NASEM Grapevine Viruses Committee Meeting
March 4, 2024
Davis, CA

Grapevine Red Blotch Virus and Vectors

Frank Zalom

Department of Entomology and Nematology

UC Davis

Mysore Sudarshana

USDA-ARS and Department of Plant Pathology

UC Davis

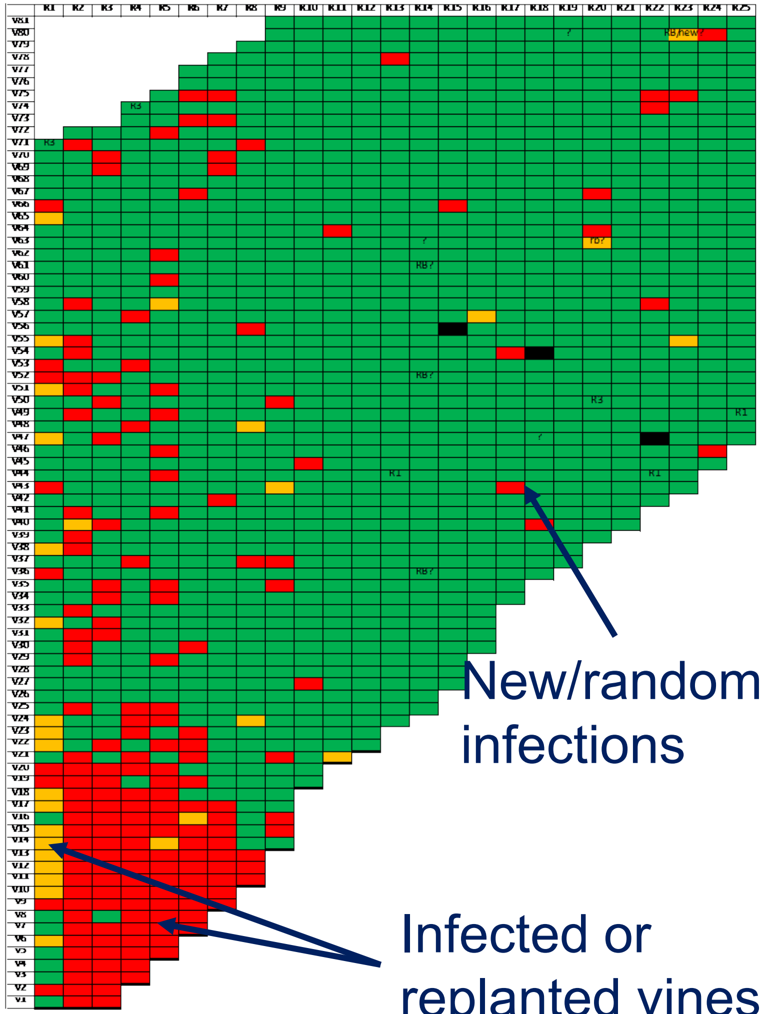
2013 → *Is red blotch disease spreading?*

Many virologists believed that GRBV was not spreading, but …

- Observed spatial patterns of diseased vines suggested spread was occurring
- Year-to-year increase in new GRBV infections was documented in several vineyards
- All known geminiviruses are insect transmitted
- Pattern of infection in vineyards indicated a motile pest might be responsible

Search for potential GRBV vectors began in 2014 - methods

- Identify vineyard sites where spread was occurring
- Collect phloem-feeding Hemiptera from symptomatic areas of the vineyards
- PCR-test the collected insects for presence of GRBV to determine proportion carrying the virus
- Identify those insect species with a high proportion of insects collected with GRBV present in body (or salivary glands)
- Identify the insect species of possible vector candidates



Transmission studies (>225 in 2014 and 2015 alone)

- Established insect colonies when possible
- Allowed insects to feed on GRBV-infected grapevines (control insects were fed on uninfected vines)
- Transferred insects to GRBV negative grapevines
- Tested plants periodically for GRBV presence

From these initial assays, only one insect was shown to transmit GRBV using standard protocols …

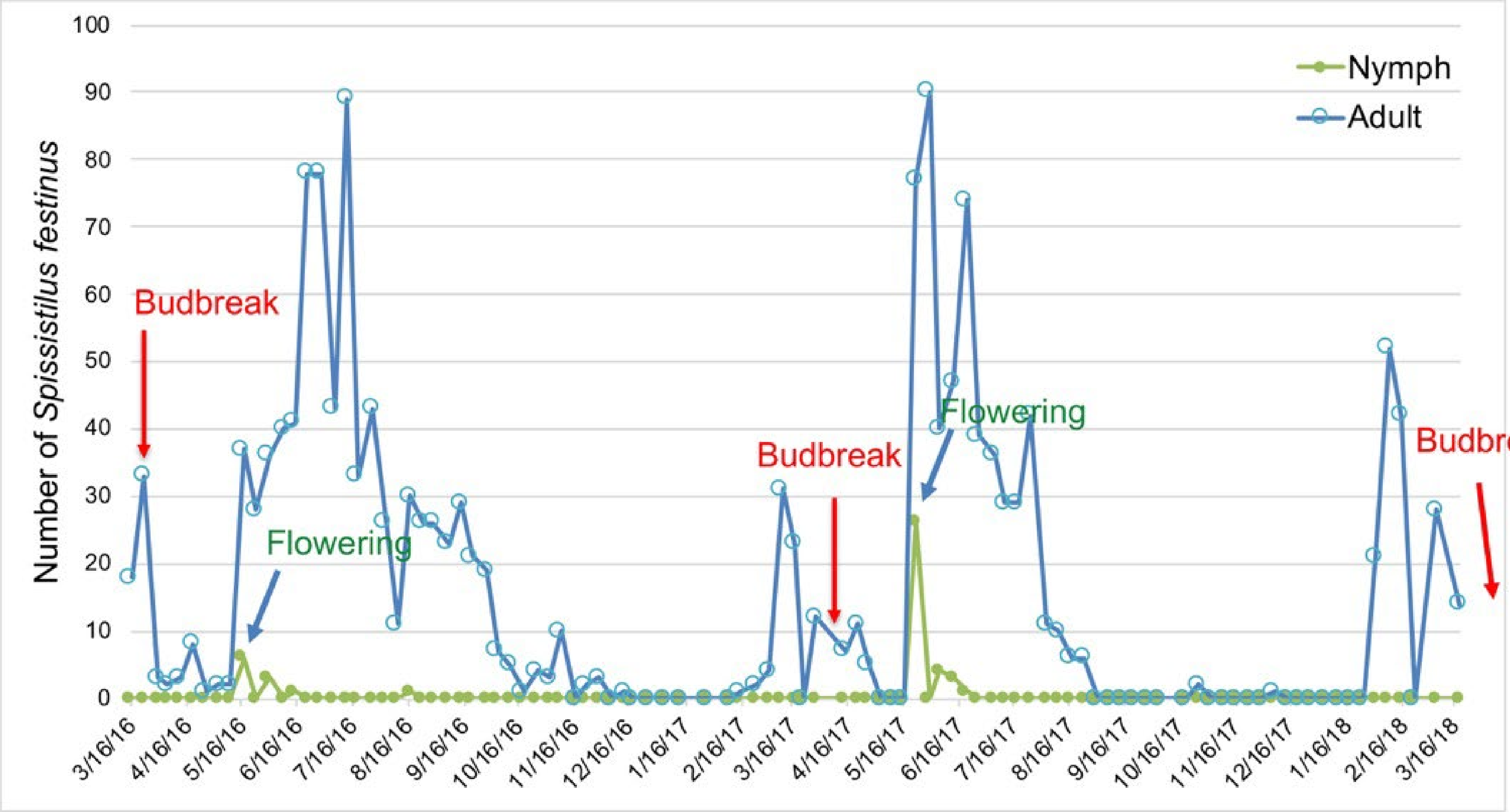
Three-cornered alfalfa hopper

Spissistilus festinus (Say)

Bahder B.W., Zalom F.G., Jayanth M., and Sudarshana M.R. 2016. Phylogeny of geminivirus coat protein sequences and digital PCR aid in Identifying *Spissistilus festinus* as a vector of Grapevine red blotch-associated virus. Phytopathology. 106:1223-1230.

Vector Management Research

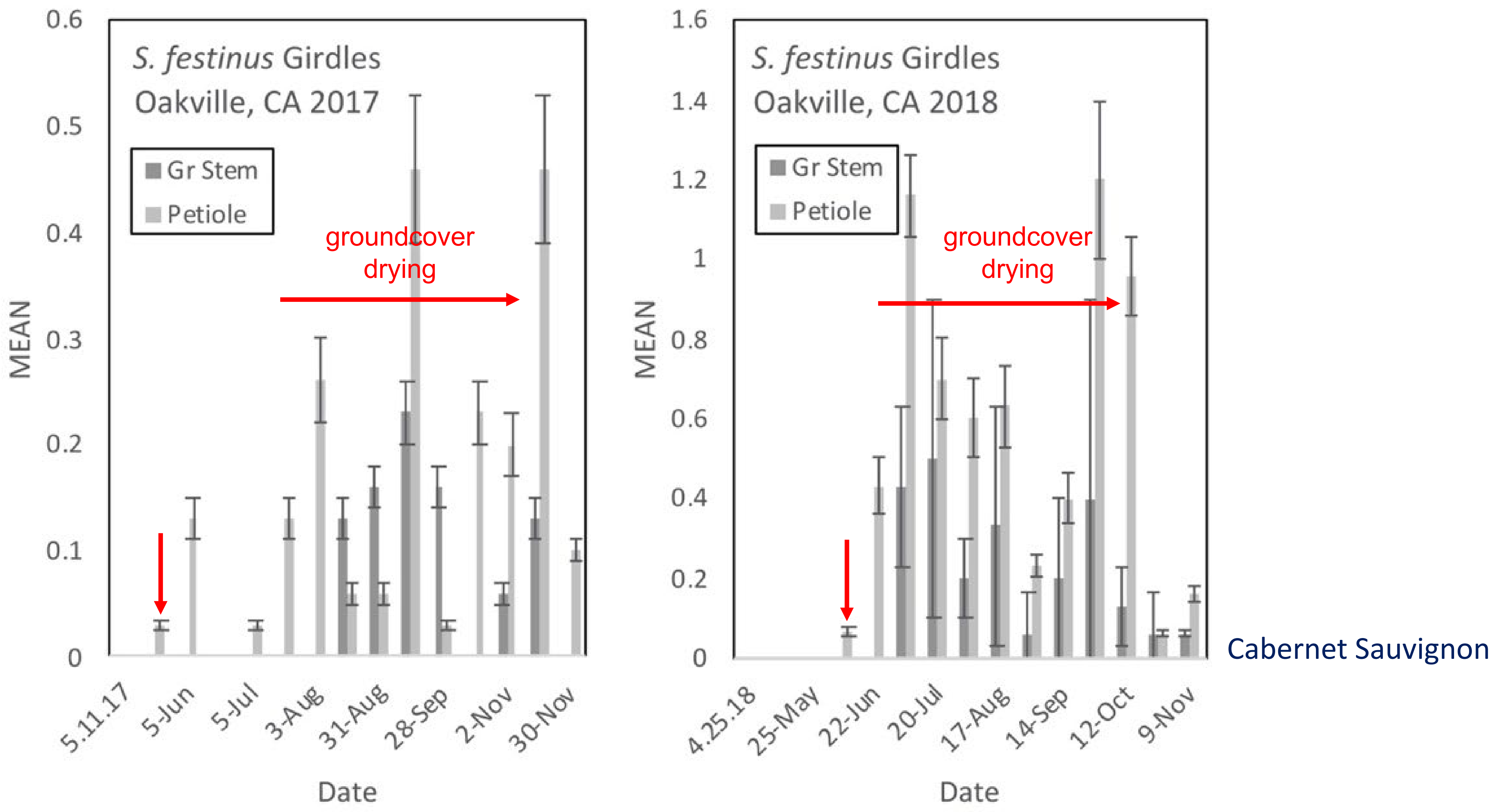
- *Spissistilus* seasonal phenology
- *Spissistilus* biology in and around vineyards
- Feeding and reproductive hosts of *Spissistilus* and vector/virus studies



Spissistilus festinus seasonal phenology

Preto, C.R., B.W. Bahder, E.N. Bick, M.R Sudarshana, and F.G. Zalom. 2019. Seasonal dynamics of *Spissistilus festinus* (Say) (Hemiptera: Membracidae) in a Californian vineyard. J. Econ. Entomol. 112(3): 1138-1144. https://doi.org/10.1093/jee/toz022

Spissistilus festinus seasonal girdling – Oakville



Bollinger, M.R. 2021. Seasonal and Within-Plant Distribution of Feeding Damage to *Vitis vinifera* caused by *Spissistilus festinus* (Hemiptera: Membracidae). Ch. 1, MS Thesis, Entomology Graduate Group, UC Davis.

Spissistilus festinus feeding and reproductive hosts

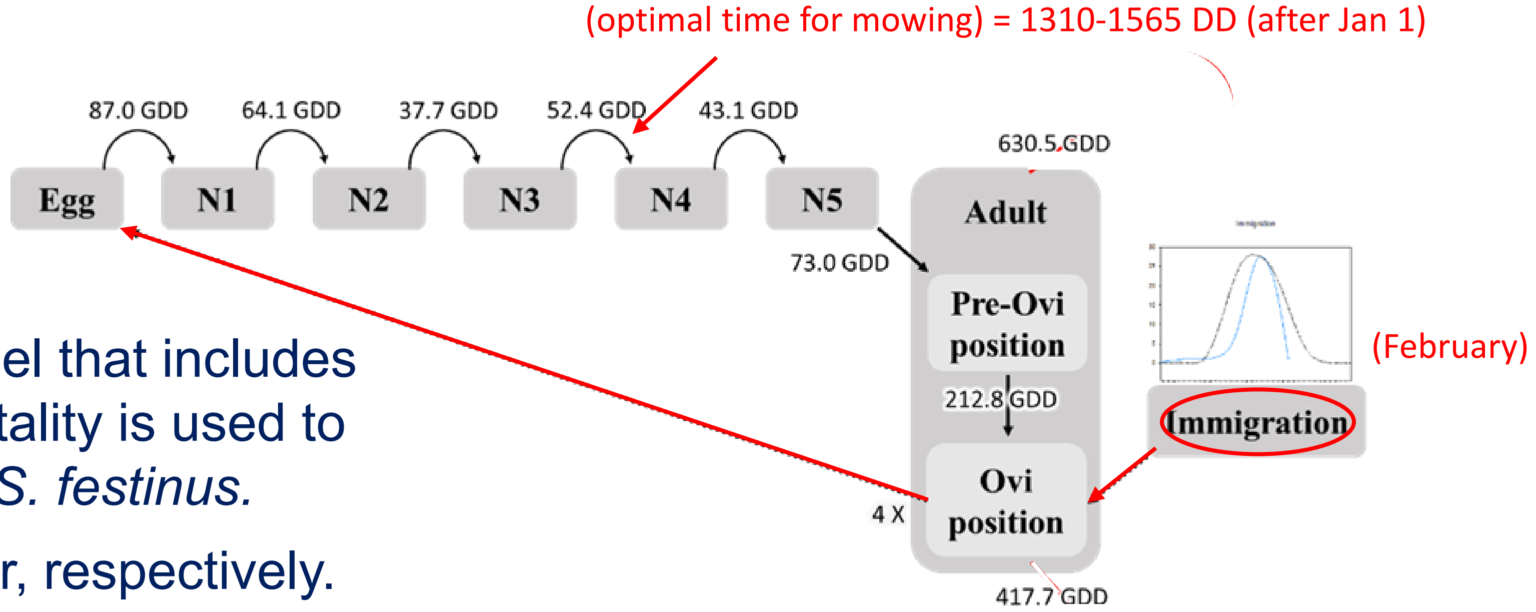
- Evaluated common vineyard weeds and cover crops as feeding and reproductive hosts of *S. festinus*
- Found that most legumes are feeding and reproductive hosts
- Very few non-legumes are reproductive hosts* (*exceptions: dandelion, common groundsel, field bindweed, blando brome)
- *S. festinus* was not found to complete its life cycle on grapevines in the field

Preto, C.R., M.R Sudarshana, and F.G. Zalom. 2018. Feeding and reproductive hosts of *Spissistilus festinus* (Hemiptera: Membracidae) found in Californian vineyards. J. Econ. Entomol. 111(6): 2531-2535. https://doi.org/10.1093/jee/toy236

Preto, C.R., M.R Sudarshana, M.L. Bollinger and F.G. Zalom. 2018. *Vitis vinifera* as a reproductive host of *Spissistilus festinus* (Say) (Hemiptera: Membracidae). J. Insect Sci. 18(6): 20, https://doi.org/10.1093/jisesa/iey129

Spissistilus festinus decision support model –

Predicts timing to initiate tilling/mowing to eliminate vineyard floor plant hosts of *S. festinus* in late winter or spring. Validated with field data collected in winter and spring 2020-21 in 5 North Coast commercial vineyard locations.



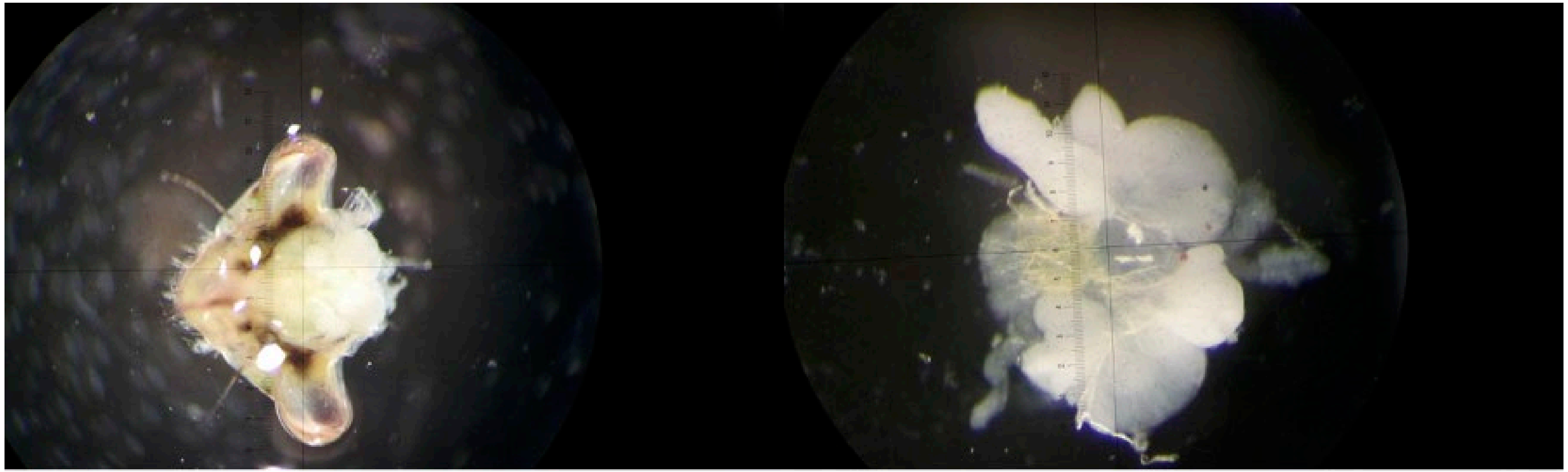
A stage-structured degree-day model that includes immigration, reproduction, and mortality is used to predict the population dynamics of *S. festinus.*

N1-N5 refers to 1st through 5th instar, respectively. Developmental thresholds = 12.8°C min and 35.0°C max.

Bick, E.N., C.R. Preto, and F.G. Zalom. 2020. Timing the implementation of cultural practices for *Spissistilus festinus* (Hemiptera: Membracidae) in California vineyards using a stage-structured degree-day model. J. Econ. Entomol. 113(5): 2558-2562. https://doi.org/10.1093/jee/toaa165

GRBV acquisition and transmission -

Since GRBV transmission is circulative, then detecting GRBV in salivary glands should be a suitable proxy for transmission studies in plants.



salivary glands within head capsule

salivary glands removed from head capsule

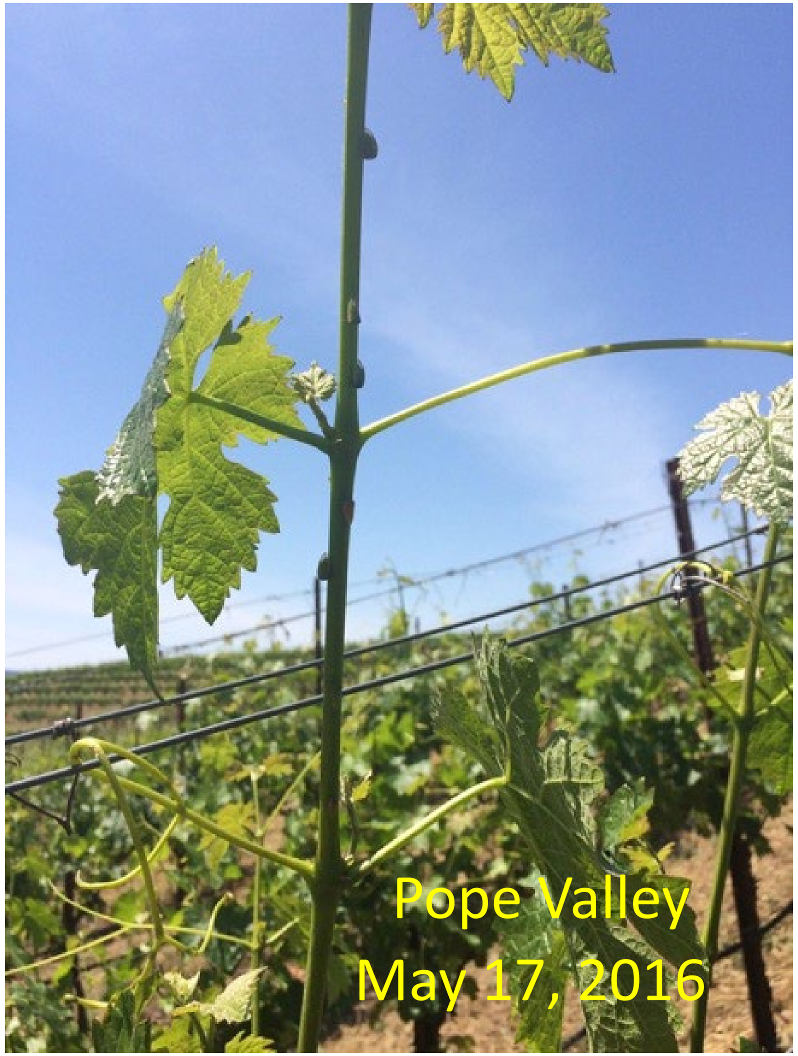
Alternative method for evaluating candidate GRBV vectors

Developed and validated with beet leafhopper adults fed on beet curly top virus

Safeguards to ensure no contamination occurs

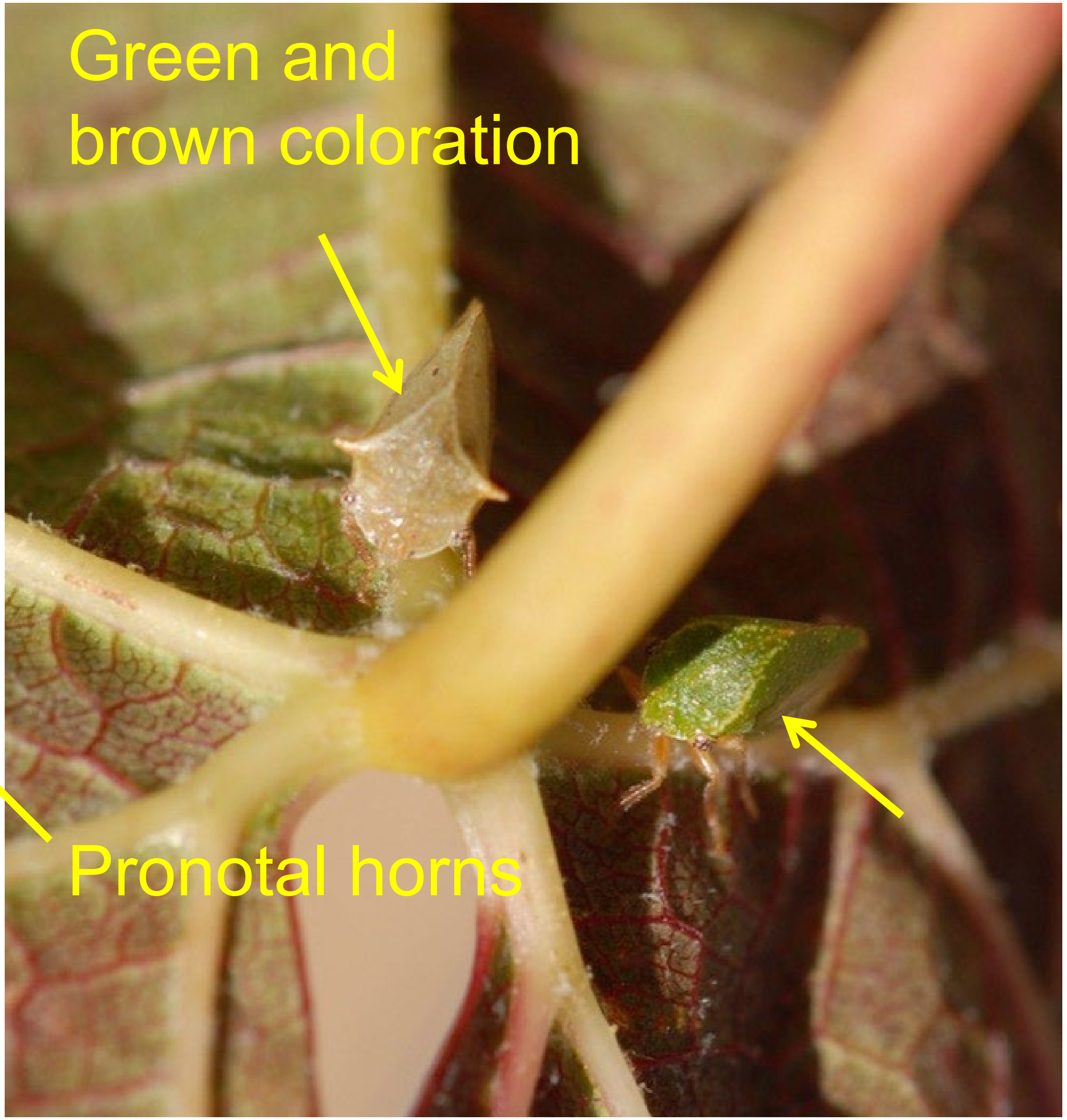
Tortistilus – another treehopper associated with California vineyards

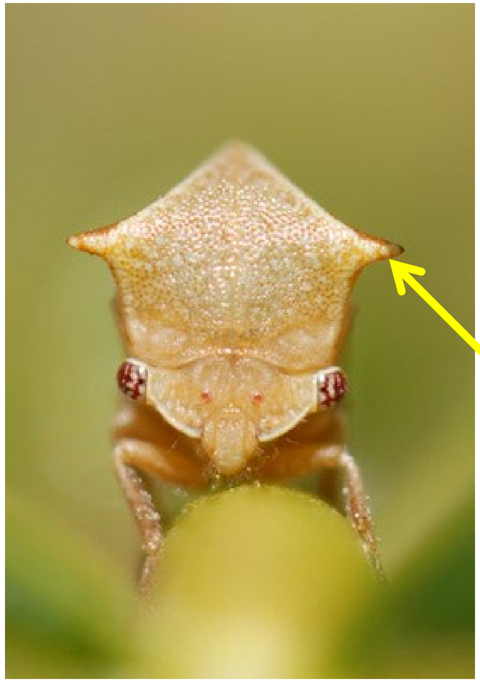
- Adults can occur in massive numbers for a few weeks
- Mainly found in sloping vineyards where *S. festinus* are absent or in very low abundance



Tortistilus spp.

Determined by Dr. Dennis Kopp, Smithsonian





542 adults collected on June 2, 2017, from grapevines at same location

Different species or morphotypes?

Tortistilus spp.

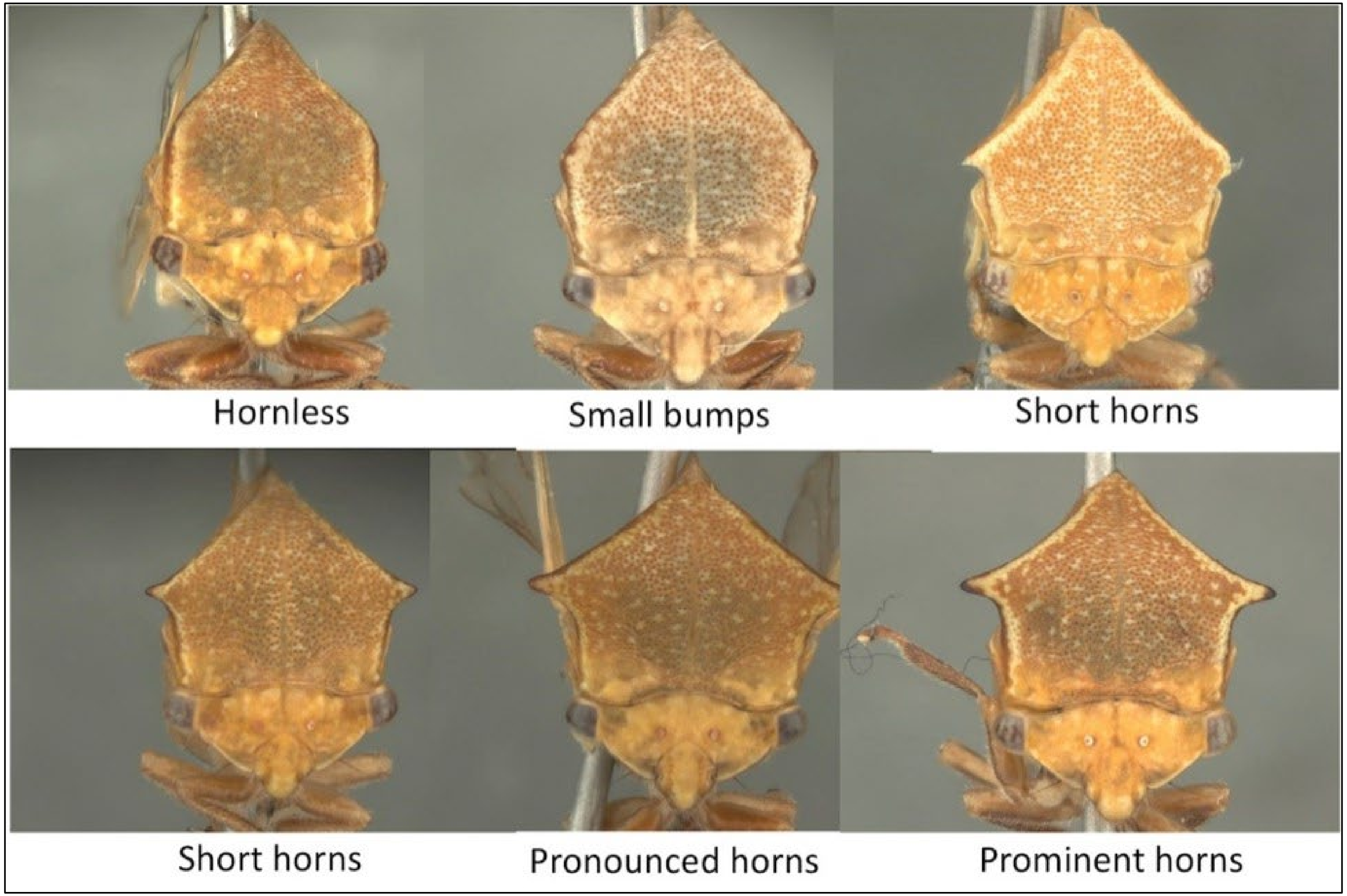
Horned -
Tortistilus albidosparsus

Without horns -
Tortistilus wickhami
Tortistilus pacificus

Removed 6 legs of 2 specimens of each of the 4 morph for DNA sequencing

CO1 sequences indicated they are one species

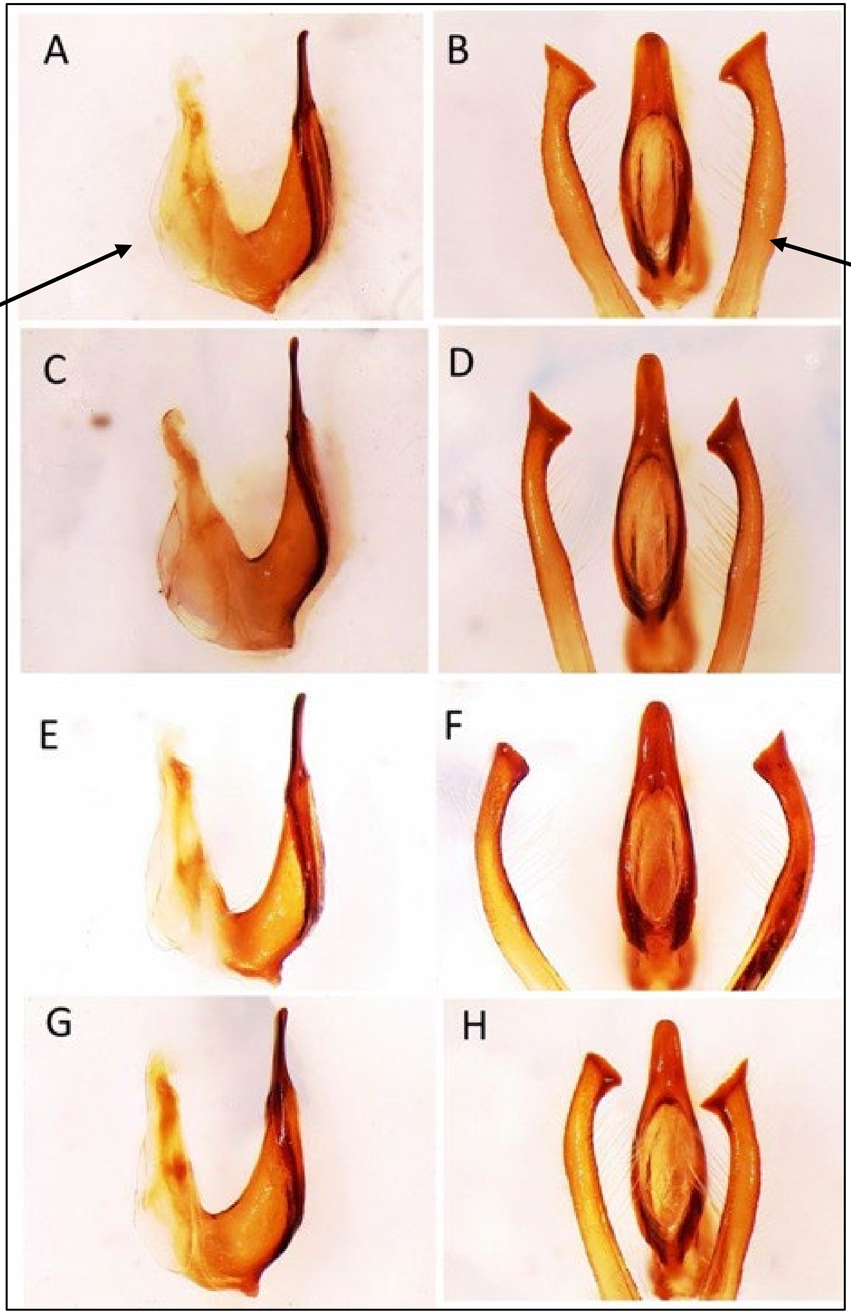
Clinal variation of suprahumeral horns in females from a single Napa County vineyard collection



Tortistilus spp.

Identified by male genitalia

Profiles of male aedeagus posterior and anterior arms



Caudal views of male posterior aedeagus and posterior style arm

A and B = horned brown
C and D = horned green
E and F = hornless brown
G and H = hornless green

Indistinguishable

Sequences indicate they are one species

Sequence reads obtained by Illumina sequencing of DNA from four morphs of insects identified as *Tortistilus* spp. and collected on the same grapevines on June 2, 2017.

Sample ID	Code	Color	Horned	Illumina reads (million)	Coverage (Gbp)
DS17-01	BH-	Brown	No	46.0	13.8
DS17-02	BH-	Brown	No	44.8	13.4
DS17-03	GH-	Green	No	47.3	14.2
DS17-04	GH-	Green	No	45.8	13.7
DS17-05	BH+	Brown	Yes	43.9	13.2
DS17-06	BH+	Brown	Yes	45.9	13.8
DS17-07	GH+	Green	Yes	44.5	13.4
DS17-08	GH+	Green	Yes	45.3	13.6

No difference between sequences for the 4 morphotypes

Tortistilus spp. mating study -

Matings of all combinations of horned and unhorned morphotypes collected as nymphs in late May 2018; individually reared to adults; morphotypes mated and allowed to oviposit on oak saplings; molted to adults from April to May 2019.

Group	Male parent with horns?	Female parent with horns?	Male progeny		Female progeny	
			Horns	No horns	Horns	No horns
1	Y	Y	11	none	3	none
2	Y	N	none	none	none	none
3	Y	N	1	8	none	8
4	N	Y	none	none	1	1
5	N	Y	none	none	none	none
6	N	N	none	none	1	2

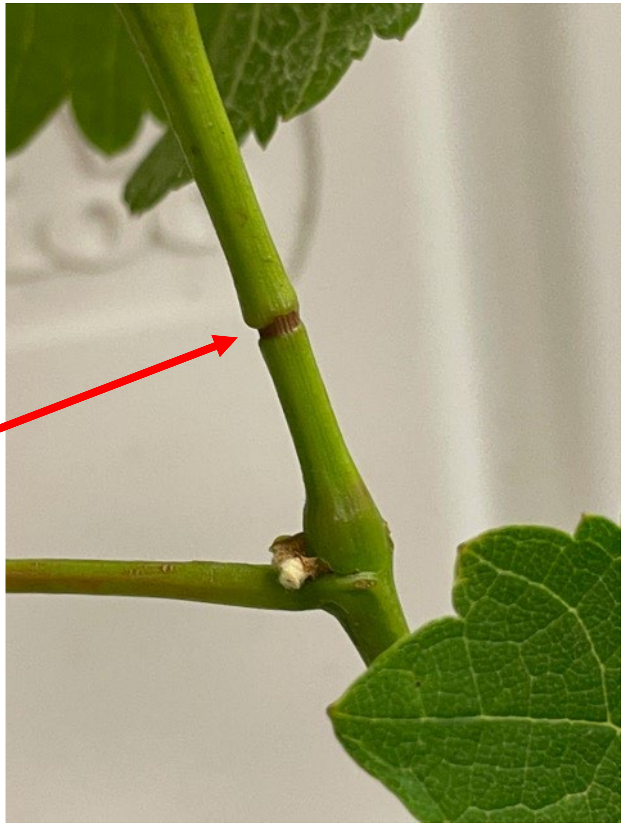
All are *Tortistilus albidosparsus*

Oviposition scar on oak

Tortistilus albidosparsus

- Horned and hornless morphotypes
- One generation a year
- Overwinter as eggs in woody host (e.g. oaks)
- Girdles similar to those of TCAH

Necrotic girdle



Swollen girdle

Artificial Transmission System

Kahl et al., 2021

"An inherent feature of the ATS is its elimination of plant-specific barriers to transmission, which should result in higher rates of transmission than one might expect in a greenhouse or in nature."

ATS uses a microcapillary tube with sucrose solution behind a membrane in lid of the tube.

A

B

Insects collected from vineyards in British Columbia

Kahl et al. (2021) Identification of candidate insect vectors of *Grapevine red blotch virus* by means of an artificial feeding diet. Canadian Journal of Plant Pathology. 43: 905-913
doi.org/10.1080/07060661.2021.1930174

Artificial Transmission System

Kahl et al., 2021

Taxonomically identified species	Total ID'd	PCR +ves
Campylenchia rugosa	21	0
Stictocephala basalis	1	**1**
Stictocephala bisonia	58	**8**
Stictocephala wickhami	1	0
Stictocephala brevitylus	1	0

- Both *Stictocephala* and *Spissistilus* are members of the treehopper tribe Ceresini
- Other insects evaluated (includes 32 leafhopper species, 5 sharpshooters and 2 aphids) did not transmit virus by ATS
- *Tortistilus* are also members of the treehopper tribe Ceresini

Table 1. Insects collected for use in the artificial transmission system (ATS), taxonomic IDs of a subset of collected insects, and ATS PCR results.

Generic name	Superfamily, Family	Total assessed in ATS	Taxonomically identified species	Total ID'd	PCR +ves
Treehopper	Membracoidea, Membracidae	82	*Campylenchia rugosa*	21	0
			Stictocephala basalis	1	**1**
			Stictocephala bisonia	58	**8**
			Stictocephala wickhami	1	0
			Stictocephala brevitylus	1	0
Leafhopper	Membracoidea, Cicadellidae	238	*Amblysellus grex*	7	0
			Athysanus argentarius	1	0
			Ceratagallia sp.	15	0
			Ceratagallia siccifolia	2	0
			Chlorotettix unicolour	1	0
			Colladonus montanus	1	0
			Colladonus reductus	6	0
			Deltocephalinae sp.	2	0
			Dikraneura absenta	3	0
			Doratura stylata	1	0
			Elymana inornata	1	0
			Empoasca sp.	1	0
			Endria inimicus	1	0
			Erythroneura ziczac	2	0
			Euscelidius sp.	14	0
			Euscelidius maculipennis	11	0
			Euscelidius variegatus	8	0
			Exitianus exitiosus	8	0
			Gyponana sp.	1	0
			Gyponana hasta	2	0
			Hebecephalus occidentalis	1	0
			Kybos exiguae	1	0
			Macrosteles quadrilineatus	5	0
			Muirodelphax arvensis	1	0
			Neocoelidia lineata	4	0
			Osbornellus borealis	1	0
			Psammotettix	7	0
			Psammotettix alienus	3	0
			Psammotettix lividellus	2	0
			Scaphytopius sp.	2	0
			Scaphytopius acutus	4	0
			Xerophloea peltata	6	0
Sharpshooter	Membracoidea, Cicadellidae	36	*Graphocephala confluens*	4	0
Froghopper	Cercopoidea, Cercopidae	35	*Aphrodes* sp.	1	0
			Athysanus argentarius	1	0
			Graphocephala hieroglyphica	1	0
			Philaenus spumarius	21	0
Aphid	Aphidoidea, Aphididae	2	*Acyrthosiphon pisum*	1	0
Not ID'd		123		0	0

Spissistilus festinus

Caged on GRBV+ vines in lab for 10 days for use in transmission assays.

GRBV+ source grapevine

Spissistilus festinus, 2022
ATS validation

4-day ATS directly following 10-day AAP on a GRBV+ vine, or after IAP on cowpea or alfalfa

AAP 10 days	IAP 6 days	IAP 4 days	GRBV+	GRBV?	GRBV-	Total TCAH
GRBV+		ATS	5	0	5	10
GRBV+		ATS	3	0	11	14
GRBV+	Alfalfa	ATS	3	0	7	10
GRBV+	Cowpea	ATS	3	2	10	15

Tortistilus albidosparsus

Collected from GRBV-infected vines in Pope Valley.

Caged on vines in field for 8 days and transferred directly to lab for use in transmission assays.

ATS

Bean plant

GRBV - grapevine

Tortistilus albidosparsus, 2022

ATS results

Tortistilus 8-day (caged) and 0-day (not caged) field AAP with 4-day ATS

AAP days	GRBV+	GRBV?	GRBV-	Total TCAH
Caged - 8	4	1	9	14
No cage - 0	2	3	7	12

Tortistilus albidosparsus, 2022
ATS results

8-day (caged) and 0-day (not caged) field AAP with 5-day IAP on GRBV- grapevine then 6-day ATS

AAP days	GRBV+	GRBV?	GRBV-	Total TCAH
Caged - 8	4	3	8	15
No cage - 0	5	1	13	19

Tortistilus albidosparsus, 2022
ATS results

Tortistilus 8-day field AAP and 5-day or 13-day lab supplemental lab AAP (13- or 21- days total) on either GRBV+ Pope Valley canes or GRBV+ Plymouth potted vines with 3-day IAP on GRBV- grapevine and 3-day ATS

Source Vine	GRBV+	GRBV?	GRBV-	Total TCAH
Pope Valley (13 d)	5	2	6	13
PV + Plymouth (13 d)	6	0	4	10
PV + Plymouth (21 d)	5	0	2	7

Tortistilus albidosparsus, 2022

Salivary glands results

Tortistilus 8-day field AAP and 5-day lab supplemental lab AAP (13-days total) on either GRBV+ Pope Valley canes or GRBV+ Plymouth potted vines with 3-day IAP on GRBV- grapevine and 3-day ATS

Source Vine	GRBV+	GRBV?	GRBV-	Total TCAH
Pope Valley	5	3	4	12
PV + Plymouth	9	1	0	10

Tortistilus albidosparsus studies in 2023

Validate and refine 2022 studies of GRBV transmission using an artificial transmission system and a factitious host

- Conducted transmission studies by caging uninfected nymphs collected on vetch outside of an infected vineyard on GRBV- grapevines in an untreated experimental vineyard, rearing them to adults, then caging them on GRBV+ grapevines in a commercial vineyard for AAP
- AAP of 7, 15, 24 and 33 days on the GRBV+ vines; control adults remained on GRBV- vines
- IAP of 5 days using artificial transmission system (ATS) or cowpea
- Dissected salivary glands from each insect
- qPCR assay results pending

Tortistilus albidosparsus studies in 2023

Will Tortistilus nymphs acquire GRBV and will the resulting adults transmit GRBV

- Conducted transmission studies by caging uninfected nymphs collected on vetch outside of an infected vineyard on GRBV- grapevines in an experimental vineyard, then caging them on potted GRVB+ grapevines or GRBV+ grapevines in a vineyard for a 10-day AAP
- Returned to GRBV- grapevines and allowed to mature to adults
- IAP of 4 days using artificial transmission system (ATS)
- Dissected salivary glands from each insect
- qPCR assay results pending

Avg. Ct of grape leaves on 5/15/23 = 22.9

Tortistilus albidosparsus studies in 2023

Tortistilus biology

- Adult movement into vineyards
- Can *Tortistilus* utilize grapevines as a reproductive host?

Monitored adult *Tortistilus* with yellow sticky cards on fence lines between vetch patches and vineyard, and on trellis wires

- 3 vineyard blocks, 10 traps per block, then calculated average number per trap per day

Caged adult *Tortistilus* in mesh sleeve cages tied at base of canes and recorded oviposition scars

- 2 males and 2 females per cage
- Insects fed on GRBV+ or GRBV- grapevines
- Monitored and recorded adult mortality
- Determined where oviposition occurs on cane

Tortistilus overwinter as eggs on woody hosts

One generation a year
Oak is a common host

May 15, 2023

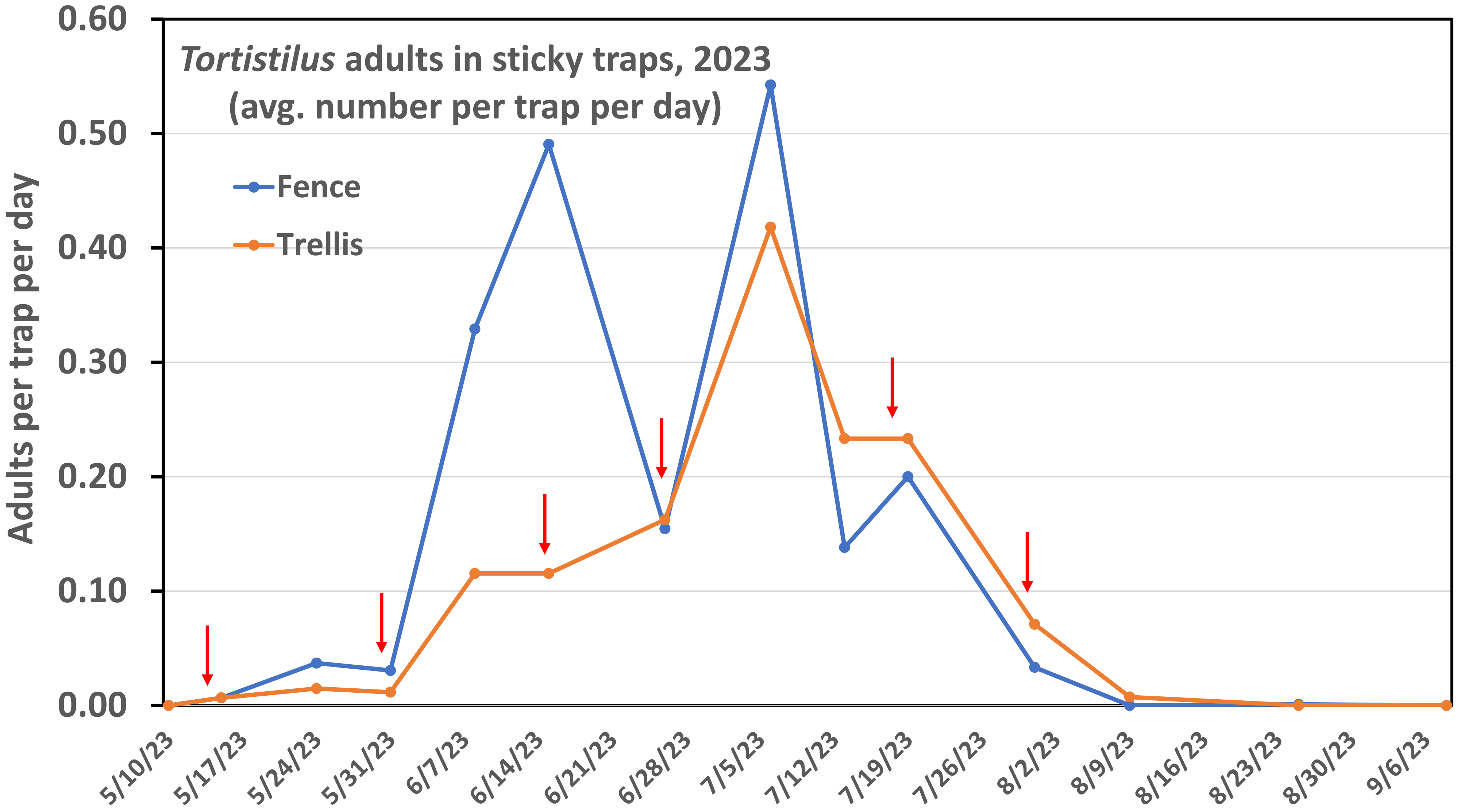
Like many other treehoppers, *Tortistilus* nymphs develop on an alternate host – especially legumes, such as vetch.

Vetch in Oak Woodland Adjacent to Vineyard

May 15, 2023
May 31, 2023
June 15, 2023
June 26, 2023
July 19, 2023
July 31, 2023

Vetch in Oak Woodland Adjacent to Vineyard

Tortistilus adults captured in yellow sticky traps
3 vineyard blocks, 10 traps per block, avg number per trap, 30 traps total



Tortistilus can oviposit on grape canes

GRBV+ 0.59 scars per cane; 19 canes
82%of scars within two buds of base

GRBV- 0.33 scars per cane; 20 canes
69% of scars within two buds of base

NASEM Grapevine Viruses Committee Meeting
March 4, 2024
Davis, CA

Grapevine Red Blotch Virus and Vectors

Acknowledgements

Cindy Preto Kron, Brian Bahder, Michael Bollinger, Emily Bick, Nicole Nicola
Cristian Olaya Arias, Kishore Reddy, Hazel Scully, Teresa Erickson,
Lucia Varela, Rhonda Smith, Lynn Wunderlich
Dennis Kopp

The work presented was funded by grants and agreements from:

CDFA/SCBGP #SCB14051
CDFA PD/GWSS Program #18-0398-000-SA
CDFA PD/GWSS Program #23-0379-000-SA
California Grape Rootstock Commission
USDA-NIFA-SCRI #2019-51181-30020
USDA-ARS NP303 National Program