Red Blotch Vectors

Buffalo treehopper
Stictocephala bisonia
(not confirmed)

Threecornered alfalfa treehopper
Spissistilus festinus
(confirmed)

Tortistilus albidosparsus
(not confirmed)

Doug Walsh, Entomologist

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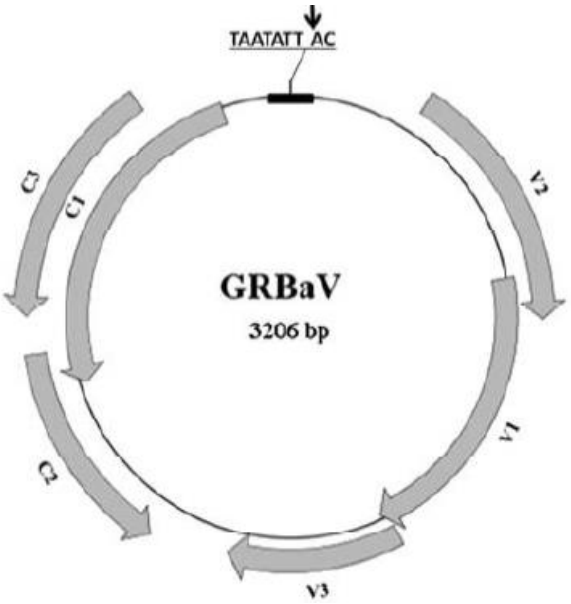
Special thanks to:
Frank Zalom, UC Davis & Vaughn Walton, Oregon State University

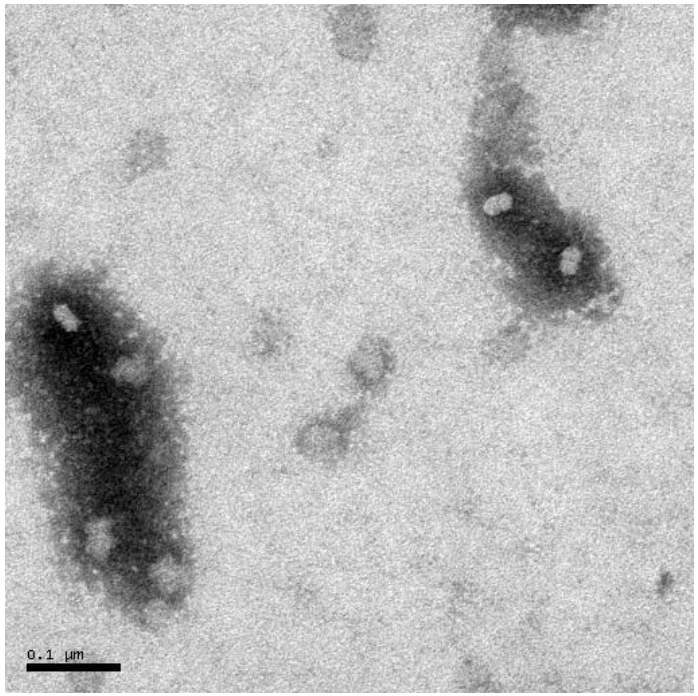
Background of GRBaV

- 2008: Leafroll-like symptoms didn’t fit exactly, investigations began
- 2011-2013: Novel virus discovered and genome sequenced independently at Cornell and UC Davis
- 2014: Data demonstrates spread occurring in CA vineyard
- 2014 – 2018: Vectors investigated in California, Oregon, California, Washington State, and New York.

Grapevine Red Blotch-associated Virus (GRBaV)

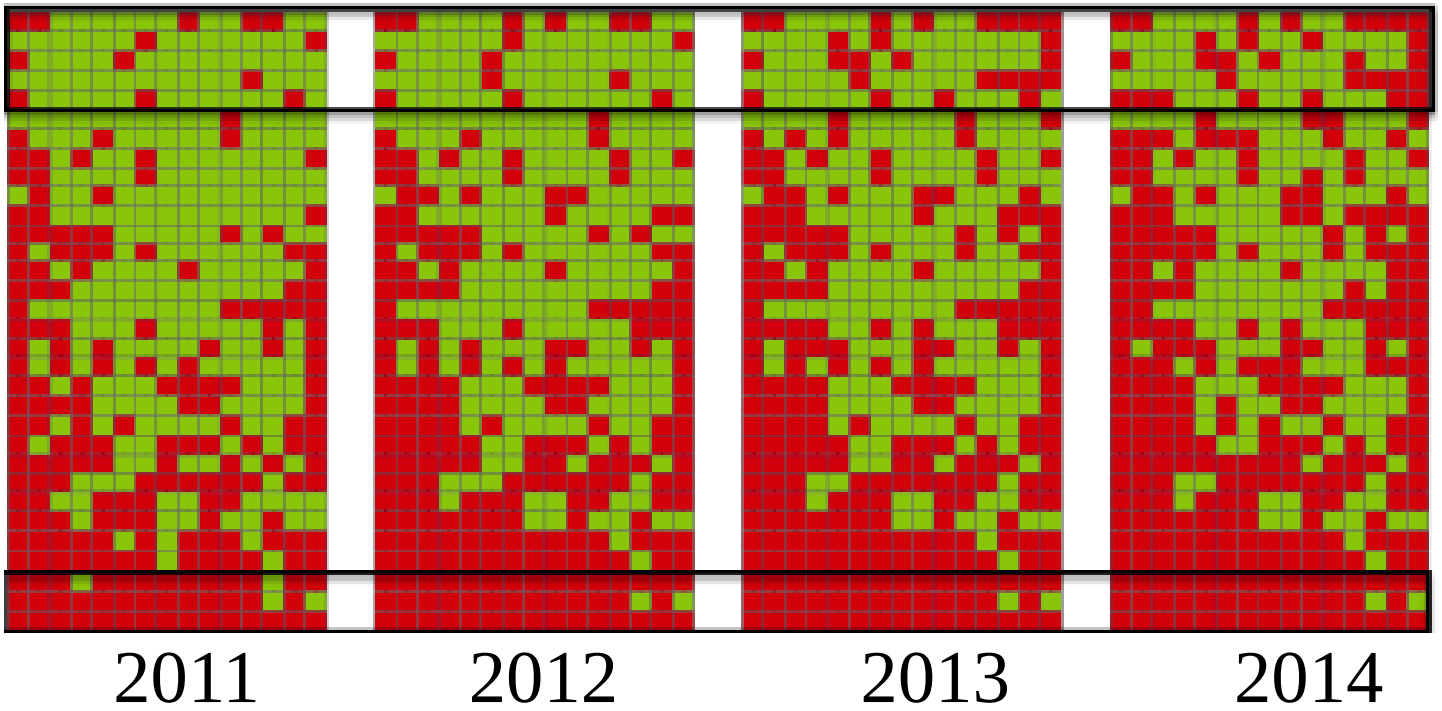
- Genome: circular, ssDNA
- Belongs to the Geminiviridae
- Causal agent of grapevine red blotch disease (GRBD)





Distribution and Spread of GVRBaV

- Widespread in North America, present in all major grape growing regions
- Spreading rapidly in CA 2011-2018



Detail of rapid spread of GRBaV in UC research vineyard in the Napa Valley. This vineyard was pulled in 2018 due to disease

Membracid vectors of grapevine red blotch-associated virus

WSU alumni Brian Bahder determined that the threecornered alfalfa treehopper was a key vector of GRBaV in California.

There are no state records for this insect in Washington State. (I'm the alfalfa entomologist too.)

Transmission Assays of Insects Commonly found in California Vineyards in 2015

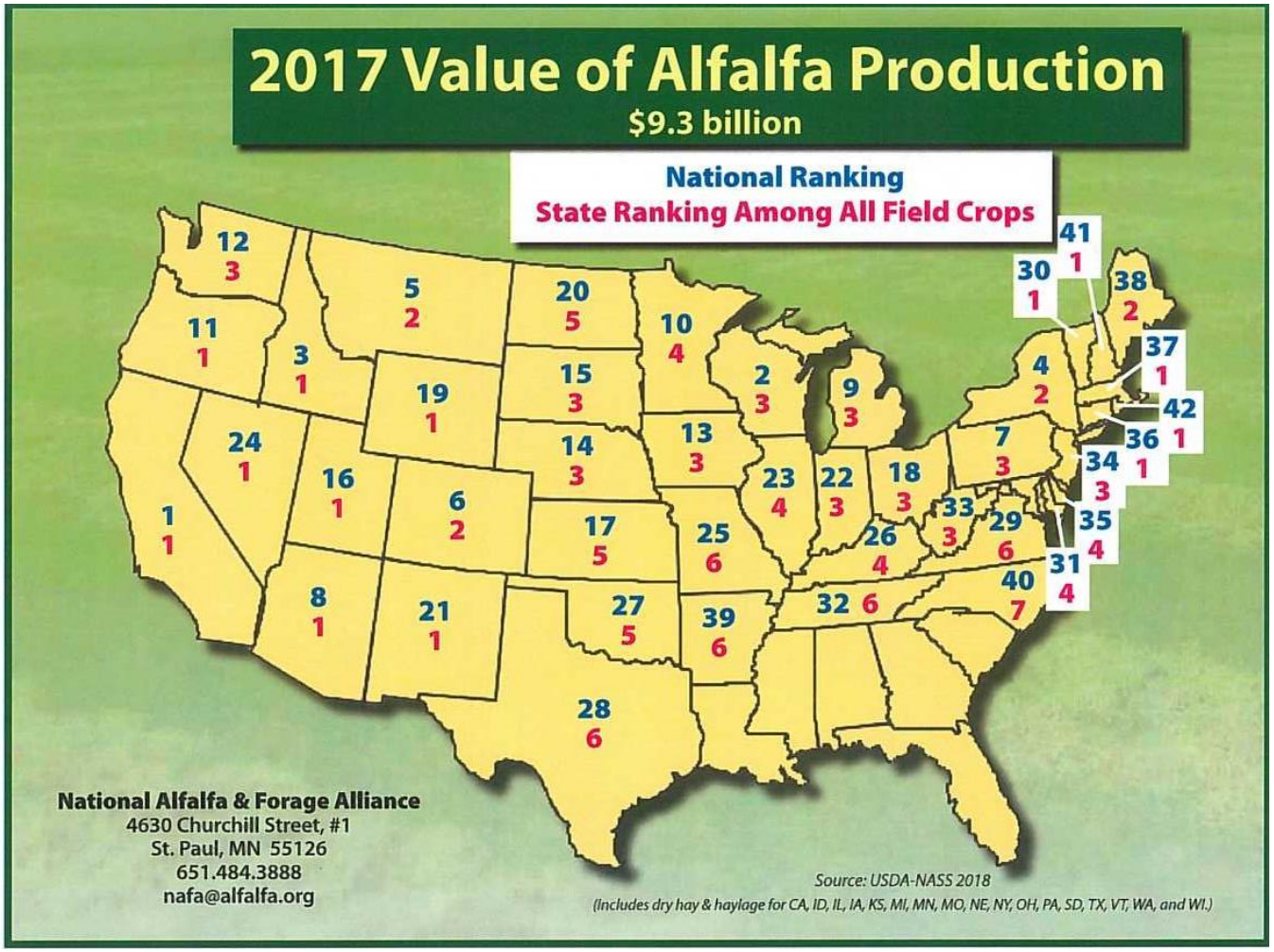
Species	9-month post-inoculation
Erythroneura elegantula	0/15
E. variabilis	0/15
E. ziczac	0/15
Spissistilus festinus	14/30
Bactericerca cockerelli	0/10
Scaphytopius acutus	0/20
Melanoliarus sp.	0/5
Delphacidae	0/10

Spissistilus festinus as a Vector

- Native species to North America
  - Present in the southern United States and California
  - No state records in WA
  - In OR present in the Rogue River Valley. Not Present in the Willamette Valley
- Historically a minor pest in grapes
- Research is ongoing to understand the biology of this species in CA to aid in management strategies
- In the Southeast US this insect is a pest in soybeans and peanuts.

Threecornered alfalfa treehopper

California is the largest cheese producing state in the US
Alfalfa makes up 2/3 of the dietary rations of a lactating cow



There are 1,000,000 acres of alfalfa grown in California
Threecornered alfalfa treehopper is present in all these fields.

Genetically engineered alfalfa

In 2012 over 32% of the “roadside feral” alfalfa patches sampled in Fresno County, California tested positive for the Round up Ready™ transgene. Walla Walla County was 9%. It is likely much greater greater by now.

(Note that this roadside was sprayed with round-up!)

Alternate hosts:

- *Vitis californica*, with common names California wild grape, Northern California grape, and Pacific grape, is a wild grape species widespread across much of California as well as southwestern Oregon.
- Brian Bahder found GRBaV in *V. californica* in the foothills in and around Sonoma and Napa Valleys

Ironically this is a photo from a commercial ornamental nursery in California.
These plants were for sale.
5 gal pot is $26.99
1 gal pot is $8.99

Vitis riparia

- There are reports of isolated populations in the northwestern USA, but these are probably naturalized and not native.

Vitis labrusca aka Concords

- Virologists in New York are presently determining the status of GRBaV in Concords.
- Stay tuned

California Group

BIOLOGY AND ROLE OF TREEHOPPERS IN GRAPEVINE RED BLOTCH DISEASE

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Project Objectives

1. Monitor the population dynamics of 3CAH in vineyards and surrounding landscapes over the season.
2. Conduct GRBV transmission studies using treehoppers collected from vineyards with red blotch disease, and detect GRBV in the salivary glands of insects collected. Monitor field transmission by 3CAH.
3. Determine transmission efficiency of 3CAH to identify virus acquisition periods and persistence in the insect.
4. Evaluate the role of cover crops on the 3CAHs in vineyards.
5. Determine status of common weed and cover crops as feeding and reproductive hosts for 3CAH.

Seasonal Phenology in Napa

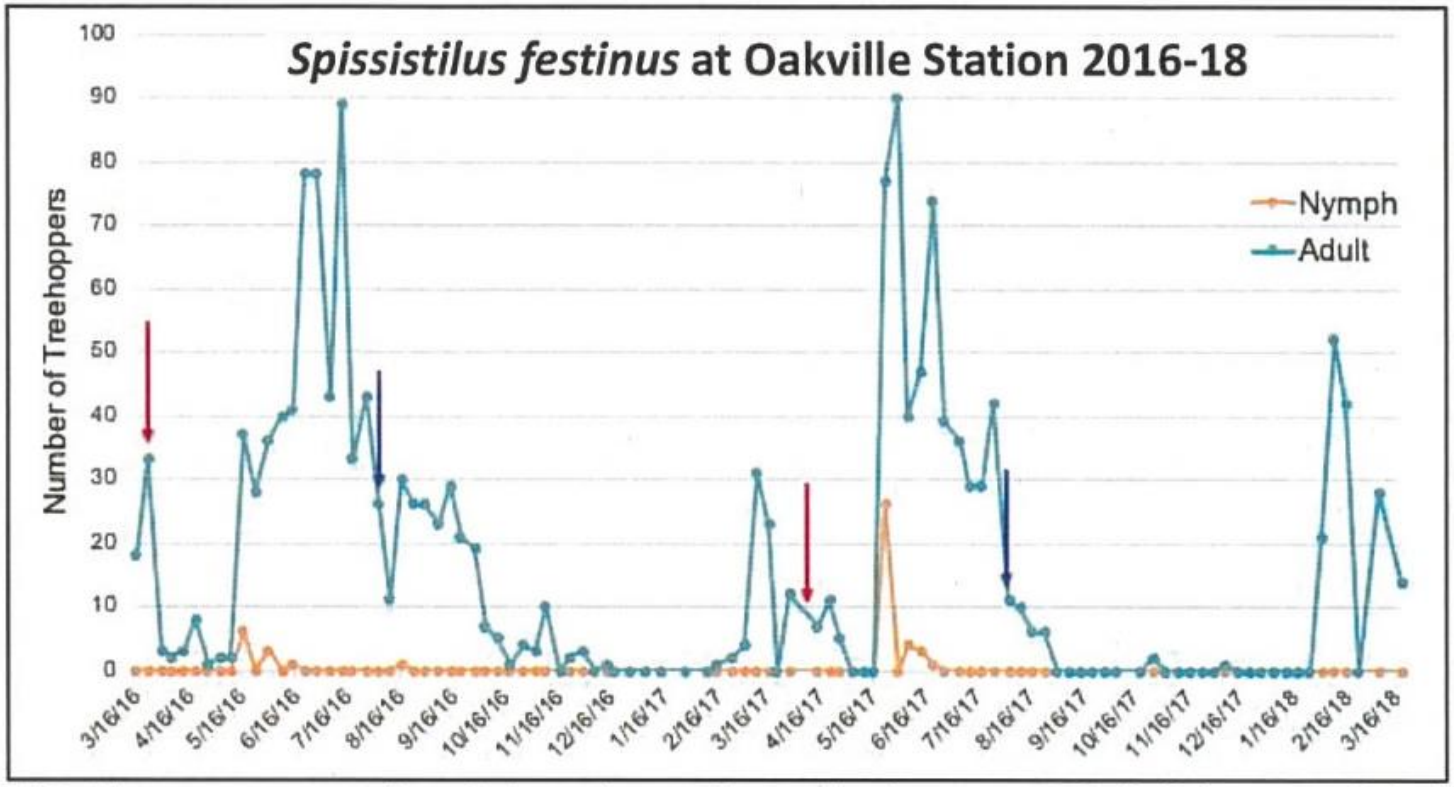

Figure 1. Weekly sweep net sampling of vineyard ground cover for three-cornered alfalfa hopper at Oakville, 2016-2018. Red arrows indicate bud break and purple arrows indicate time when ground cover was completely dried.

Adults invade vineyards in late winter and lay eggs in grape twigs. Eggs hatch in mid May and the threecornered alfalfa treehopper nymphs feed on weeds on the vineyard floor. Adults migrate out of the vineyard when it dries down in August.

Salivary glands were extracted from the 3CAH collected at the Oakville vineyard to test for presence of GRBV biweekly beginning March 3, 2017, just prior to bud break, and throughout the season. A total of 96 usable samples were collected. Salivary glands from 3CAH reared from eggs were dissected on each collection date, and these served as negative controls. The salivary glands were removed, placed in 180uL ATL and 20uL proteinase K incubated 4 h at 56°C, and stored in a -80°C freezer until they were analyzed by qPCR for GRBV detection in February 2018. None of the salivary glands tested positive for GRBV.

Figure 3. Three-cornered alfalfa hopper salivary gland dissections showing salivary glands within head capsule (left) and removed from head capsule (right).

Transmission assays with Tortistilus spp.

- This California group has attempted several methods for transmitting GRBaV in small single vine cages with 1 insect, 8 vine cages with multiple insects, and in field caged vines in 2017.
- These tests have been inconclusive.
- Studies were repeated in 2018. They are waiting for results.

Alternative weed and cover crop hosts for *S. festinus* in California
No choice tests

Weeds common in California vineyards as well as some managed cover crop plants were greenhouse grown.

Three adult males and 3 adult females were placed in each cage.

The cages were opened weekly to confirm adult survival, girdling, and nymph hatch.

Purple vetch was used as the “positive control”.

Table 1: Weed species tested as feeding and reproductive hosts for *Spissistilus festinus*.

Scientific Name	Common Name	Family	Girdles	Nymphs	% Survival[a]
Acmispon americanus	Spanish clover	Fabaceae	Yes	Yes	92
Taraxacum officinale	Dandelion	Asteraceae	Yes	Yes	71
Lotus corniculatus	Birdsfoot trefoil	Fabaceae	No	Yes	58
Poa pratensis	Kentucky bluegrass	Poaceae	Yes	No	25
Senecio vulgaris	Common groundsel	Asteraceae	Yes	Yes	21
Plantago lanceolata	Buckhorn plantain	Plantaginaceae	No	No	8
Daucus carota	Wild carrot	Apiaceae	Yes	No	4
Convolvulus arvensis	Field bindweed	Convolvulaceae	Yes	Yes	4
Kickxia elatine	Sharppoint fluvellin	Plantaginaceae	No	No	0
Cynodon dactylon	Bermuda grass	Poaceae	No	No	0

[a] Survival of adults for first 2 weeks on plants

Table 2: Cover crop species tested as feeding and reproductive hosts for *Spissistilus festinus*.

Scientific Name	Common Name	Family	Girdles	Nymphs	% Survival[a]
Pisum sativum	Magnus Peas	Fabaceae	Yes	Yes	92
Vicia faba	Bell beans	Fabaceae	No	Yes	83
Bromus hordeaceus	Blando brome	Poaceae	Yes	Yes	33
Vicia benghalensis	Purple vetch	Fabaceae	Yes	Yes	30
Medicago lupulina	Black medick	Fabaceae	Yes	Yes	25
Trifolium subterraneum	Subterranean clover	Fabaceae	Yes	Yes	17
Trifolium incarnatum	Crimson clover	Fabaceae	Yes	Yes	13
Vicia villosa ssp. varia	Woollypod vetch	Fabaceae	Yes	Yes	13
Brassica sp.	Mustard	Brassicaceae	No	No	0
Avena sativa	California red oats	Poaceae	No	No	0

[a] Survival of adults for first 2 weeks on plants

Choice tests were completed with the plants that were considered host plants as determined by the no choice tests. Four “host plants” and a purple vetch plant were placed in cages. 10 adult male and 10 adult female *S. festinus* were put in each cage. The insects were removed after 1 week.

Nymphs emerging from each plant were counted at weeks 2, 3, and 4.

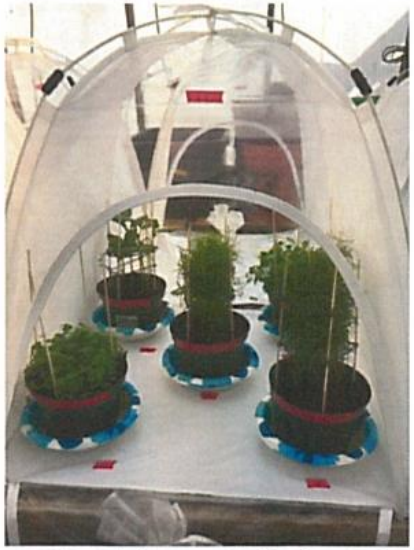

Figure 10. Four reproductive hosts plus purple vetch as a standard caged with three cornered alfalfa hoppers in a greenhouse preference study at UC Davis.

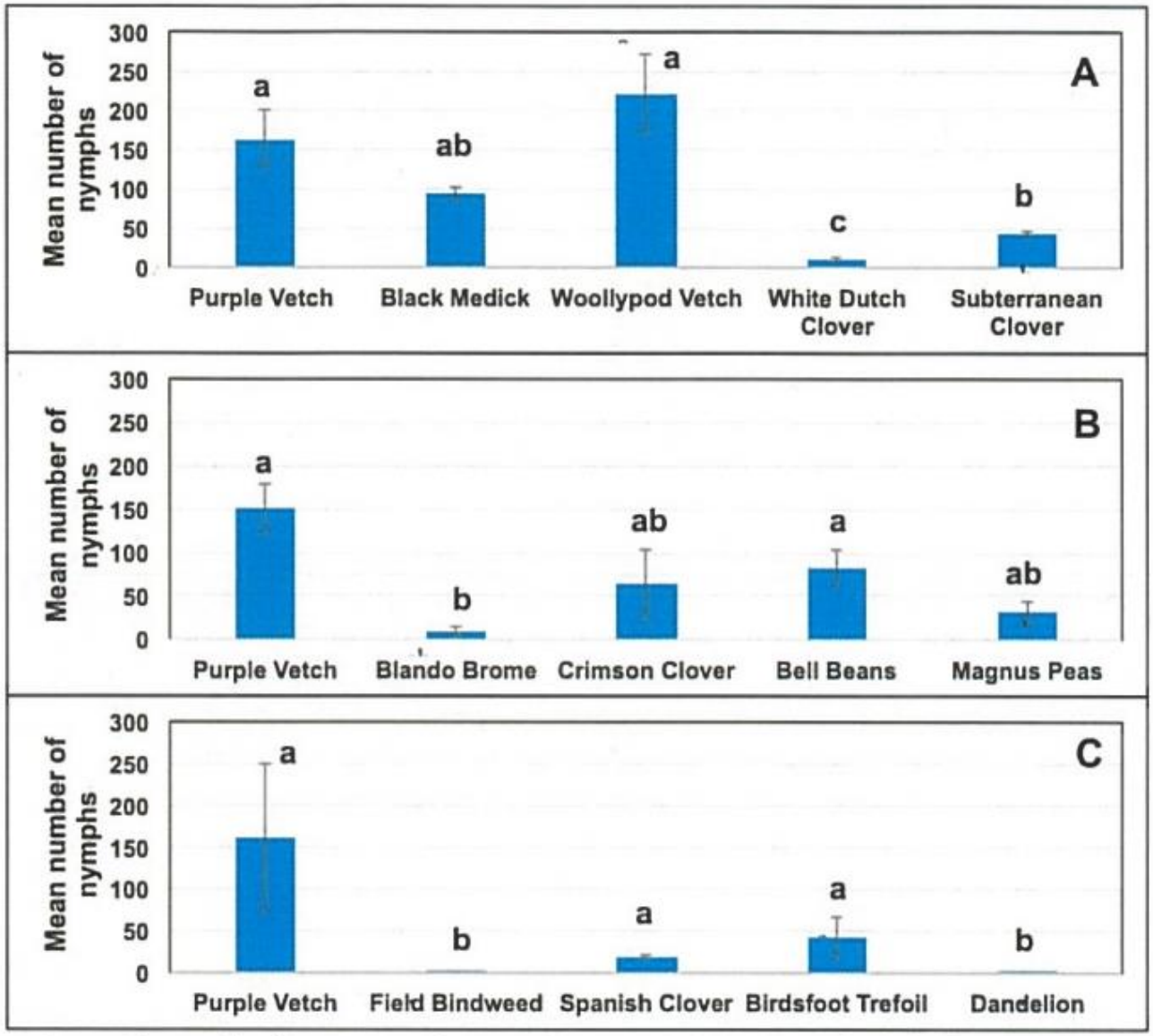

Figure 11. Results of post-hoc pairwise comparisons of mean number (±SEM) of *Spissistilus festinus* nymphs emerging from A) cover crop - group 1 (purple vetch, black medick, woollypod vetch, white Dutch clover, subterranean clover), B) cover crop - group 2 (purple vetch, blando brome, crimson clover, bell beans, magnus peas) and C) weeds (purple vetch, field bindweed, Spanish clover, birdsfoot trefoil, dandelion). Means followed by the same letter are not significantly different (Tukey HSD test, $P \leq 0.05$).

The Oregon Group

- Vaughn Walton, Professor, Entomologist, Department of Horticulture, OSU
- Achala N KC, Assistant Professor, Southern Oregon Research and Extension Center
- Rick Hilton, Faculty Research Assistant, Oregon State University, Southern Oregon Research & Extension Center
- Clive Kaiser, Assoc. Prof., Oregon State University, Umatilla County Extension Service,

- Unfortunately scientists in fall 2016 observed substantial spread of GRBaV in the Willamette Valley in the absence of *S. festinus* (Threecornered alfalfa treehopper).

Oregon Objectives

- 1. Follow insect vector distribution, and disease progression in relation to management.
- 2. Conduct controlled transmission biology experiments.
- 3. Obtain baseline information on current levels and extent of Red Blotch.
- 4. Extension of information of grapevine red blotch-associated virus, and insect vectors.

Insect Surveys

T. wickhami, mostly in Southern Oregon

Insect Surveys

T. albidosparsus, mostly in Willamette Valley

Three-cornered alfalfa hopper
Spissistilus festinus (Say)

K. Garvey, UC Davis

Table 1. Treehopper collection method and species from AV (southern Oregon) during 2017 and from nine southern Oregon sites in 2018.

	2017			2018		
Sampling method	***S. festinus***	***T. albidosparsus***	***T. wickhami***	***S. festinus***	***T. albidosparsus***	***T. wickhami***
Hand	0	20	3	1	74	340
Sweep	0	51	3	33	53	28
Vacuum	2	1	0			
Beating	0	0	2			
Sticky cards	0	52	2	0	0	76
Total	**2**	**124**	**10**	**34**	**127**	**444**

Pinned specimens of *T. albidosparsus* (horned) and *T. wickhami* (non-horned) captured and pinned in southern Oregon.

In a California study 2 morphs of both "species" (brown horned, brown unhorned, green horned and green unhorned) were subjected to shotgun DNA sequencing. Collectively their DNA varied <2%. Sudarshana et al. (in press) recommended all morphs be designated as T. albidosparsus.

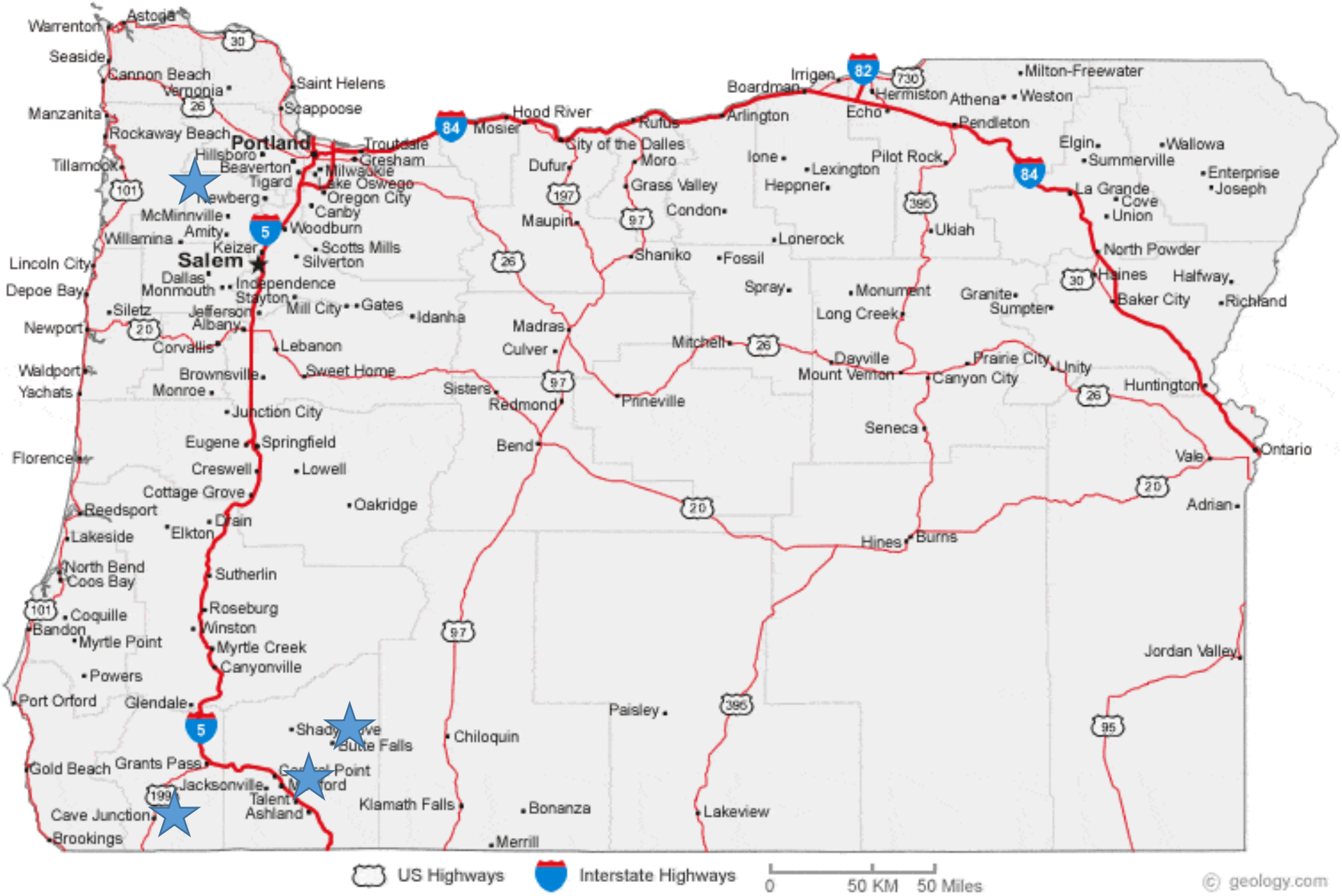
Warrenton
Astoria
Seaside
Cannon Beach
Vernonia
Saint Helens
Scappoose
Manzanita
Rockaway Beach
Portland
Hillsboro
Beaverton
Tigard
Tillamook
Newberg
Milwaukie
Lake Oswego
Oregon City
Canby
McMinnville
Amity
Woodburn
Willamina
Keizer
Scotts Mills
Lincoln City
Salem
Silverton
Dallas
Independence
Depoe Bay
Monmouth
Stayton
Siletz
Jefferson
Mill City
Gates
Newport
Albany
Idanha
Corvallis
Lebanon
Waldport
Brownsville
Sweet Home
Yachats
Monroe
Junction City
Eugene
Springfield
Florence
Creswell
Lowell
Cottage Grove
Reedsport
Oakridge
Drain
Lakeside
Elkton
North Bend
Coos Bay
Sutherlin
Coquille
Roseburg
Bandon
Winston
Myrtle Point
Myrtle Creek
Canyonville
Powers
Port Orford
Glendale
Shady Cove
Butte Falls
Gold Beach
Grants Pass
Central Point
Jacksonville
Medford
Talent
Ashland
Cave Junction
Brookings
Klamath Falls
Bonanza
Merrill
Chiloquin
Paisley
Lakeview
Jordan Valley
Mosier
Hood River
Troutdale
Gresham
City of the Dalles
Dufur
Moro
Grass Valley
Maupin
Condon
Shaniko
Fossil
Madras
Culver
Sisters
Redmond
Bend
Prineville
Mitchell
Spray
Monument
Long Creek
Dayville
Mount Vernon
Canyon City
Prairie City
Unity
Seneca
Hines
Burns
Rufus
Arlington
Boardman
Irrigon
Hermiston
Echo
Athena
Weston
Milton-Freewater
Pendleton
Pilot Rock
Ione
Lexington
Heppner
Ukiah
Lonerock
Elgin
Wallowa
Summerville
Enterprise
Joseph
La Grande
Cove
Union
North Powder
Haines
Halfway
Baker City
Richland
Granite
Sumpter
Huntington
Vale
Ontario
Adrian
US Highways
Interstate Highways
0
50 KM
50 Miles
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Table 1. Treehoppers species collected in key winegrape growing regions of Oregon in 2017.

Location	Eggs	Nymphs	*S. festinus*	*T. wickhami*	*T. albidosparsus*
AV	8	23	2	10	124
CJV	0	13	0	180	0
CRV	55	0	0	0	0
EPV	0	0	0	65	0
JV	0	0	11	1	0
TV	0	0	0	17	0
YV	129	289	0	0	58
Misc. sites	0	0	1 (SOREC sticky trap) 5 (alfalfa fields)	1 (Beat tray) 1 (SOU sticky trap)	1 (Sticky trap)
Total	192	325	19	275	183

Ashland (AV), Cave Junction (CJV), Oregon Coast Range (CRV), Eagle Point (EPV), Jacksonville (JV), Talent (TV), and Yamhill (YV)

Table 4. Woody hosts of *Tortistilus albidosparsus* eggs at study sites in YV and CRV (Willamette Valley) in 2017.

Site	Season	Species	Number buds	Number buds with eggs	Total eggs	% Buds with eggs
YV	spring	apple	1906	61	79	3.20%
YV	spring	white oak	533	14	15	2.63%
YV	spring	rose	458	7	8	1.53%
YV	spring	hawthorn	270	2	2	0.74%
YV	spring	plum	190	1	1	0.53%
YV	spring	grapevine	480	2	3	0.42%
YV	spring	thistle	8	0	0	0%
YV	fall	white oak	56	9	16	16.1%
YV	fall	grapevine	189	2	5	1.06%
YV	**2017**	**total**	**4090**	**98**	**129**	**2.40%**
CRV	fall	white oak	347	29	53	8.36%
CRV	fall	red oak	185	2	2	1.08%
CRV	fall	apple	213	0	0	0%
CRV	fall	cherry	231	0	0	0%
CRV	fall	plum	882	0	0	0%
CRV	fall	rose	81	0	0	0%
CRV	**2017**	**total**	**1939**	**31**	**55**	**1.60%**

In the spring collections (April through June) 3,845 buds from YV were examined under a microscope. Findings of eggs from the highest to lowest proportion were apple (highest), oak,

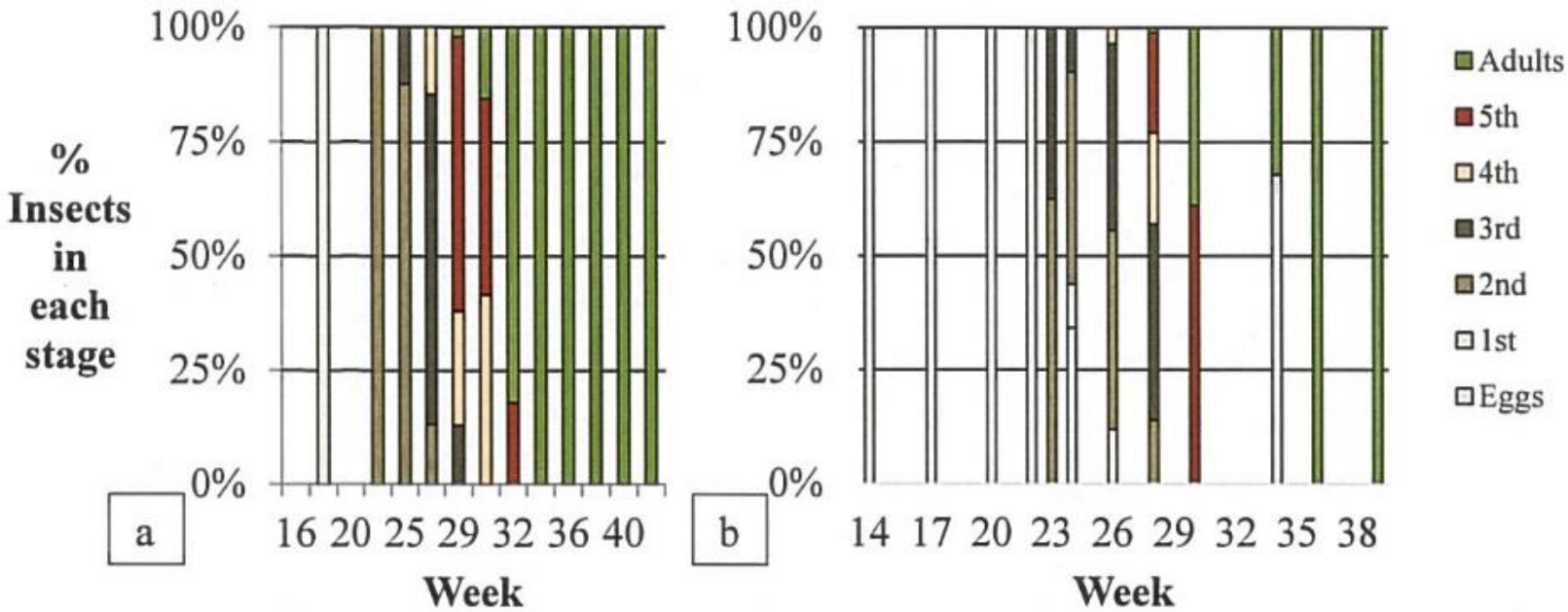
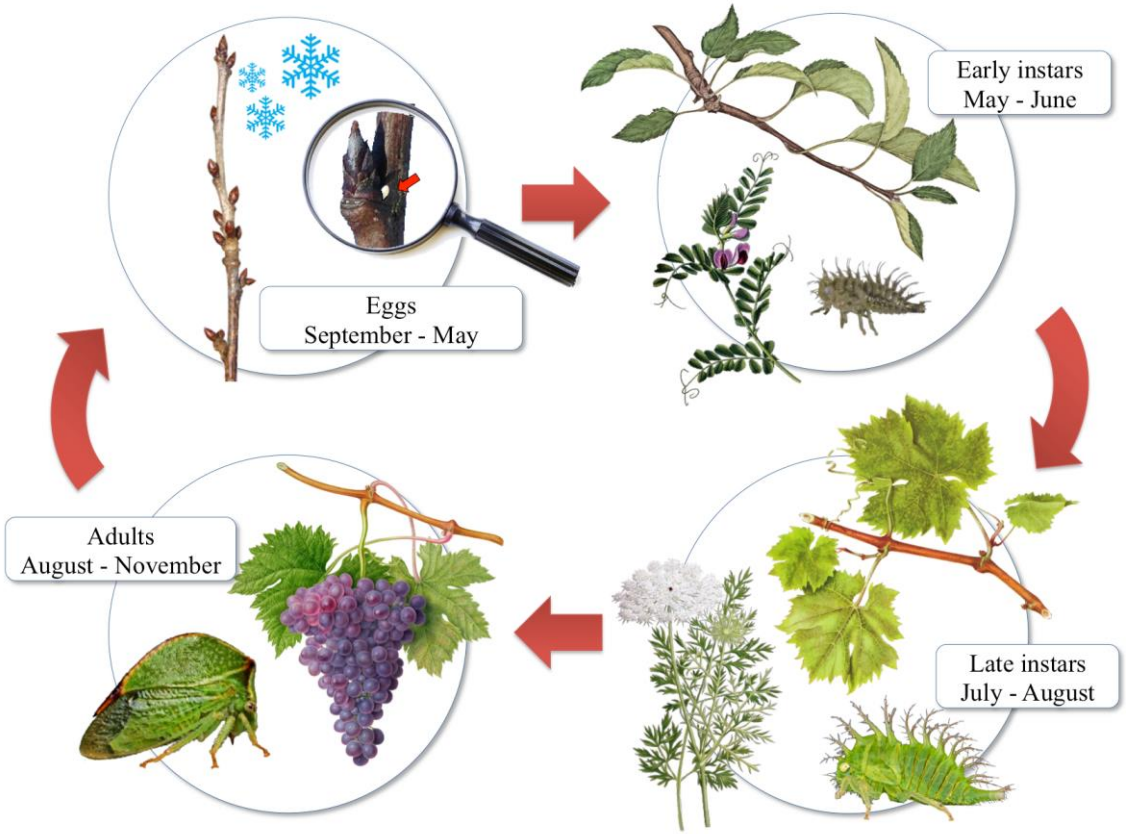


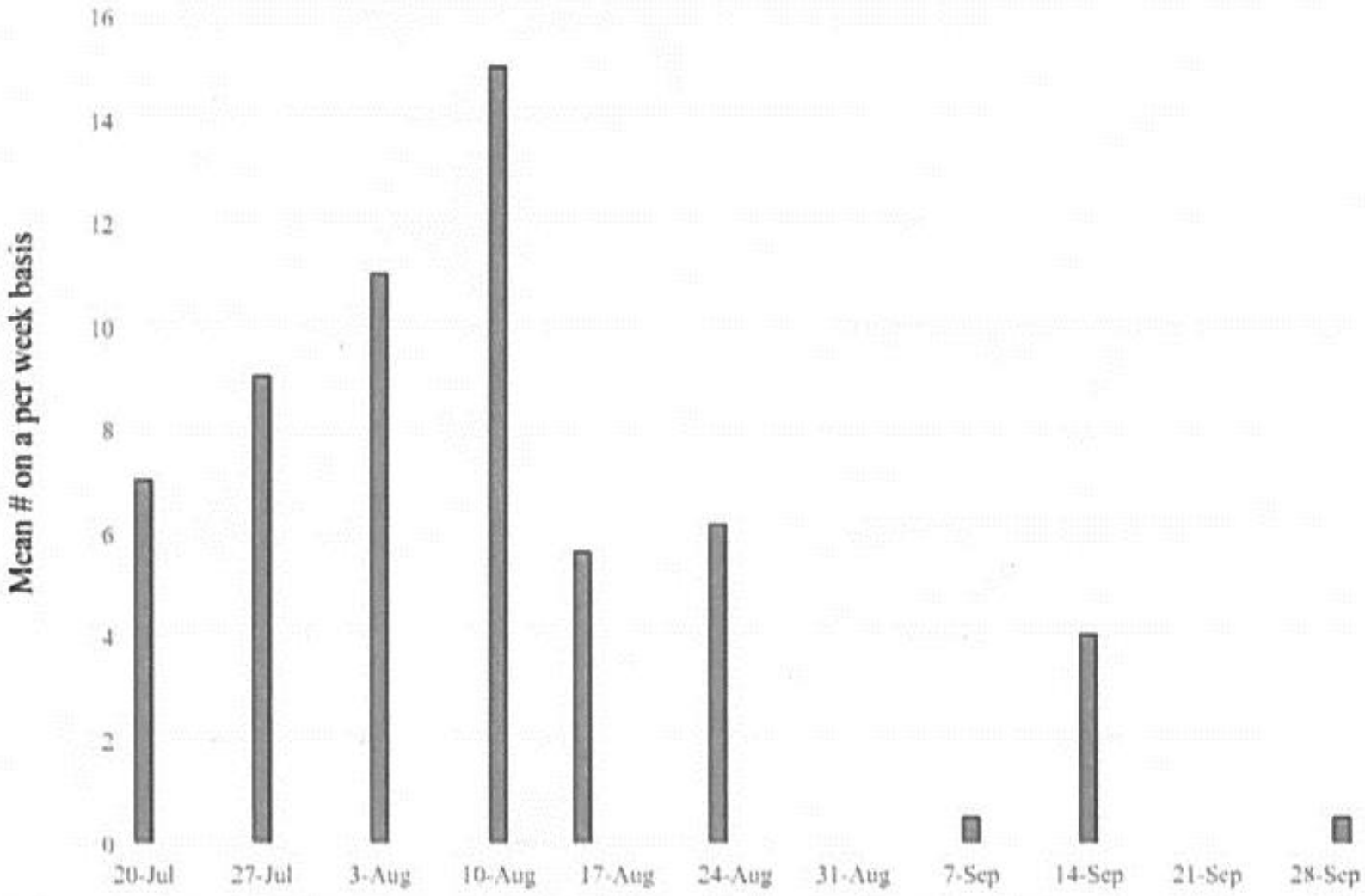
Figure 1. Proportion of *Tortistilus albidosparsus* life stages observed throughout the growing season at YV during 2017 (a) and during 2018 (b).

Week	2017 Date
16	April 1
20	May 13
25	July 16
29	August 13
32	September 3
36	October 1
40	October 29

Week	2017 Date
14	April 1
17	April 22
20	May 3
23	June 3
26	June 24
29	July 15
32	August 5
35	August 26
38	September 16

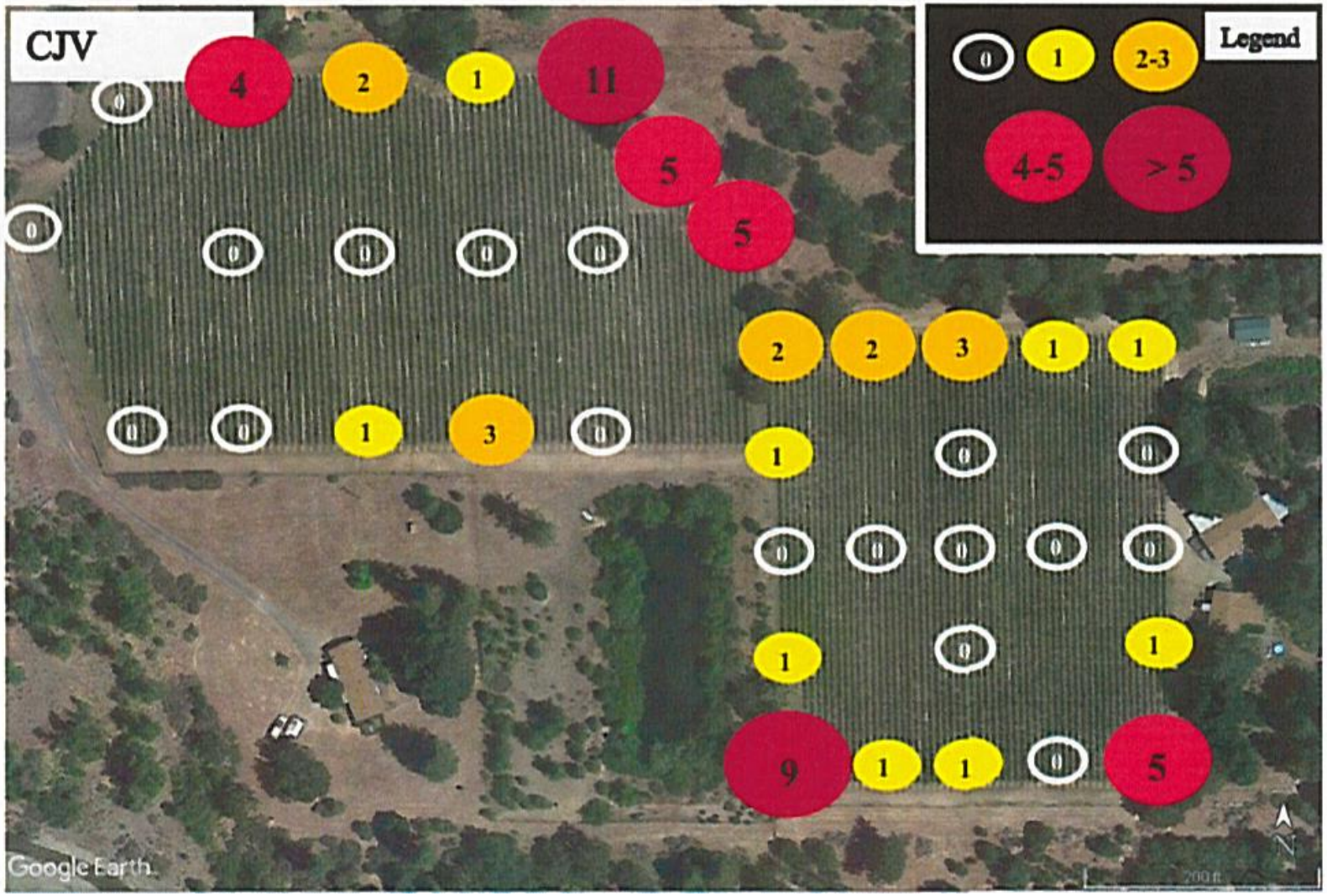


The seasonal lifecycle of *Tortistilus albidosparsus* as observed in the Willamette Valley. The insect overwinters in the egg stage hidden in dormant bud scales of suitable perennial host plants. In spring, the early instar emerges and drops onto suitable green herbaceous plant hosts. In mid-summer, the later instar migrates to drought-hardy herbaceous or woody host vegetation. In late summer, the adult emerges and mates, and eggs are laid into the buds of suitable perennial host plants in the fall.



Yellow sticky trap collections of *T. wickhami (albidosparsus)* found in CJV (Southern Oregon) during summer 2017

Placement of yellow rectangular sticky traps in CJV (Southern Oregon) during 2017.



Tortistilus wickhami (albidosparsus) collections in CJV (Southern Oregon) during 2017. Numbers within ovals show cumulative number of *T. wickhami* adults collected from corresponding yellow sticky card placement sites.
This obviously demonstrates a strong edge effect for *T. wickhami*

Membracids in Washington State.

- In 2017 and 2018 we completed a comprehensive survey of most AVAs in WA State
- Never captured *Spissistilus festinus* or *Tortistilus albidosparsus* in WA
- Found the buffalo treehopper (*Stictocephala bisonia*) in abundance.
- Did not find *S. bisonia* in any wine grape vineyards in either year.
- Most frequent detections in pear orchards, alfalfa patches, & riparian areas.
- Found *Palonica, (*possibly *P. pyramidata)* in one riparian location in 2017. (Yakima River). Willows are listed as a host plant.

Stictocephala bisonia
Bufallo Treehopper

Palonica spp.

Transmission Assays

- In 2018 we established a colony of Buffalo treehoppers.
- Transmission assays were completed in summer 2018
- We're in a wait and see mode and will complete PCR tests in spring 2019

All reports from Califonia and Oregon can be seen at the California Department of Food and Agriculture's Pierce's Disease website.

http://www.piercesdisease.org/reports/2018

Walsh funding has been provided by the Wine Research Advisory Committee and the Washington Commission on Pesticide Registration

Jonathan O'Hearn and Peter Forrence have completed the survey and transmission work in Washington State.