RENEWAL PROGRESS REPORT OF CDFA 17-0418-000-SA

Project Title: Integrative studies of vector-related field epidemiology for grapevine red blotch-associated virus. UGMVE proposal number,.

Principal Investigator/Cooperator(s):
Vaughn Walton, Professor, Horticultural Entomologist, Department of Horticulture, Oregon State University, Tel. (541) 740-4149, vaughn.walton@oregonstate.edu
Kent Daane, Extension Specialist, Environmental Science, Policy and Management, University of California, Berkeley, CA 94720, Tel. (559) 646-6522, kdaane@ucanr.edu
Daniel Todd Dalton, Graduate Research Assistant, Department of Horticulture, Oregon State University, Tel. (541) 737-3913, daniel.dalton@oregonstate.edu
Rick Hilton, Faculty Research Assistant, Oregon State University, Southern Oregon Research & Extension Center, 569 Hanley Rd, Central Point, OR 97502, Tel. (541) 772-5165, richard.hilton@oregonstate.edu
Achala N KC, Assistant Professor, Southern Oregon Research and Extension Center
569 Hanley Rd, Central Point, OR 97502, achala.kc@oregonstate.edu, Tel. (541) 772-5165
Clive Kaiser, Assoc. Prof., Oregon State University, Umatilla County Extension Service, 418 N Main St, Milton-Freewater, OR, 97862 Tel. (541) 938-5597, clive.kaiser@oregonstate.edu
Sudarshana Mysore, Plant Pathologist, USDA-ARS, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616, Tel. (530) 752-3621, mrsudarshana@ucdavis.edu
Frank Zalom, Professor, Dept. of Entomology and Nematology, Univ. of California, Davis, CA 95616, Tel: (530) 752-3687, fgzalom@ucdavis.edu

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Introduction

Grapevine virus diseases are of serious concern for vineyard managers and winemakers in all Western production regions. Grapevine leafroll-associated virus (GLRaV) and *Grapevine red blotch virus* (GRBV) impact grape berry quality. Berries of infected vines have lower °Brix at harvest (Al Rwahnih et al. 2013, Sudarshana et al. 2015), necessitating in removal of symptomatic vines from vineyards. GRBV is spreading; ecological mapping of GRBV-positive vines, as verified by qPCR during 2013-2016 (Dalton et al. *submitted*), showed a significant trend of virus increase over time in two of three areas studied in Oregon.

Objective(s) and Experiments Conducted to Meet Stated Objective(s):

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.

Description of activities conducted

1. Follow insect vector distribution, and disease progression in relation to management.

Follow insect vector distribution and incidence. In 2017, vineyards in Ashland (AV), Cave Junction (CJV), Oregon Coast Range (CRV), Eagle Point (EPV), Jacksonville (JV), Talent (TV), and Yamhill (YV) were surveyed for presence of treehoppers (Hemiptera: Membracidae). Additional sites where treehoppers were trapped included Southern Oregon University Sustainability Farm (SOU) and Southern Oregon Research and Extension Center (SOREC) (Table 1). Adults of three treehopper species: *Spissistilus festinus*, *Tortistilus wickhami*, and *T. albidosparsus* were found in Oregon vineyards and closely proximate orchards in 2017. The taxonomic identification for the two *Tortistilus* species is tentative. For the purposes of this report, all adult *Tortistilus* treehoppers without pronotal horns are considered to be *T. wickhami* and horned individuals are *T. albidosparsus*.

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

Several surveying techniques were used based on the time of season and host plant in order to improve collection efficiency (Tables 2, 3). The most effective methods during early season collection were microscopic examination of dormant tissues allowing determination of the presence of younger life stages (eggs and 1st instar nymphs), caging (young instars), visual surveys coupled with hand collection (2nd and 3rd instar nymphs, adults), vacuum sampling (4th and 5th instar nymphs), sweep netting (adults), and deploying sticky cards (adults). Beat sheeting was ineffective and only yielded three insects across all sites during 2017.

Table 2. Treehopper collection method and species from AV (Southern Oregon) during 2017.

Collection method	*S. festinus*	*T. wickhami*	*T. albidosparsus*
Hand-collection	0	3	20
Sweep netting	0	3	51
Vacuuming	2	0	1 (w/ D-vac)
Beat sheeting	0	2	0
Sticky cards	0	2	52

Table 3. Treehopper collection by host plant/crop in Southern Oregon and the Willamette Valley during 2017.

Region	Plant/Crop	Eggs in buds	Nymphs	*S. festinus*	*T. wickhami*	*T. albidosparsus*
Southern Oregon	Pear	0	11 from caged limbs 7 w/ Vortis vacuum	0	2 by beating 3 by sweep	48 by sweep
	Apple	8 (visual inspection)	4 w/ Vortis vacuum	0	1 in sticky trap	3 by sweep 20 in sticky trap
	Grape	----		2 w/ Vortis vacuum	61 in sticky traps 206 by hand	20 by hand 7 in sticky traps
	Cherry	----	----	0	0	24 in sticky trap
	Other	----	1 w/ Vortis vacuum	15 by sweep 1 (D-vac) 1 in sticky trap	1 by beating 1 in sticky trap	1 w/ D-vac 2 in sticky traps
Willamette Valley	Apple	79 (visual inspection)	15 (rearing and hand)	0	0	2 (hand)
	Blackberry	----	3 (D-vac)	0	0	8 (hand)
	Carrot	----	192 (hand and D-vac)	0	0	5 (hand)
	Grape	8 (visual inspection)	25 (hand and D-vac)	0	0	33 (hand)
	Hawthorn	2 (visual inspection)	1 (hand)	0	0	3 (hand)
	Oak	104 (visual inspection)	7 (hand and D-vac)	0	0	0
	Plum	1 (visual inspection)	0	0	0	3 (hand)
	Rose	8 (visual inspection)	4 (rearing and D-vac)	0	0	0
	Thistle	0	1 (D-vac)	0	0	0
	Undetermined	----	23 (D-vac)	0	0	4 (D-vac, hand)
	Vetch	----	18 (hand)	0	0	0

Seasonal observations of treehoppers in the Willamette Valley, Oregon. YV is a commercial vineyard in the Willamette Valley and was surveyed every fourteen days from spring through fall. Several vineyard blocks are at YV, ranging in age and size. The study area in 2017 was a block of Pinot noir grapevines and the adjacent surrounding habitat. To the west of the vineyard block was a mix of riparian habitat at the bottom of steep, heavily vegetated slopes. Riparian habitat was dominated by Oregon ash (*Fraxinus latifolia*) and wild blackberry (*Rubus armeniacus*).

Dominant woody species above the riparian areas included wild apple (*Malus domestica*), Oregon white oak (*Quercus garryana*), wild plum (*Prunus domestica*), wild blackberry, bigleaf maple (*Acer macrophyllum*) and hawthorn (*Crataegus* spp.). Minor species included wild rose (*Rosa* spp.), poison oak (*Toxicodendron diversilobum*), and wild hazelnut (*Corylus cornuta*). Herbaceous species in the adjacent habitat included wild carrot (*Daucus carota*), vetch (*Vicia* spp.), Canada thistle (*Cirsium arvense*) and unidentified grasses. Alleys between grapevine rows were maintained as wild-growing grass with occasional seedling blackberry and oak plants. Infrequent mowing and herbicide applications were used to control weeds. No irrigation or insecticide sprays were applied to the vineyard block. CRV is an experimental vineyard managed by Oregon State University and was surveyed in October. Habitat adjacent to a young planting of winegrapes (planted in 2015) was primarily grass, and 10-15 yards to the east and southeast of the block was a stand of oak trees (*Quercus* spp.) that contained rose and blackberry.

Tortistilus albidosparsus lays its eggs behind the bud scales of woody hosts (Yothers 1934). Collection of woody materials from study vineyards and surrounding habitat provided a reading of the percent of buds infested with treehopper eggs (Table 4).

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,

rose, hawthorn, plum, and grapevine (lowest). During fall 2017 bud collections at YV (245 examined buds), oak contained higher rates of eggs compared to grapevine. Overall, buds that did contain eggs hosted a single egg. At CRV, oak, heritage apple, cherry (*Prunus avium*) and plum trees were recorded growing 30 yards away from the vineyard edge and 5-50 yards from woody surrounding habitat. Here, rose, Oregon white oak, and red oak were the dominant species in the surrounding habitat. Upon microscopic examination of 1,939 buds collected in the fall from CRV, treehopper eggs were found only on samples of the two respective oak species. No other treehopper life stages were observed during the October field visit.

The YV site was surveyed repeatedly from spring through fall in order to track the phenological progression of treehopper life stages. A clear seasonal progression of the *T. albidosparsus* lifecycle was recorded (Fig. 1). Collected nymphs of all instar stages developed into adult *T. albidosparsus* in the laboratory. First instar treehopper nymphs emerged from apple wood cuttings (n=12) and rose cuttings (n=1) held in a walk-in cold room (44 ºF) between 5 May and 18 May. The first field-collected 2nd instar nymphs were discovered on apple (n=3) and oak (n=3) on 2 June; after this date 2nd instar nymphs were found on vetch (n=9), wild carrot (n=5), or from a D-vac vacuum sample (Rincon-Vitova Insectaries Inc., Ventura, CA) of undetermined understory vegetation (n=1). No 2nd instar nymphs were caught after 6 July. Third instar nymphs were caught on 6 July and 20 July on herbaceous vegetation including wild carrot (n=36), in vacuum samples of understory vegetation (n=14), and on vetch (n=6). Third instar nymphs were also found on oak (n=3), rose (n=3), and thistle (n=1 on 22 June). Fourth instar nymphs were captured from 6 July through 28 July. Wild carrot hosted the majority of the 4th instar nymphs (n=55), followed by grapevine (n=10), undetermined understory vegetation (n=6), and blackberry (n=2). The presence of 5th instar nymphs was documented from 20 July through 10 August. The majority of captured 5th instar nymphs also occurred on wild carrot (n=96), followed by grapevine (n=15), vetch (n=3), undetermined understory vegetation (n=2), blackberry (n=1), hawthorn (n=1), and oak (n=1).

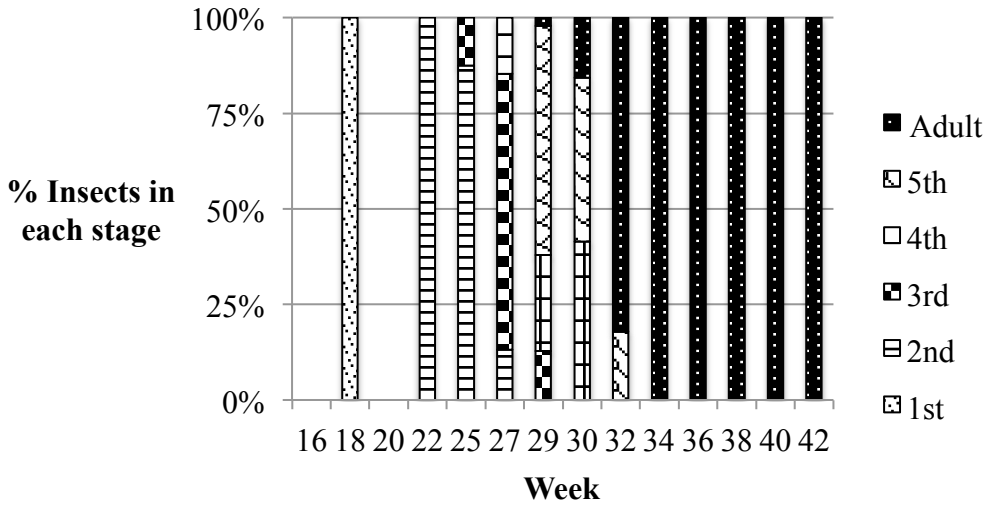

Figure 1 Proportion of *Tortistilus albidosparsus* life stages observed throughout the growing season during 2017 at YV (Willamette Valley).

The above observations can be summarized as follows to describe the seasonal lifecycle of *T. albidosparsus* treehoppers at YV (Fig. 2). Insects overwinter in woody vegetation as eggs and start to emerge in early May. Immature nymphs molt five times (juvenile instar stages 1-5), eventually giving rise to winged adults. The early instar stages may remain on the woody host for a period of time but will eventually drop to the understory vegetation. Juvenile insects feed on lush green tissue such as vetch until the host plant dries out in early summer. Later instar nymphs will migrate to drought-hardy or evergreen perennial plants, including grapevines, which can provide a nutritional or water resource. Adults mate toward the latter portion of the season and females lay eggs on suitable perennial woody host plants. The first adults were captured from the field on 20 July and were present in the field through the final field visit of the season on 17 October. Grapevine was the host for the majority of captured adults (n=33), followed by blackberry (n=8), wild carrot (n=5), undetermined understory vegetation (n=4), hawthorn (n=3), plum (n=3), and apple (n=2). In total, 347 insects were collected from the field at the YV site (see Table 1).

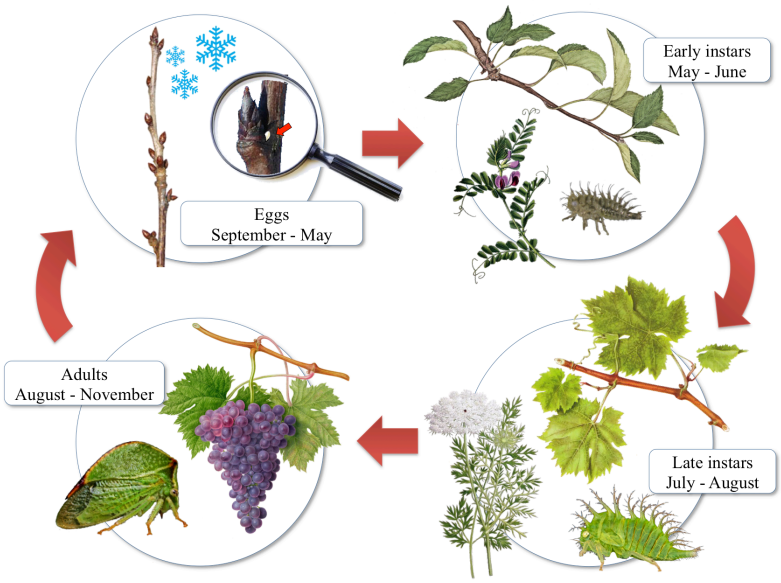

Figure 2 The seasonal lifecycle of *Tortistilus albidosparsus* as observed during 2017 at YV (Willamette Valley). The insect overwinters in the egg stage hidden in dormant bud scales of suitable perennial host plants. In spring, the early instar emerges and drops onto suitable green herbaceous plant 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.

Treehopper feeding produces characteristic girdling damage on affected leaves and stems. In addition to surveying for phenology of *T. albidosparsus*, feeding damage was documented at YV on the edge rows in summer and fall. From the first observed girdle on July 20 until October 17 the two edge rows of the study block were surveyed six times, and incidence of girdling was noted. All girdled tissues were removed upon observation. In the lab, the caliper of the damaged tissue was measured above the girdling point (Fig. 3a). Most vines (84.9%) in Row 1 nearest the field edge had at least one girdle, whereas 58.6% of the vines in Row 2 were affected. Up to 10 girdles were found on individual grapevines over the course of the season (Fig. 3b). Girdling consistent with treehopper feeding damage was also observed on other woody and herbaceous hosts, including hawthorn, apple, wild carrot, and vetch. In many cases a treehopper was found above the girdling point of damaged tissue.

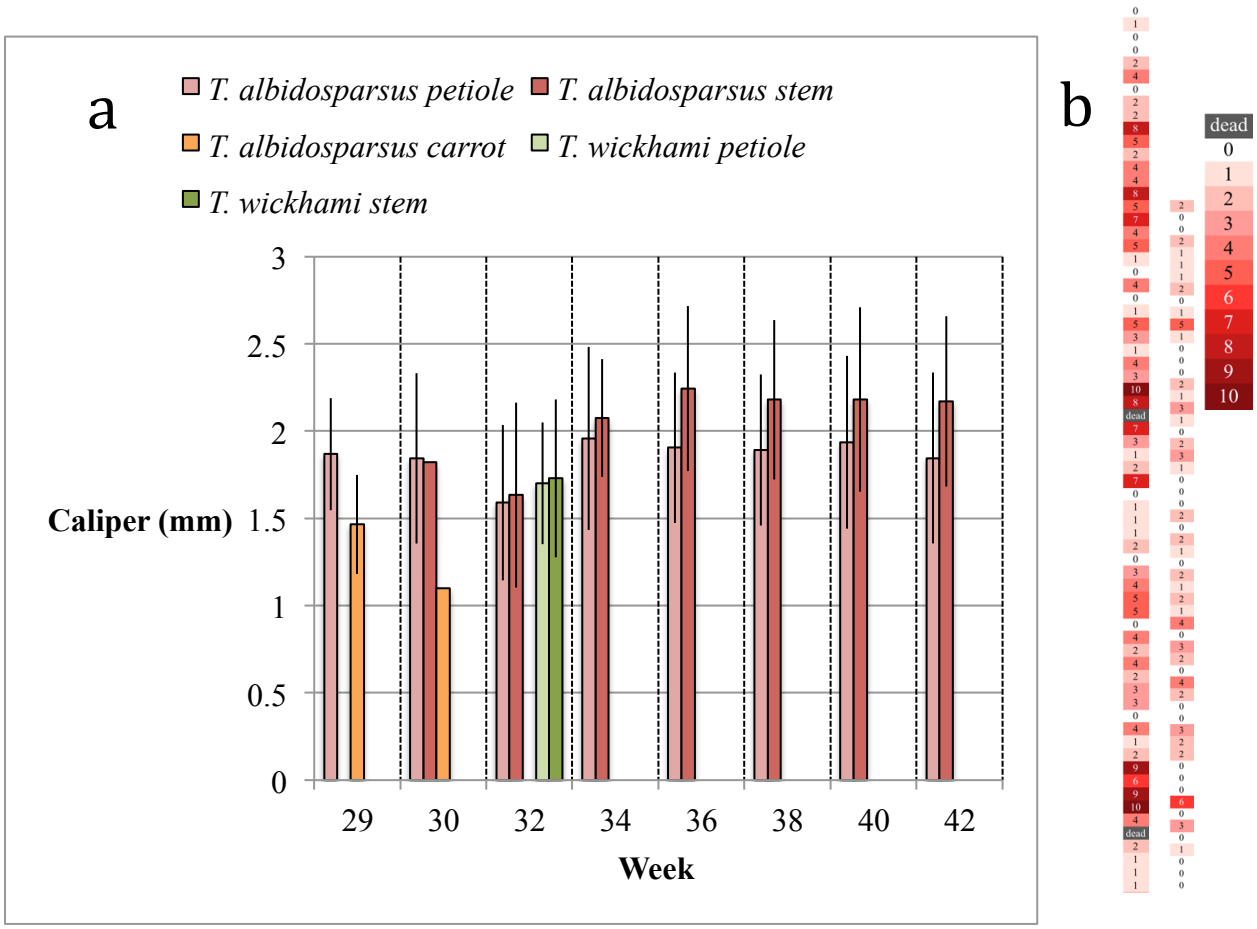

Figure 3. Caliper of treehopper-damaged leaf and stem tissue damaged by *Tortistilus albidosparsus* from YV (Willamette Valley) and *T. wickhami* from CJV (Southern Oregon), as measured above the girdling point (a). Cumulative numbers of girdles observed on edge rows from 20 July through 17 October at Yamhill (b).

Seasonal observations of treehoppers in Southern Oregon. A total of 19 *S. festinus* adults were found in Southern Oregon and were either associated with vineyards or from sampling in alfalfa fields. Most of the *S. festinus* were collected from JV by sweeping the groundcover vegetation. Two adult *S. festinus* were found at AV by sampling the groundcover with a Vortis vacuum sampler (Burkard Manufacturing Co. Ltd, Hertfordshire, UK). One *S. festinus* was also found in a clear sticky trap placed next to a small vineyard at SOREC. Sampling in alfalfa fields resulted in *S. festinus* collected on a single date in two adjacent alfalfa fields. Four adults were collected by sweep netting while one was collected with a D-vac. One nymph was also collected at the same time in an alfalfa field with the Vortis sampler.

All but one of the 125 *T. albidosparsus* collected in Southern Oregon in 2017 were from AV and a mixed orchard adjacent to the vineyard. Both the orchard and vineyard are being farmed organically. Sweeping and sticky traps were the most productive methods of sampling, especially sweeping the groundcover in pear blocks and sticky traps placed in the orchard. Twelve yellow

sticky cards (3” x 5”) were placed in a vineyard. Ten of these sticky cards were placed along the vineyard edge. Seven *T. albidosparsus* adults were collected in sticky cards on the vineyard edge. Two larger yellow sticky traps (5½” x 9”), one in cherries and one in apples (targeting western cherry fruit fly and apple maggot, respectively), caught a total of 44 *T. albidosparsus* adults. Twenty *T. albidosparsus* were found in the vineyard using visual searching. No *T. albidosparsus* were collected using beat trays even though they were collected in the orchard groundcover in sweep net samples.

The most abundant treehopper collected (n=275) in Southern Oregon 2017 was *T. wickhami* (see Table 1). Approximately two thirds of those specimens were from a vineyard near CJV. *Tortistilus wickhami* were found in a total of six vineyards at varying levels. Additionally, one individual was found in a beat tray sample along the edge of a pear orchard and one other was found in a clear sticky trap associated with a small tree fruit planting at SOU. At the AV location where 10 *T. wickhami* were found, insects were fairly evenly collected using sweep netting, beat tray, hand collecting and sticky cards.

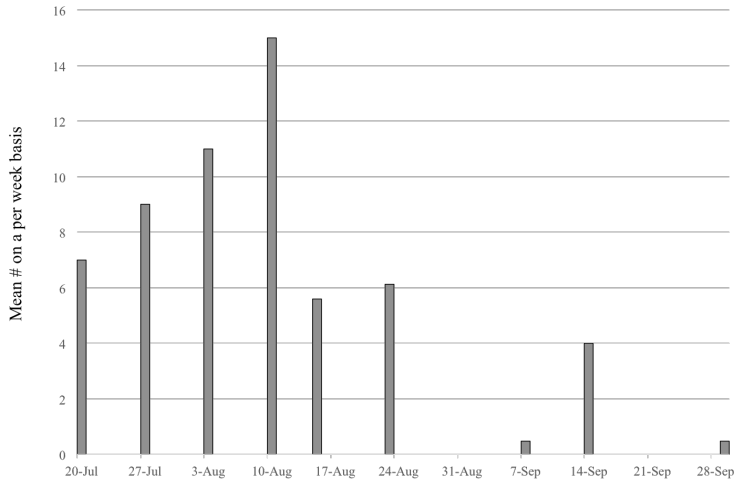

Figure 4. Yellow sticky trap collections of *T. wickhami* found in CJV (Southern Oregon) during 2017.

Figure 5. Placement of yellow rectangular sticky traps in CJV (Southern Oregon) during 2017.

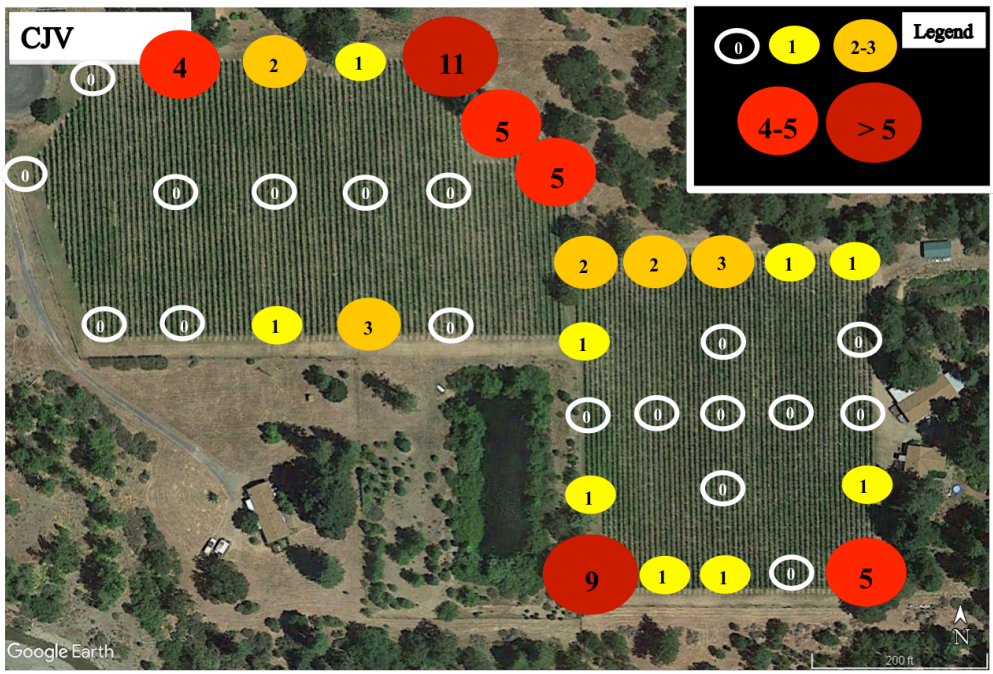

Figure 6. *Tortistilus wickhami* collections in CJV (Southern Oregon) during 2017. Numbers within ovals show cumulative number of *T. wickhami* adults collected from corresponding yellow sticky card placement sites.

2. Conduct controlled transmission biology experiments.
We initiated controlled greenhouse trials in 2016 to determine whether GRBV could be successfully transmitted by *T. wickhami* or *T. albidosparsus*. The initial virus status of all plant material was verified using qPCR. Field-collected live insects were placed on known GRBV-infected plant material for a 48-hour acquisition access feeding period (AAFP) and single insects were then transferred to GRBV-free plants for a 48-hour inoculation access feeding period (IAFP). All surviving insects were subsequently transferred individually at one-week intervals to new plants with no virus infection. This process was repeated weekly until all insects had died. In total, 113 initially GRBV-free grapevines were infested with *T. wickhami* in 2016, and 90 vines were infested with *T. albidosparsus*. Control vines (n=120) that were not infested with treehoppers were also maintained. Plants were tested periodically with qPCR in 2017, and as of the latest testing date (September 21) no plants from the 2016 greenhouse transmission bioassay had tested positive for GRBV.

The greenhouse bioassay was repeated in 2017 with modified methodology. In August, rooted cuttings of GRBV-infected plants were infested with *T. wickhami* and *T. albidosparsus* adults. Following a six-day AAFP, cohorts of five insects of the same species were put onto disease-free vines. The IAFP was seven days, after which all insects of each cohort were placed onto previously uninfested GRBV-negative vines. At the end of each IAFP, one cohort of each species was collected directly from a randomly selected infested vine and stored in 95% EtOH at -9 ºF until genetic analysis. Grapevines were infested with *T. albidosparsus* (n=62 vines) and with *T. wickhami* (n=53 vines) over a 6-week period. In addition, control plants (n=60 vines) were never infested with treehoppers. Insects that survived the greenhouse infestation period were transferred to potted alternate host plants. The alternate hosts were *M. domestica* var. Liberty, *Pyrus communis* var. D'Anjou, Douglas hawthorn (*Crataegus douglasii*), and Oregon white oak.

Cohorts of insects were tested for presence of GRBV using qPCR to evaluate whether the virus can be uptaken by the treehopper species under examination, as well as to determine the persistence of the virus in the insect body. Laboratory-reared insects of *S. festinus*, a confirmed treehopper vector of GRBV (Bahder et al. 2016), served as a positive control and were placed onto GRBV-positive material for a 6-day AAFP, at which point they were collected and frozen at -112 ºF until genetic analysis.

Insects were prepared for genetic analysis as per Bahder et al. (2015). Individual insects were placed in microcentrifuge tubes and immersed in a Proteinase K +ATL buffer solution. Insects were incubated at 133 ºF for 3 days, at which point the supernatant was transferred to a new microcentrifuge tube. DNA was then extracted using the DNEasy kit (Qiagen), standardized to a concentration of 4ng/µl, and then tested by qPCR for presence of GRBV using primers F1580 and 1693R to amplify virus DNA.

The results show that treehoppers provided an AAFP of 6 days will uptake GRBV particles. As expected, the buffer control tested negative for GRBV. The virus persisted for the entire 5-week period in both *T. albidosparsus* and *T. wickhami* (Table 5). Currently, virus transmission by the

two *Tortistilus* species has not been confirmed; however, persistence of GRBV within the insects provides indirect support that they may be vectors of the virus.

Table 5. Treehopper species tested in 2017 using qPCR to assess uptake and persistence of GRBV. Cohorts of insects were removed from randomly selected vines at the conclusion of each IAFP. Date indicates when insects were removed from host vines.

Species	Date	Gender	Positive	Total	Percent infected
S. festinus	10/26/17	female	4	5	80%
		male	3	4	75%
	Subtotal		**7**	**9**	**78%**
T. albidosparsus	8/29/17	female	2	3	67%
		male	0	2	0%
	9/5/17	female	3	3	100%
		male	2	2	100%
	9/12/17	female	2	3	67%
		male	2	2	100%
	9/19/17	female	2	3	67%
		male	1	2	50%
	9/28/17	female	3	3	100%
		male	1	1	100%
	Subtotal		**18**	**24**	**75%**
T. wickhami	8/29/17	female	1	3	33%
		male	1	1	100%
	9/5/17	female	3	3	100%
		male	2	2	100%
	9/12/17	female	2	3	67%
		male	2	2	100%
	9/19/17	female	0	3	0%
		male	0	2	0%
	9/28/17	female	1	2	50%
		male	1	1	100%
	Subtotal		**13**	**22**	**59%**
	Total		**38**	**55**	**69%**

3. Obtain baseline information on current levels and extent of GRBaV

In Southern Oregon, five Pinot noir blocks in different vineyards and exhibiting varying amounts of GRBV foliar symptoms were systematically surveyed following harvest and symptomatic vines were recorded. The percentage of symptomatic vines ranged from 72% to 0.55%. Testing is underway to confirm the extent to which the symptomatic vines test positive for the presence of GRBV. Additional testing for the presence of grapevine leafroll-associated virus is also being conducted to assess the level of potential co-occurrence of the viruses. These vineyards will be followed in 2018 and will be monitored periodically for treehopper activity using a variety of

sampling methods: vacuum sampling for nymphs, sweep net, sticky cards, and visual observations for adults.

Limited samples were received in 2017 from a grower in Eastern Oregon. These samples tested negative for GRBV.

In 2016, girdled leaf petiole materials collected at YV were tested for GRBV and compared with non-girdled petiole tissue from the same vines. Results from 2016 showed no detection of GRBV within 7-10 days of feeding damage. Petioles from the same vines will be retested in 2018, as well as materials from CRV and from established Southern Oregon sites.

4. **Extension of information of grapevine red blotch-associated virus, and insect vectors**

Results were presented a total of thirteen times in-person to growers, grape industry representatives and OSU Cooperative Extension personnel through grower reports, seminars, and national webinars. We organized a regional vineyard workshop on vectors and vineyard disease transmission for growers and industry in 2017. Vaughn Walton, Clive Kaiser and Rick Hilton are the statewide and regional extension agents in the affected regions. They have given numerous presentations on grape insect pests at grower and research symposia. A recently submitted manuscript documents the spread of virus over successive years of genomic analysis. Several extension outreach activities were conducted during 2017. Additional results will also be published in popular and scientific journals. Walton, Kaiser, and Hilton are strongly committed to the grape industry and have a good relationship with growers, consultants and industry personnel that will aid in research and extension. Several presentations have been given at scientific meetings and public research expositions (see report for the list of applicable publications).

Publications

- Dalton, Daniel T., Richard J. Hilton, Clive Kaiser, Kent M. Daane, Mysore R. Sudarshana, Julia Vo, Frank Zalom, Jessica Z. Buser, and Vaughn M. Walton. *Submitted*. *Grapevine red blotch virus* spread and associative mapping of Grapevine leafroll-associated virus-3 in Oregon vineyards. Plant Disease.

Presentations

- Walton, V. M., Dalton, D. T., Hilton, R. J., Kaiser, C., Yamhill-Carlton Winegrowers' Association annual meeting, "Vectors of Red Blotch," Yamhill. Local, Invited. (January 2018).

- Walton, V. M., Dalton, D. T., Hilton, R. J., Kaiser, C., Red Blotch Workshop, "Identifying Insect Vectors for Red Blotch Disease in Oregon," Salem Oregon. Regional, Invited. (November 2017).

- Walton, V. M., Dalton, D. T., Hilton, R. J., Kaiser, C. Walton, V. M., Dalton, D. T., Hilton,

R. J., Kaiser, C. (February 22, 2017). Red blotch in Oregon vineyards. Oregon Wine Industry Symposium, Portland, Oregon, Face to Face, Adult Contacts: 350, Gave talk on the newest finds on Red Blotch, an important disease in Oregon vineyards. State and agency collaboration, Regional, Invited.

- Dalton, Daniel T., Richard J. Hilton, and Vaughn M. Walton. 2017. Red Blotch Virus Status and Update. Webinar, Oregon Wine Research Institute, March 2, 2017. https://media.oregonstate.edu/media/t/0_vj8femfy.

- Walton, V. M., Dalton, D. T., Hilton, R. J., Kaiser, C. Red Blotch and Spotted Wing Drosophila, Blue Mountain Horticultural Society, Milton Freewater, Face to Face, Adult Contacts: 20, Invited. Local. February 2017

- Walton, Vaughn, Presenter. 3rd Annual Southern Oregon Grape Symposium, Central Point, OR, Face to Face, Adult Contacts: 100. Local Workshop, March 2017.

- Walton, Vaughn M., Presenter. IPM class for Tree Fruit and Winegrapes, County Courthouse in Roseburg Oregon, Face to Face, Adult Contacts: 20, Renquist, Steve. November 1, 2017.

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- Dalton, Daniel T., Richard Hilton, and Vaughn Walton. 2017. Virus transmission ecology and phenology of treehoppers in Oregon vineyards. Presentation, ESA 65th Annual Meeting, Denver, CO. Scheduled November 7, 2017. [*Role: data acquisition, preparation of presentation.*]

- Hilton, Richard, Vaughn Walton, and Daniel Dalton. 2017. Treehoppers associated with vineyards in southern Oregon. Presentation, ESA 65th Annual Meeting, Denver, CO. Scheduled November 7, 2017. [*Role: data acquisition, presentation review.*]

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Research relevance statement
This research has identified characteristics of candidate insect vectors of GRBV that will help growers determine whether their vineyards are at risk of virus spread. The lifecycle of candidate insect vector species is confirmed, and common host plants are identified. Through distribution of feeding damage and insect collections, growers can focus their sampling on observations of feeding symptoms to determine presence of potential vector populations. We identified that GRBV is persistent in greenhouse-infected treehoppers, strongly pointing toward these insects as vectors in the field.

Layperson summary of project accomplishments
Distribution, non-crop host plants, and seasonal phenology of candidate vector insects of *Grapevine red blotch virus* (GRBV) were confirmed during 2017. Adult treehopper insects (Hemiptera: Membracidae) feed and lay eggs in the fall on suitable perennial host plants including grapevines. These insects overwinter as eggs in oak, apple and pear. Nymphs hatch from eggs, move to annually growing vetch and wild carrot plants as soon as temperatures become suitable for development in the spring, and develop to adults. Late instar nymphs move to perennial host plants as soon as annual plants dry out in mid-late summer. This life cycle was observed in 2017 by a combination of collection techniques including vacuum sampling, sweep netting, sticky trap monitoring, and observing feeding symptoms on vines.

Feeding damage and distribution of treehoppers is concentrated on vineyard edges in close proximity to suitable wild habitat. Feeding on grapevines typically can be found on green canes with a diameter of up to 0.08 inches. This information will help growers identify potential host plants, assess whether the vineyard landscape is favorable to candidate vector insects, and determine whether such vectors are present.

The candidate insect vector treehopper species *Tortistilus albidosparsus*, *T. wickhami*, and *Spissistilus festinus* showed persistence of GRBV for at least five weeks after acquisition in greenhouse transmission trials. Additional transmission biology experiments were conducted in the greenhouse in 2017. Additional virus testing of the greenhouse plants is needed in order to confirm transmission with these insects.

Regionally, we found treehoppers in Southern Oregon, the Willamette Valley and Columbia Gorge. Earlier work showed spread of virus over successive years of genomic analysis (Dalton et al. submitted). GRBV can be found in all winegrape production regions of the Pacific coast, indicating the significant magnitude of this problem.
Several extension outreach activities were conducted during 2017.

Fund Status: Include a general summary of how funds were spent.

All funds were spent based on the project budget.

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