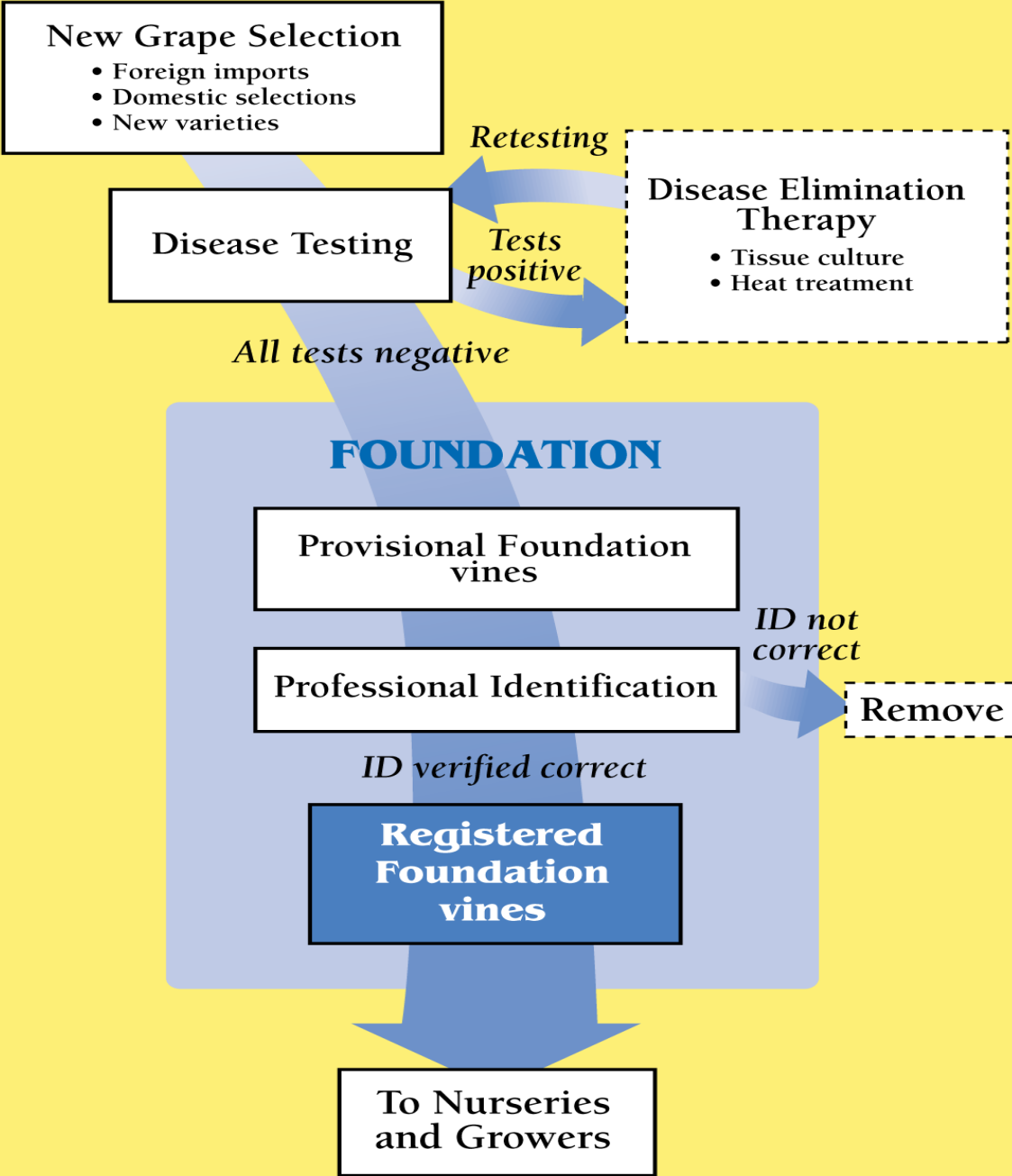
<http://fps.ucdavis.edu>

Foundation Plant Services

UCDAVIS

Foundation Plant Services:

- Produces, tests, maintains and distributes elite disease-tested plant propagation material
- Provides plant importation and quarantine services, virus testing and elimination
- Coordinates release of UC patented horticultural varieties
- Links researchers, nurseries, and producers



FPS Target Grapevine Diseases

Grapevine Degeneration

- Fanleaf

Grapevine Decline

- Tomato Ringspot Virus

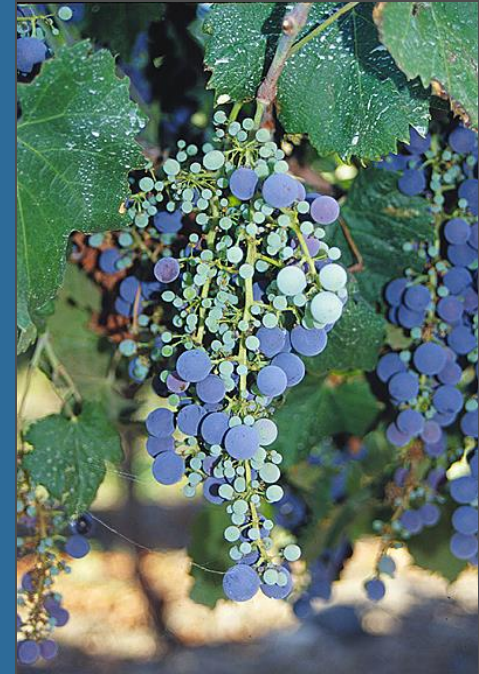
Leafroll

Rugose Wood Complex

- Kober Stem Grooving
- Corky Bark
- LN33 Stem Grooving
- Rupestris Stem Pitting

Fleck

Minor Viruses



Pathogen Elimination

Meristem shoot tip culture



10.0 mm



Cut to < 0.5 mm

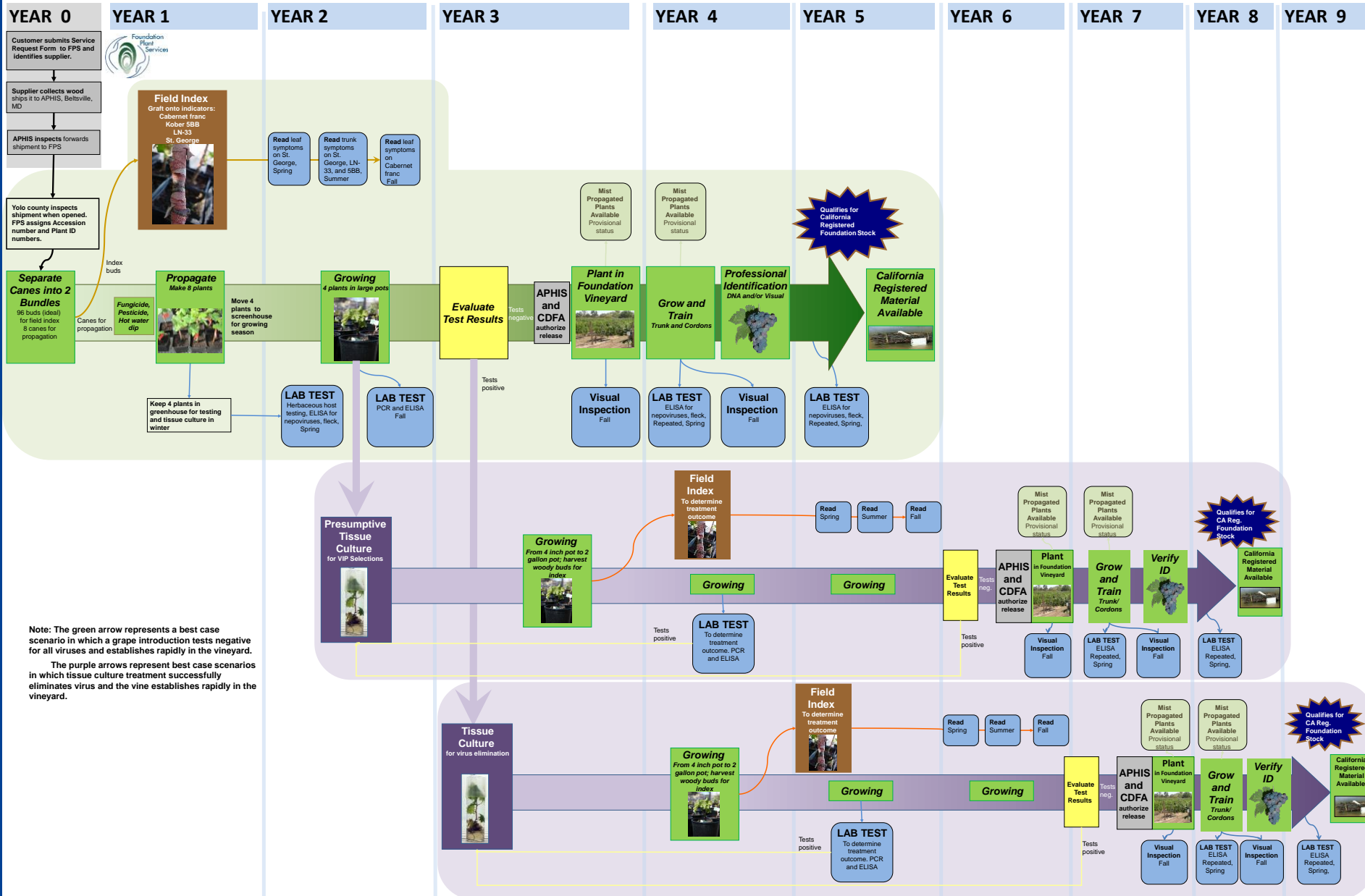


7 months



Process Description: Grapevine Importation through Foundation Plant Services, UC Davis (Simplified)

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Association of a Circular DNA Virus in Grapevines Affected by Red Blotch Disease in California

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Leafroll

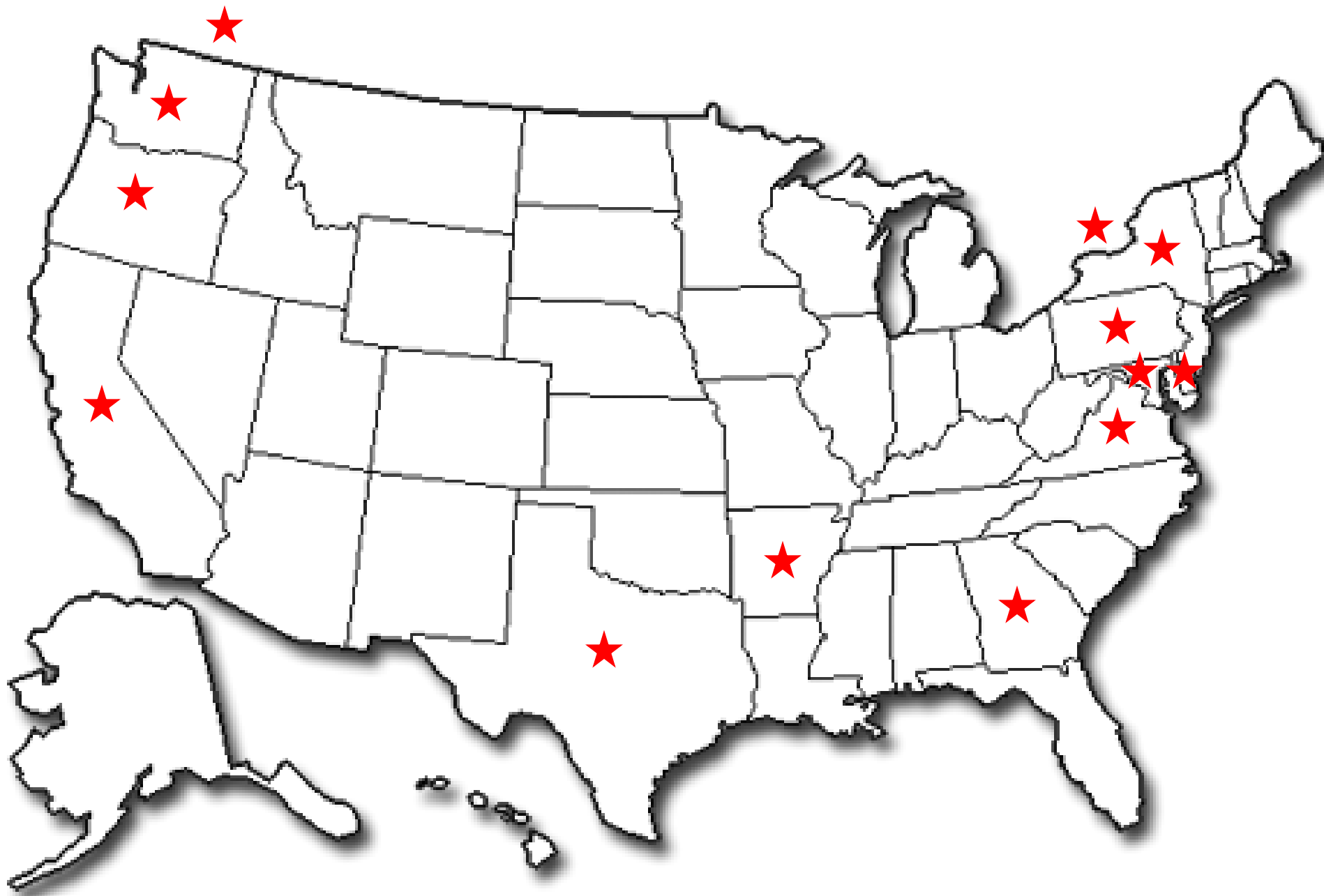


Red blotch

Vitis vinifera cv. Cabernet franc

Distribution of GRBaV

- Wine grapes
 - Red
 - White
- Table grapes
- Raisin grapes
- Rootstocks



Distribution of *GRBaV*-infected vines

Red Blotch: What Do We Know?

- Red blotch is a recently recognized disease
- Fruit ripening of diseased vines can be delayed in some vineyards
 - Brix can be substantially reduced
- GRBaV, a newly discovered DNA virus, is associated with symptomatic grapevines
- GRBaV is the causal agent of red blotch
- Microshoot tip culture is not efficient at curing

Red Blotch: What Do We Know?

- GRBaV is graft-transmissible
- Increased incidence of GRBaV in some vineyards over time
- Virginia creeper leafhopper (*Erythroneura ziczac*) may be able to transmit GRBaV from grapevine to grapevine in the greenhouse
- Symptoms can be misleading. Test, don't guess! A PCR assay is available for diagnosis
- Two distinct genetic variants of GRBaV

Red Blotch: What Don't We Know?

1. Ecology
 - Vector(s)
 - Transmission from grapevine to grapevine in vineyards
2. Detection
 - Maximize Sampling Efficiency
3. Interaction of GRBaV and other viruses
 - Synergistic/antagonistic/commensalistic relationships
4. Effect of GRBaV on vine health
 - Comparative performance evaluation
 - Tolerant cultivars/ rootstocks
5. Management
 - Clean stocks

**What about Red Blotch in
the Classic and Russell
Ranch Foundation
Vineyards?**

Variety	Area	Block	Row	Plant #	RB-PCR	pH	Brix
Orange Mus. 02	BKN	A	15	1	Neg	4.2	26.5
Orange Mus. 02	BKN	A	15	2	Pos	4.2	27.0
Chardonnay 68	BKN	D	3	7	Neg	3.9	31.5
Chardonnay 68	BKN	D	3	8	Pos	3.9	27.0
Thomcord 02	BKN	E	8	4	Neg	3.8	26.0
Thomcord 02	BKN	E	8	3	Pos	3.9	19.0
Chardonnay 49	NYL	C	17	9	Neg	4.0	29.0
Chardonnay 49	NYL	C	17	8	Pos	4.0	30.0
Chardonnay 39	NYL	C	17	15	Neg	3.9	28.0
Chardonnay 39	NYL	C	17	16	Pos	4.1	28.0
Chardonnay 41	NYL	C	18	6	Neg	4.0	28.0
Chardonnay 41	NYL	C	18	7	Pos	4.1	29.0
Chardonnay 37	NYL	C	18	15	Neg	4.0	28.5
Chardonnay 37	NYL	C	18	16	Pos	4.1	29.0
Ruby Cab. 02	NYL	D	3	4	Neg	3.6	25.0
Ruby Cab. 02	NYL	D	3	3	Pos	3.6	23.0
Marsanne 574	NYL	I	13	1	Neg	4.0	23.0
Marsanne 574	NYL	I	13	2	Pos	4.1	24.5



Orange Muscat



Chardonnay 68



Chardonnay 41



Ruby Cabernet 02

Woody index Procedure

- Disbud, wax, root and pot up indicator plants



Woody index Procedure

- Chipbud 2 buds/plant, 6 plants/variety



Woody index Procedure

- Wrap with budding rubber bands



Woody index Procedure

- Harden off



Woody index Procedure

- Read budtake
- Rub off candidate buds
- Plant in field



- Late Fall yrs 1 &2 – Observe Cabernet franc for leaf symptoms



Field Index on Cab. Franc

Plant ID	LR-PCR	GRBaV-PCR	Cab. F. Index
52429	Pos	Pos	Pos
52289	Neg	Pos	Pos
52415	Neg	Pos	Pos
52674	Neg	Pos	Pos
52702	Pos	Pos	Pos
52814	Pos	Pos	Pos
52345	Neg	Pos	Pos
52373	Neg	Pos	Pos
52856	Neg	Pos	Pos
52940	Pos	Pos	Pos
52387	Pos	Pos	Pos



The National Clean Plant Network

New Russell Ranch Foundation Vineyard

established 2010, Davis California



October, 2012

Qualification of Russell Ranch Foundation Vines – Grapevine Disease Testing Protocol 2010

- 1) Microshoot tip tissue culture therapy**
- 2) Negative test results - long list of pathogens - index, herbaceous, ELISA and PCR tests**

**What about Red Blotch in
Russell Ranch Foundation
Vineyard?**

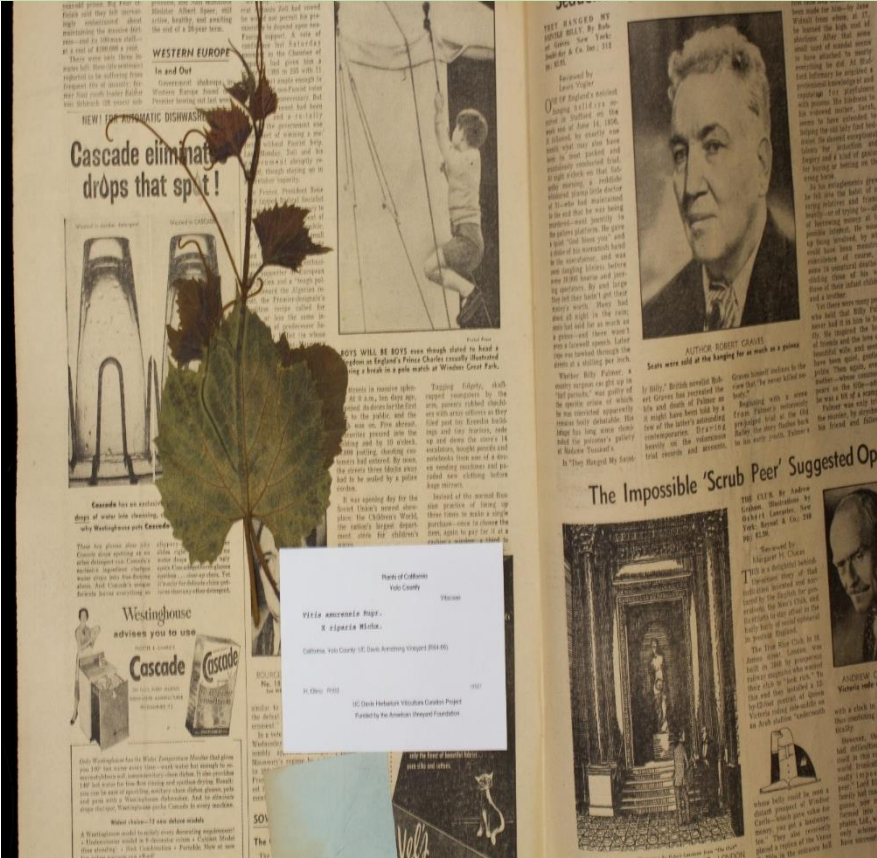
**Is Red Blotch a new
disease?**

UC Davis Center for Plant Diversity/ Herbarium

- The Herbarium has over 300,000 dead plants called herbarium specimens.
- Each specimen consists of a flattened and dried plant glued onto an archival paper with a label.
- The label has specimen data: cultivar, place, date and the collector name.



The herbarium has few un-mounted grape samples from Yolo County



Example of a specimen records



Accession Detail Results

[UCD is the home institution for this record](#)

Please cite data retrieved from this page: Data provided by the participants of the Consortium of California Herbaria (ucjeps.berkeley.edu/consortium/; Fri Oct 31 15:16:31 2014).

Records are made available under the [CCH Data Use Terms](#).

Specimen number	UCD17955
Determination	<i>Vitis vinifera</i> <small>More information: Jepson Online Interchange</small>
Collector, number, date	H. Olmo, s.n., 08 11 1940
County	Fresno
Locality	Wahtoke Vineyard in Sanger
Coordinates	36.7042 -119.5553 <small>BerkeleyMapper [or without layers, here]</small>
Datum	WGS84; ER = 3.612 km
Coordinate source	Geolocate (copied from UCD17950)
Annotations and/or curatorial actions	<i>Vitis vinifera</i> L. cv. Murocain 2010-10-06 Original determination

Sample collection

- Fifty six grapevine specimens were collected (Approximately 0.5 g of leaf/petiole tissue).
- specimens were originally harvested and pressed between 1937-1950.
- Pieces of foil containing each sample were placed in individual Ziplock bags to prevent cross contamination.
- The 56 sample bags were transported to a lab in which research on grapevine had NOT previously been conducted.



Sample collection in the herbarium

List of samples- Group 1: *Vitis* cultivars from Napa or Sonoma County

Sample #	Herbarium ID#	Variety/cultivar	Location (County)	Year of collection	Collector	Tissue
1	DAV202170	Vitis vinifera L. cultivar Early Burgundy	Sonoma	1940	H. Olmo	Leaves
2	DAV202866	Vitis vinifera L. cultivar aff. Napa Gamay	Napa	1939-1940	H. Olmo	Leaves
3	DAV202172	Vitis vinifera L. cultivar Petite Bouschet	Sonoma	1937	H. Olmo	Leaves
4	DAV202202	Vitis vinifera L. cultivar Rosetta	Sonoma	1938?	H. Olmo	Leaves and petioles
5	DAV202196	Vitis vinifera L. cultivar Zinfandel	Napa	1935	H. Olmo	Leaves and petioles
6	DAV202174	Vitis vinifera L. cultivar Black Malvoisie	Sonoma	1935	H. Olmo	Leaves and petioles
7	DAV202201	Vitis vinifera L. cultivar Burger	Napa	1935	H. Olmo	Leaves and petioles
8	DAV202171	Vitis vinifera L. cultivar Carignane	Sonoma	1937	H. Olmo	Leaves and petioles
9	DAV202870	Vitis vinifera L. cultivar aff. Merlot	Napa	1939-1940	H. Olmo	Leaves and petioles;
10	DAV202872	Vitis vinifera L. cultivar aff. Gamay	Sonoma	1939-1940	H. Olmo	Leaves and petioles
11	DAV202227	Vitis vinifera L. cultivar Grey Riesling	Sonoma	1938	H. Olmo	Leaves and petioles
12	DAV202860	Vitis vinifera L. cultivar Early Burgundy	Napa	1939-1940	H. Olmo	Leaves and petioles

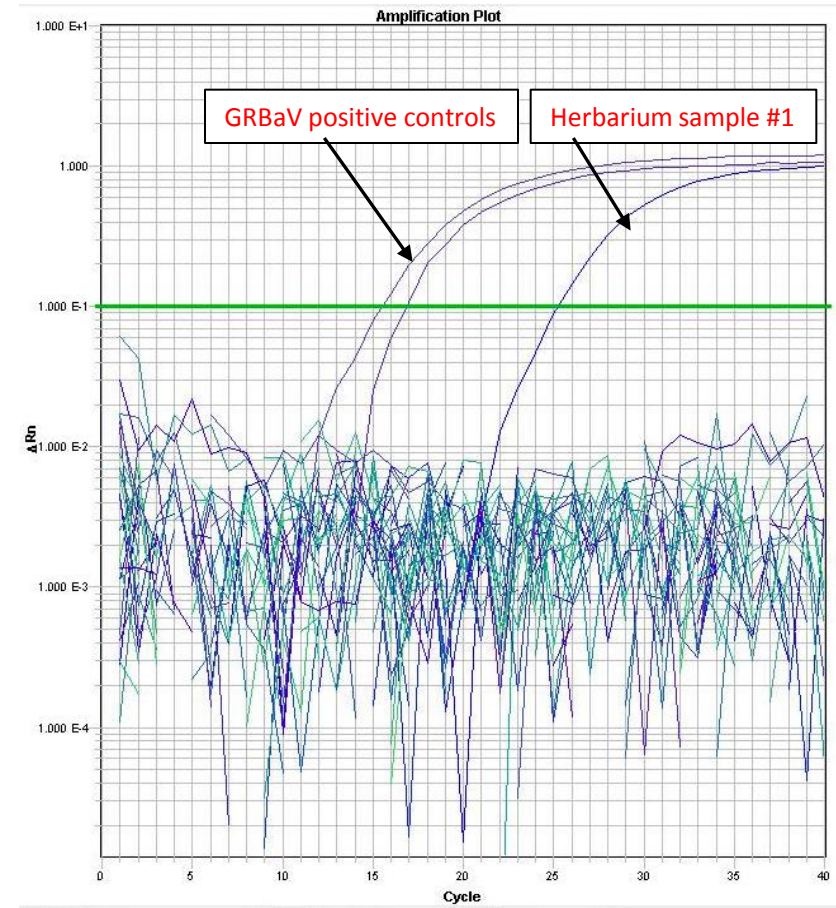
Precautions to prevent cross-contamination

- Extractions were conducted in an isolated lab (The Michelmore Lab)
- Samples processed with ALL new materials and reagents.



GRBaV PCR Results

- All herbarium samples were tested for GRBaV using both conventional and Quantitative PCR assays
- Only one sample found to be positive for GRBaV (sample #1)



Sample #1

Cultivar: Early Burgundy

Location: Sonoma

Collector: H. Olmo

Year of collection: 1940



What came Next: Genomic analysis

We obtained the full genome sequence of GRBaV herbarium isolate and compared it with the recently sequenced GRBaV isolates.

Summary of results

The results suggest that GRBaV was present in the Sonoma wine grape growing area for at least 74 years before the virus was identified and correlated to specific symptoms.

Red Blotch: Challenges and opportunities

* With much thanks to Marc Fuchs

	<u>Fanleaf</u>	<u>Leafroll</u>	<u>Red blotch</u>
First description	1841	1905	2008
Graft transmission	1962	1935	2012
Virus recognition	1960	1979	2012
Vector transmission	1958	1984	2013
Diagnostic assays	1960	1984	2012
Koch's postulates	1962	n/a	2013

Flowchart of Mist Propagated Plants (MPPs) from Foundation Plant Services to Nurseries to Growers

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YEAR 1



Mist Propagated Plants (MPPs) Available at FPS
Spring to Fall

1 plant

YEAR 2

At nursery

Nursery Grows and Propagates MPP; Plants in Increase Block

Grow MPP; continuously take cuttings and root (10 to 30 possible)

Plant 10 to 20 vines in Increase Block, (IB)

Grow IB vines

Winter

Spring

Summer

10 - 30 plants

YEAR 3

At nursery



California Registered Green - growing Benchgrafts Available

Harvest cuttings from IB, (10 to 20 cuttings/ vine), make benchgrafts for sale

Continue to Grow IB vines

Winter

Spring Summer

80 - 450 plants

YEAR 4

At nursery



California Registered Green - growing and Dormant Benchgrafts Available

Harvest cuttings (50 to 100 cuttings/vine); make benchgrafts

Winter

Spring

400 - 2,500 plants



Thank you
American Vineyard Foundation
Fruit Tree, Nut Tree and Grapevine Improvement
Advisory Board (IAB)
California Grape Nurseries