2017 Sonoma County Grape Day
UC Cooperative Extension, Santa Rosa, CA
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Grapevine red blotch disease: the virus, its spread and a vector

Frank Zalom
Dept. of Entomology and Nematology, UC Davis

Mysore Sudarshana
USDA-ARS, Dept. of Plant Pathology, UC Davis

Grapevine red blotch-associated virus

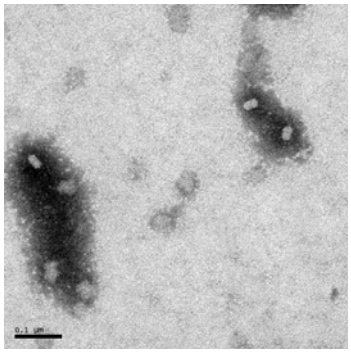
2011
- Next generation sequencing and analysis identified a new virus in grapevines
- Virus was detected in six commercial vineyards and at the UC Davis Oakville station

2012
- Identical virus was found in New York
- Virus was associated with symptomatic grapevines
- "Grapevine red blotch-associated virus (GRBaV)"

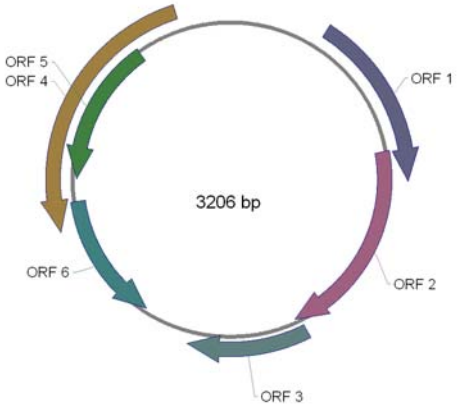
2013
- Identical virus was found in Washington State and several other states in the US and Canada.

Grapevine red blotch-associated virus

GRBaV - a circular ssDNA genome (3,206 nt) closely related to Geminiviridae



Negatively stained virions of GRBaV



(Rwahnih et al. 2013)

Learning about GRBaV, 2013-14

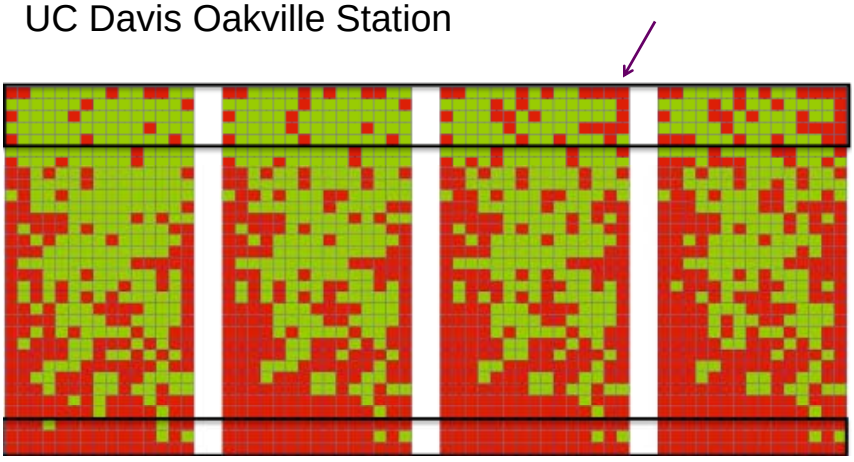
- 'New'? - found in herbarium sample collected from Sonoma County in 1940
- Distribution? - no formal survey was conducted, but found to be widespread
- Why? - Became widespread by the propagation of infected planting stock, grafting non-infected vines using infected budwood, and using infected rootstock
- Is spread occurring?
- If so, is there a vector?

Is red blotch disease spreading?

That the disease was associated with a virus and that spread occurs was not universally accepted, however…

- Observed patterns of incidence that would suggest spread was occurring
- Year-to-year increase in new GRBaV infections was documented
- All known geminiviruses are insect transmitted

UC Davis Oakville Station

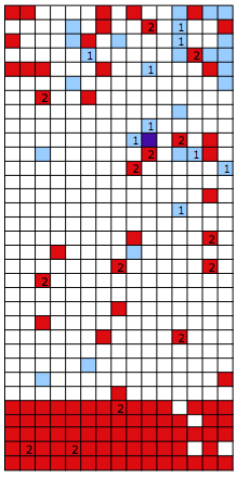


2011	2012	2013	2014
44%	+5.4%	+4.8%	+5.4%

Red squares are infected vines, green squares are healthy vines, all vines located within black rectangles were tested using qPCR

UC Davis Oakville Station

In 2013, a new genotype was found for the first time associated with the northwest corner of the vineyard; consistent with the introduction of the new genotype by a vector



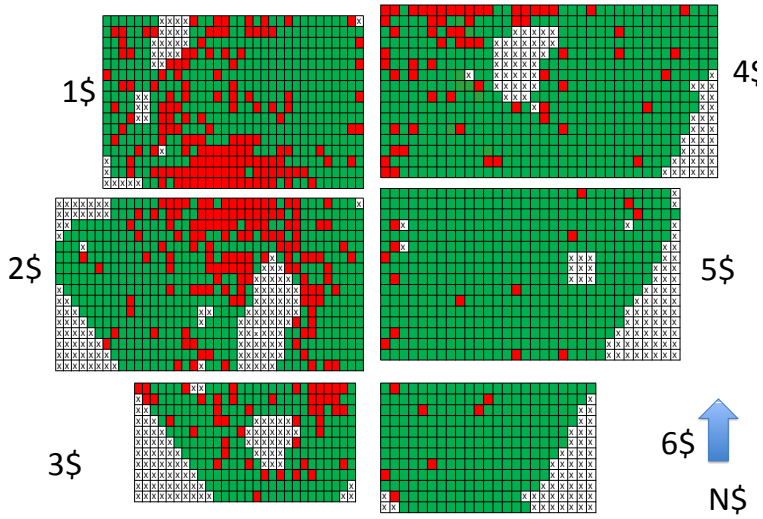
Blue squares = genotype one
Red squares = genotype two
Purple square = mixed infection

Now called ‘clade 1’ and ‘clade 2’; primers are available for both

Amador Co. - self-rooted Zinfandel

(F. Zalom)

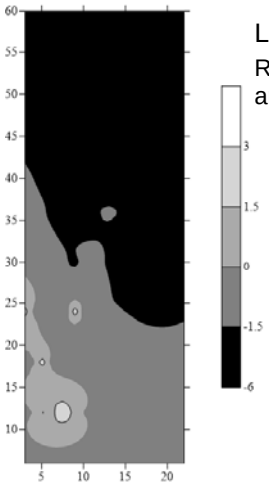
Number of infected vines increased by 18% from 2015 to 2016



Source of cuttings - a Zinfandel block planted in 1928

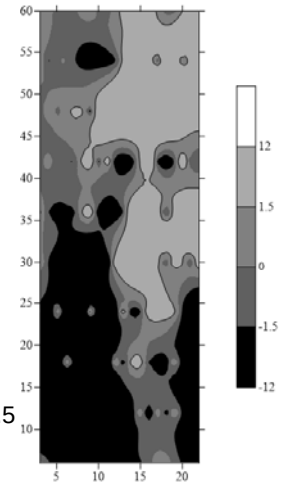
Oregon

Red blotch distribution differs from leafroll distribution
- Vaughn Walton et. al, OSU



Leafroll
Results >1.5 or <-1.5 are significant

Red blotch
Results >1.5 or <-1.5 are significant



Oregon

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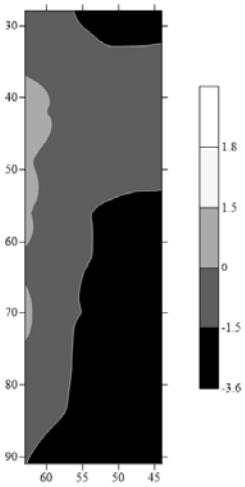
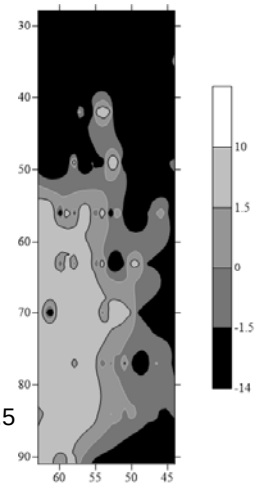


Leafroll = mealybug
Results >1.5 or <-1.5 are significant

Pattern suggests a motile vector is involved

Red blotch = ??
Results >1.5 or <-1.5 are significant



Is an insect involved?

A 2013 study at Washington State University implicated the Virginia Creeper Leafhopper, *Erythroneura ziczac* as a vector in a greenhouse study… - Poojari et al. (2013)

… but no other labs were able to repeat the study results.

(C. Preto)

VCLH as a vector?

Nice story, but problematic -

- VCLH were not present in some vineyards where disease spread was suspected
- VCLH was present in vineyards with red blotch, but disease not spreading
- VCLH feeds primarily on mesophyll, but monopartite geminiviruses are phloem limited

Vector Transmission Bioassays

- 2014: 100 transmission bioassays
- 2015: 125 new transmission bioassays

No transmission detected

VCLH as a vector?

All 3 *Erythroneura* species tested positive for presence of GRBaV after feeding on infected plants, but they had lower virus concentration and a lower percentage tested positive than other Hemiptera tested

Fewer positive Lower virus conc.

Species	No. positive/ No. tested	Mean (Ct ± SE)	Mean virus Qty./µl±SE
E. elegnatula	2/20	28.9±0.81	1.79E+01
E. variabilis	1/20	32.1±0.0	1.00E+01
E. ziczac	1/20	26.5±0.0	1.55E+01
Spissistilus festinus	→ 15/20	→ 16.1±0.34	1.86E+05
GRBaV-infected[1]	5/5	14.1±0.63	1.34E+06
Healthy grapevine[1]	0/5	No Ct	N/A
Buffer control[1]	0/5	No Ct	N/A

VCLH as a vector?

2014-16: Recipient plants were tested monthly for 1.5 years post inoculation:

- *Erythroneura ziczac*: 0/10 positive for GRBaV
- *Erythroneura elegantula*: 0/10 positive for GRBaV
- *Erythroneura variabilis*: 0/10 positive for GRBaV

2015-16: Recipient plants were tested monthly post inoculation, results at 5 month:

- *Erythroneura ziczac*: 0/15 positive for GRBaV
- *Erythroneura elegantula*: 0/15 positive for GRBaV
- *Erythroneura variabilis*: 0/15 positive for GRBaV

Vector Transmission Bioassays

Species considered as likely candidates at end of 2015

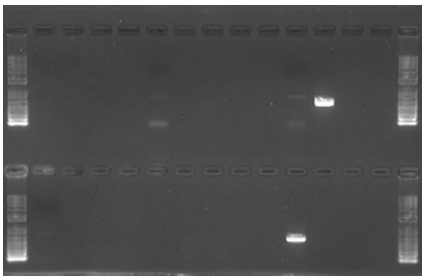
- 1 treehopper (Membracidae)
- 2 leafhoppers (Cicadellidae)
- 1 jumping plant louse (Psyllidae)
- 1 planthopper (Cixiidae)

Vector Transmission Bioassays

5 month PCR test in February, 2016 -

- Membracidae 3/15 { Clade I - 8/15 at 6 months; Clade II - 5/15 positive at 7 months
- Cicadellidae 0/50
- Cicadellidae 0/20
- Psyllidae 0/10
- Cixiidae 0/10

Spissistilus festinus



PCR results at 5 months

Red Blotch Symptoms

Two of the three recipient plants also exhibited mild red blotch leaf symptoms

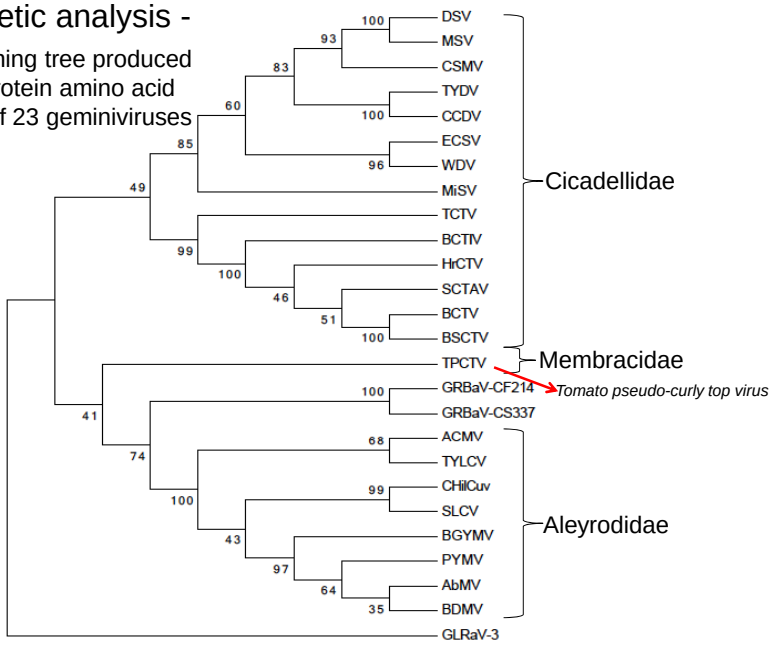
Recipient plant

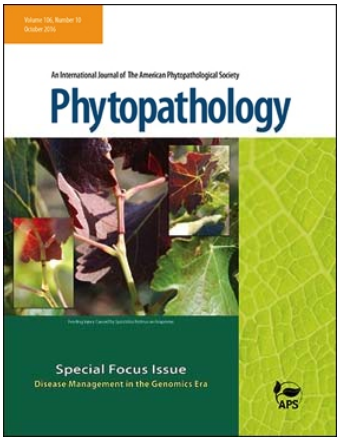
Negative control

B. Bahder, UC Davis

Phylogenetic analysis -

Neighbor-joining tree produced using coat protein amino acid sequences of 23 geminiviruses





For more information on the study:

Bahder, B.W., F.G. Zalom, and M.R. Sudarshana. 2016. Phylogeny of geminivirus coat protein sequences and digital PCR aid in identifying *Spissistilus festinus* (Say) as a vector of Grapevine red blotch-associated virus. Phytopathology 100(10): 1223-1230.

Three-cornered alfalfa hopper
Spissistilus festinus (Say)

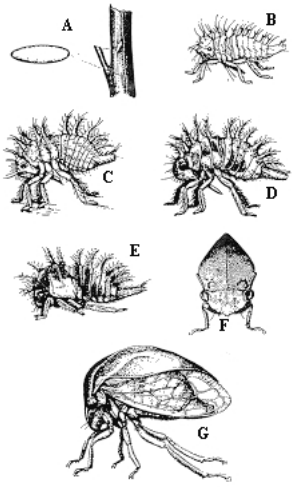
K. Garvey, UC Davis

Spissistilus festinus (Say)

- Adults - 6.0 to 6.5 mm long
- Eggs - white, oblong-oval and 0.9 to 1.3 mm long; slightly larger at one end; laid in stems of host plant
- Nymphs - wedge-shaped and heavily spined

Distribution – present from California and northern Mexico across the south to the Atlantic Ocean and as far north as Canada

Life Cycle

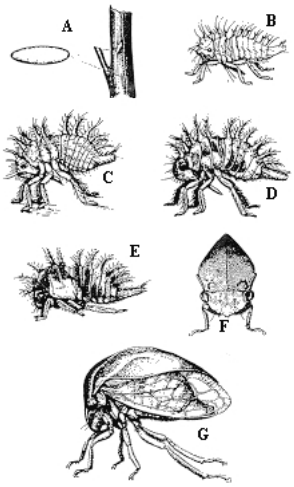


A, egg; B-F, nymphs; G, adult

Spissistilus festinus (Say)

C. Preto, UC Davis

Life Cycle

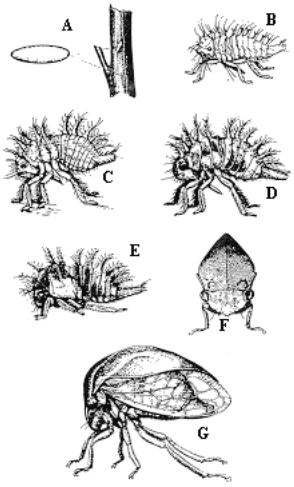


A, egg; B-F, nymphs; G, adult

Spissistilus festinus (Say)

Host preference - leguminous plants such as alfalfa, peanut, soybean, bean, cowpea, and sweet clover; occasionally infest tomato, melon, cotton, wheat, barley, oat, Bermuda grass, and Johnson grass, as well as some trees and shrubs

Life Cycle



A, egg; B-F, nymphs; G, adult

Spissistilus festinus (Say)

Oviposition scars and eggs

C. Preto and F. Zalom

Spissistilus festinus (Say)

Life history - (during summer)

- Eggs are deposited in the stems of a host plant and hatch in 7-10 days
- Nymphs reach the adult stage in ~24 days
- “There are ‘several’ generations annually” (2 to 4 generations; probably 3 to 4 in California)

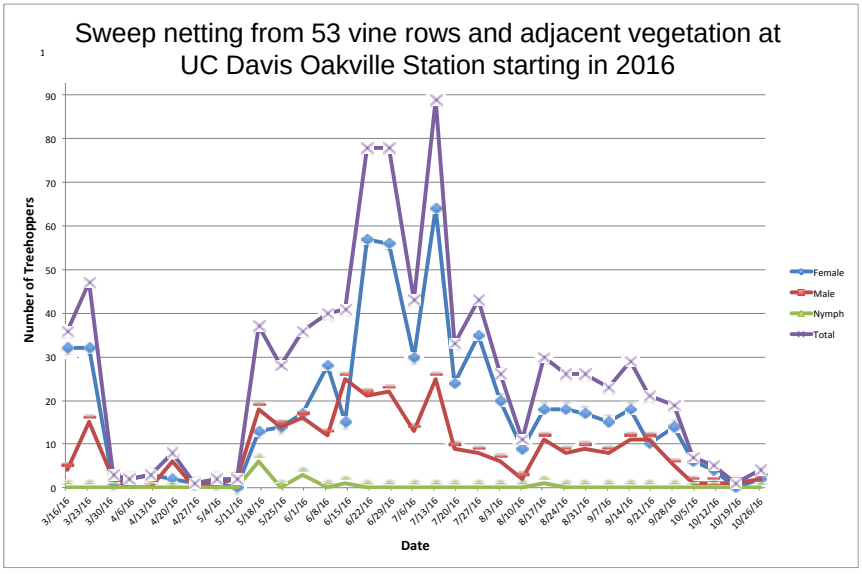
“Overwinter as eggs in plant tissues or as adults protected by clumps of grasses; overwintering adults are active…”

Damage - complete girdling of stems of the host plant; the girdle is the result of many punctures made in a ring around the stem of the plant

Spissistilus festinus (Say) - Damage

F. Zalom

Spissistilus festinus (Say) – Seasonal phenology



Other vineyard treehoppers?

May 17, 2016

Other vineyard treehoppers?

Tortistilus spp.

B. Bahder

S. Sudarshana

Vector status, unknown, but being evaluated

Three cornered alfalfa hopper

Tortistilus vs
Spissitilus festinus

C. Preto

Virus and Vector Management Research

- Additional vectors
- Alternate plant hosts for vector and virus
- Virus development cycle in plants
- Vector transmission requirements
- Better methods for virus and vector detection
- Characterize virus spread
- 3CAH seasonal phenology and overwintering
- Cultural controls – vegetation management, cover crops, roguing, pruning
- Chemical controls – insecticides, antifeedants

Acknowledgements

- Cooperating growers, nurseries and managers
- CDFA SCBG Program
- California Tree Fruit, Nut Crops and Grapevine Industry Board
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- Lucia Varela
- Michael Anderson
- USDA-ARS NP303 National Program

S. Sudarshana

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