Summary Final Progress Report for CDFA Agreement Number 2017-0418-000-SA

Project Title: Integrative studies of vector-related field epidemiology for grapevine red blotch-associated virus. UGMVE proposal number, 2017-0418-000-SA.

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

Time period covered by report: July 1, 2017 – June 30, 2019

- *Grapevine red blotch virus* (GRBV) is the causal agent of grapevine red blotch disease affecting winegrape production in Oregon and California. The threecornered alfalfa hopper *Spissistilus festinus* (Hemiptera: Membracidae), was shown to vector GRBV under greenhouse conditions in California. While *S. festinus* was found in low numbers in certain winegrape production regions of Oregon, its apparent absence in areas where GRBV appears to be spreading suggested that one or more related treehopper species may vector the virus in different geographical regions.

- Research efforts from Oregon State University (OSU) in 2017-19 included field trials to determine whether applications of pesticides or deterrents may affect treehopper presence in a vineyard. Treehopper sampling built upon previously collected phenology data. Plants used in greenhouse infestation trials were tested for presence of GRBV using quantitative PCR (qPCR), and further controlled infestation trials on grapevine and cover crop species were conducted in the greenhouse and in growth chambers.

- The life cycle of *Tortistilus* cf. *albidosparsus* (Hemiptera: Membracidae) was observed in the Willamette Valley in 2017 and confirmed in 2018. Immature treehopper nymphs emerged in April from eggs laid in woody hosts and dropped to the understory vegetation.

Feeding first took place on lush understory plants, and nymphs migrated to drought-hardy herbaceous vegetation through early-mid summer. Late-instar nymphs occasionally fed on woody perennial host species including blackberry and grapevine. Adults emerged beginning in July, fed, and mated into the fall on woody host plants. Insects overwintered as eggs in host plants such as oak, apple and hawthorn.

- Insect collections and observations of feeding damage indicated that distribution of treehoppers is concentrated at vineyard edges near suitable wild habitat. Feeding on woody hosts occurred on petiole or stem tissue about 0.08 inches (2 mm) in diameter. Diagnosis of plant damage will assist growers to search for vineyard-inhabiting treehoppers and determine whether the vineyard landscape is suitable to complete the treehopper life cycle.

- Diverse morphospecies of treehoppers were found in the Willamette Valley and in southern Oregon over the study period. GRBV is present in all winegrape production regions of the Pacific coast, and recently published work using genetic analysis showed spread of virus over successive years (Dalton et al. 2019). If confirmed as a vector, the widespread presence of *Tortistilus* treehoppers could magnify the risk of GRBV transmission to vineyards.

- Multiple extension outreach activities were conducted by OSU personnel in 2018. In southern Oregon, four events took place in 2018 where experts met with winegrape producers to discuss pests and pest management strategies. In a technical meeting, leafhopper and treehopper infestations in vineyards was ranked as the number 1 and 2 concerns, respectively, of growers. Three other meetings covering integrated pest management approaches focused on control of GRBV and potential Hemipteran vectors. In the Willamette Valley, a seminar was provided to the Yamhill-Carlton Winegrowers Association to discuss GRBV and issues related to potential insect vectors. A similar seminar occurred for the Plant Improvement Committee in The Dalles, Oregon. A presentation on GRBV vector research was given to growers and scientists at the OSU Vineyard Red Blotch Workshop in November 2018. Posters covering biological and behavioral aspects of *Tortistilus* treehoppers were provided for the annual Oregon Wine Research Institute Grape Day. Findings on *Tortistilus* biology were presented at the Pacific Branch of the Entomological Society of America 2019 Meeting.

Comprehensive Final Report for CDFA Agreement Number 2017-0418-000-SA

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Kent Daane, Extension Specialist, Environmental Science, Policy and Management, University of California, Berkeley, CA 94720, Tel. (559) 646-6522, kdaane@ucanr.edu
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Introduction

Grapevine virus diseases are of serious concern for vineyard managers and winemakers in all Western production regions. GRBV infection impacts grape berry quality, resulting in berries with lower °Brix at harvest (Al Rwahnih et al. 2013, Sudarshana et al. 2015) and necessitating the removal of symptomatic vines from vineyards. GRBV is spreading in many Oregon vineyards; ecological mapping of GRBV-positive vines, as verified by qPCR during 2013-2016, showed a significant trend of virus increase over time in two of three areas studied in Oregon (Dalton et al. 2019). Spread of GRBV occurred in an additional vineyard study block examined from 2016-2018. The role of an insect vector has not been confirmed in the field, and the available information from greenhouse studies implicates treehopper insects as the most likely vectors.

Objective(s) and Experiments Conducted to Meet Stated Objective(s): The report objectives should match the objectives in the original proposal.

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 to accomplish each objective

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

Follow insect vector distribution and incidence. In 2017, vineyards in seven locations 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). In 2018, five sites were surveyed in the Willamette Valley, and nine sites were surveyed in southern Oregon. Site CP was a natural area removed from agricultural production and did not contain *Vitis* plants. Site CRV was a research vineyard managed by OSU. All other sites were in commercial vineyards. Nymphs that survived to the adult stage were tentatively identified to species. Adults of three treehopper species: *Spissistilus festinus*, *Tortistilus albidosparsus,* and *T. wickhami*, were found.

Table 1. Treehopper species collected in surveyed vineyards of Oregon in 2017 and 2018. Region SO is Southern Oregon. Region WV is Willamette Valley. All sites were vineyards except CP. Letters in superscript indicate .emerged species: [a]=*T. albidosparsus*; [w]=*T. wickhami*.

Year	Region	Location	Eggs	Nymphs	*S. festinus*	*T. albidosparsus*	*T. wickhami*
2017	SO	AV	8	23	2	124	10
	SO	CJV	0	13	0	0	180
	SO	EPV	0	0	0	0	65
	SO	JV	0	0	11	0	1
	SO	TV	0	0	0	0	17
	SO	Misc. sites	0	0	1 (SOREC sticky trap) 5 (alfalfa fields)	1 (Sticky trap)	1 (Beat tray) 1 (SOU sticky trap)
	WV	CRV	55[a]	0	0	0	0
	WV	YV	129[a]	289[a]	0	58	0
	Subtotal	**Subtotal**	**192**	**325**	**19**	**183**	**275**
2018	SO	SO1	0	0	0	3	0
	SO	SO2	0	0	33	3	5
	SO	SO3	0	0	0	2	1
	SO	SO4	0	0	1	42	1
	SO	SO5	0	0	0	0	253
	SO	SO6	0	0	0	0	1
	SO	SO7	0	0	0	0	92
	SO	SO8	0	0	0	0	79
	SO	SO9	0	0	0	72	12
	WV	CP	0	59[a]	0	1	0
	WV	CRV	32[a]	8[a]	0	0	0
	WV	CV	0	21[w]	0	2	85
	WV	LV	0	5[a]	0	0	0
	WV	YV	199[a]	308[a]	0	24	0
	Subtotal	**Subtotal**	**231**	**401**	**34**	**149**	**529**

2-Year Total	**2-Year Total**	**423**	**726**	**53**	**332**	**804**

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 and from nine southern Oregon sites in 2018.

Year, Species / Method	**2017** ***S. festinus***	**2017** ***T. albidosparsus***	**2017** ***T. wickhami***	**2018** ***S. festinus***	**2018** ***T. albidosparsus***	**2018** ***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**

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

Region	**Plant/Crop**	**Eggs in buds**	**Nymphs**	***S. festinus***	***T. albidosparsus***	***T. wickhami***
Southern Oregon	Pear	0	18	0	48	5
	Apple	8	4	0	23	1
	Grape	----	----	2	27	267
	Cherry	----	----	0	24	0
	Other	----	1	17	3	2
Willamette Valley	Apple	79	15	0	2	0
	Blackberry	----	3	0	8	0
	Carrot	----	192	0	5	0
	Grape	8	25	0	33	0
	Hawthorn	2	1	0	3	0
	Oak	104	7	0	0	0
	Plum	----	0	0	3	0
	Rose	8	4	0	0	0
	Thistle	0	1	0	0	0
	Undetermined	----	23	0	4	0
	Vetch	----	18	0	0	0

Description of Willamette Valley, Oregon field sites. YV is a commercial vineyard in the Willamette Valley and was surveyed in 2017 every fourteen days from spring through fall and was surveyed in 2018 every 14-21 days. Several vineyard blocks are at YV, ranging in age and size. The primary study area 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 seedling 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 in 2017, but routine mowing in 2018 effectively minimized interrow vegetation. No irrigation or insecticide sprays were applied to the vineyard study block. CRV is an experimental vineyard managed by OSU and was surveyed in October 2017 and April 2018. Habitat adjacent to a young planting of winegrapes (planted in 2015) was primarily grass, and 10-15 yards (9-14 m) to the east and southeast of the block was a stand of oak trees (*Q. garryana* and *Q. rubra*) thatcontained rose and blackberry. Heirloom apple, cherry (*Prunus avium*), and plum trees were to the northeast of the CRV study block. CP is located within a scrubby clearing of an oak forest in an unmanaged natural area within the city limits of Corvallis. The dominant vegetation included hawthorn, wild carrot, and grasses. CV is a small commercial vineyard near Carlton, Oregon located on a steep south-facing slope. The western border of the vineyard had an old grove of Oregon white oak with thickets of wild blackberry and poison oak in a grassy understory. Large established oak trees were present to the north and south of the block, and the eastern margin was vegetated with riparian vegetation along an ephemeral creek. LV is a large vineyard near Lafayette, Oregon that is located on the south face of a moderately steep hillside. The surveyed area was at the high point of the hill in an infrequently mowed grassy area containing small numbers of seedling oak and hawthorn trees 30-50 yards (27-46 m) away from cultivated grapevines. Grapevines along the edges of LV blocks were also examined for presence of treehoppers.

Seasonal observations of Tortistilus spp. in the Willamette Valley. Tortistilus albidosparsus lays its eggs behind the bud scales of woody hosts (Yothers 1934). Collection of woody materials from surrounding habitat in study vineyards CRV and YV in 2017 and 2018 provided a reading of the percentage of buds infested with treehopper eggs (Table 4). Treehopper eggs were found only behind the bud scales of deciduous trees. At CRV, treehopper eggs were found on samples of the two respective oak species in 2017, and eggs were found on red oak, Oregon white oak, and mock orange (*Philadelphus lewisii*) in spring 2018. Oak, heritage apple, cherry and plum trees were recorded growing 30 yards away from the vineyard edge and 5-50 yards (5-46 m) from woody surrounding habitat. Rose, Oregon white oak, and red oak were the dominant species immediately adjacent to the vineyard block. Nymphs were observed in April 2018, and only eggs were found at CRV during October 2017. Nymphs from CRV that survived to the adult stage resembled *T. albidosparsus*, and all eggs were laid under bud scales. Across both

years, eggs from YV were found from the highest to lowest proportion of infested buds on oak, apple, hawthorn, and plum. Overall, buds that did contain eggs tended to host a single egg. Eggs from plants infested with *T. albidosparsus* in the greenhouse (insects of YV origin) were found behind bud scales, whereas eggs from greenhouse plants infested with *T. wickhami* were found in slits along mature wood (insects of CJV origin).

Table 4. Woody hosts and relative abundance of *Tortistilus albidosparsus* eggs at study sites in CRV and YV (Willamette Valley) in 2017 and 2018.

Site	Season	Species	2017 Number buds	2017 Number buds with eggs	2017 Total eggs	2017 % Buds with eggs	2017 Number eggs/bud	2018 Number buds	2018 Number buds with eggs	2018 Total eggs	2018 % Buds with eggs	2018 Number eggs/bud
CRV	late	apple	213	0	0	0%	n/a					
CRV	late	cherry	231	0	0	0%	n/a					
CRV	late	plum	882	0	0	0%	n/a					
CRV	late	red oak	185	2	2	1.08%	1.00					
CRV	late	rose	81	0	0	0%	n/a					
CRV	late	white oak	347	29	53	8.36%	1.83					
CRV	early	apple						63	0	0	0%	n/a
CRV	early	blackberry						44	0	0	0%	n/a
CRV	early	cherry						200	0	0	0%	n/a
CRV	early	Indian plum						51	0	0	0%	n/a
CRV	early	mock orange						45	3	3	6.67%	1.00
CRV	early	plum						251	0	0	0%	n/a
CRV	early	red oak						245	8	10	3.27%	1.25
CRV	early	white oak						224	15	19	6.70%	1.27
CRV		**subtotal**	**1939**	**31**	**55**	**1.60%**	**1.77**	**1123**	**26**	**32**	**2.32%**	**1.23**
YV	late	apple						48	1	1	2.08%	1.00
YV	late	grapevine	189	2	5	1.06%	2.50					
YV	late	hawthorn						173	0	0	0%	n/a
YV	late	rose						9	0	0	0%	n/a
YV	late	serviceberry						51	1	1	1.96%	1.00
YV	late	white oak	56	9	16	16.10%	1.78	313	16	18	5.11%	1.13
YV	early	apple	1906	61	79	3.20%	1.30	591	19	31	3.21%	1.63
YV	early	grapevine	480	2	3	0.42%						
YV	early	hawthorn	270	2	2	0.74%	1.00	1784	19	26	1.07%	1.37
YV	early	plum	190	1	1	0.53%	1.00	1180	6	6	0.51%	1.00
YV	early	rose	458	7	8	1.53%		116	0	0	0.00%	n/a
YV	early	thistle	8	0	0	0%						
YV	early	white oak	533	14	15	2.63%	1.07	906	73	111	8.06%	1.52
YV		**subtotal**	**4090**	**98**	**129**	**2.40%**	**1.32**	**5171**	**135**	**194**	**2.61%**	**1.49**

The YV site was surveyed repeatedly from spring through fall of 2017 and 2018 in order to track the phenology of treehoppers. A clear seasonal progression of the *T. albidosparsus* lifecycle was recorded (Fig. 1). Collected nymphs of all instar stages eventually developed into adult *T. albidosparsus* in the laboratory. First instar treehopper nymphs emerged from apple wood cuttings and rose cuttings held in a walk-in cold room (44 ºF, 7 ºC) in April 2017. First instar nymphs were found in the field in 2018 and also emerged from woody cuttings collected in late April. Second instar nymphs appeared roughly around the same time as the first instar nymphs, indicating that egg hatch likely occurred over a period of several weeks in May. Significant overlap of insect instar stages was observed in 2018 from mid-June to early July. By mid-July,

the first adult *T. albidosparsus* was collected at YV. The first adult field collection of *T. albidosparsus* in 2017 occurred two weeks later than in 2018. Females were captured for a longer period of the season than were males. Wild carrot hosted the majority of the 4th and 5th instar nymphs.

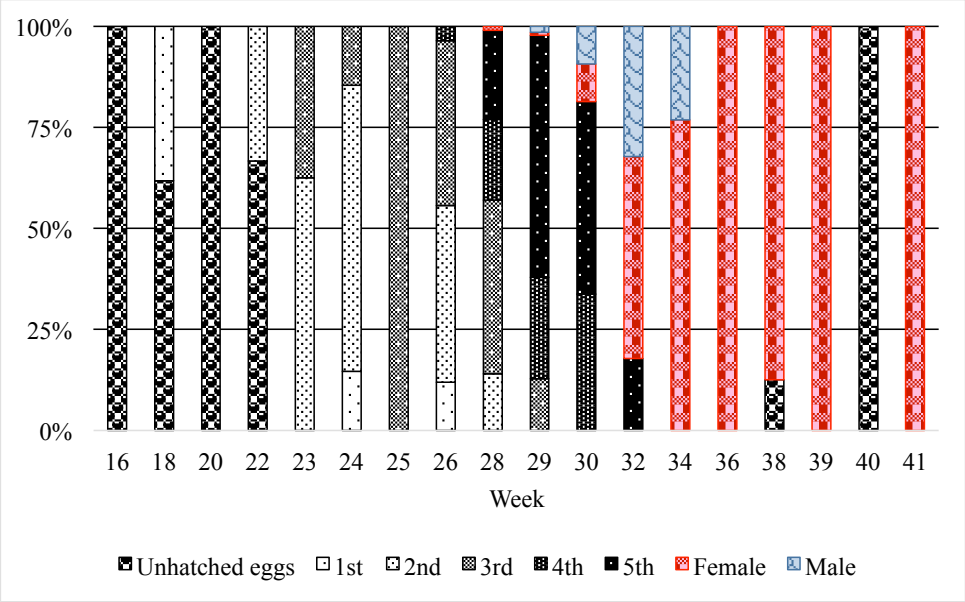


Figure 1. Proportion of *Tortistilus albidosparsus* life stages observed in the Willamette Valley from 2017-2018.

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.

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. In 2017 the two outer-most rows of the study block were surveyed six times, and incidence of girdling was noted. In the lab, the caliper of the damaged tissue was measured above the girdling point (Fig. 3).

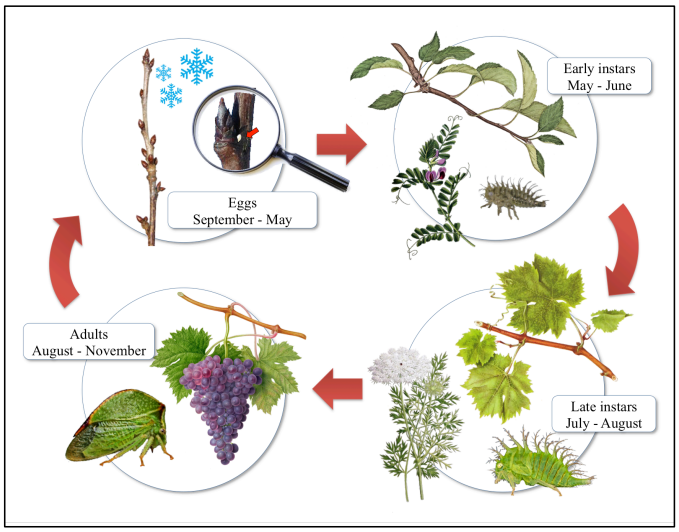


Figure 2. The seasonal lifecycle of *Tortistilus albidosparsus* at YV (Willamette Valley). The insect overwinters in the egg stage hidden in dormant bud scales of suitable perennial host plants. In spring, the early instar emerges and drops onto suitable green herbaceous plant hosts. In mid-summer, the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

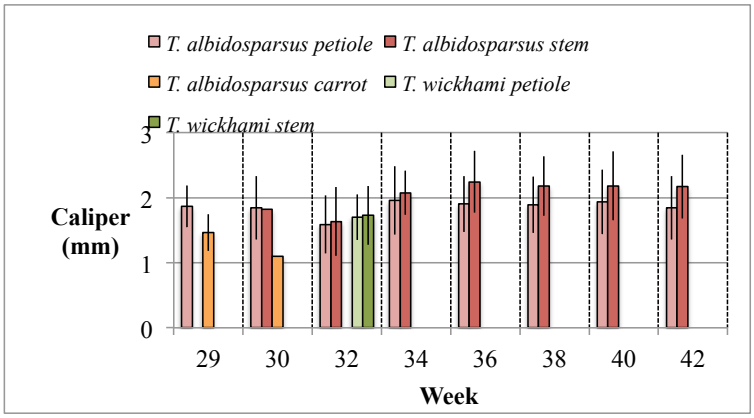
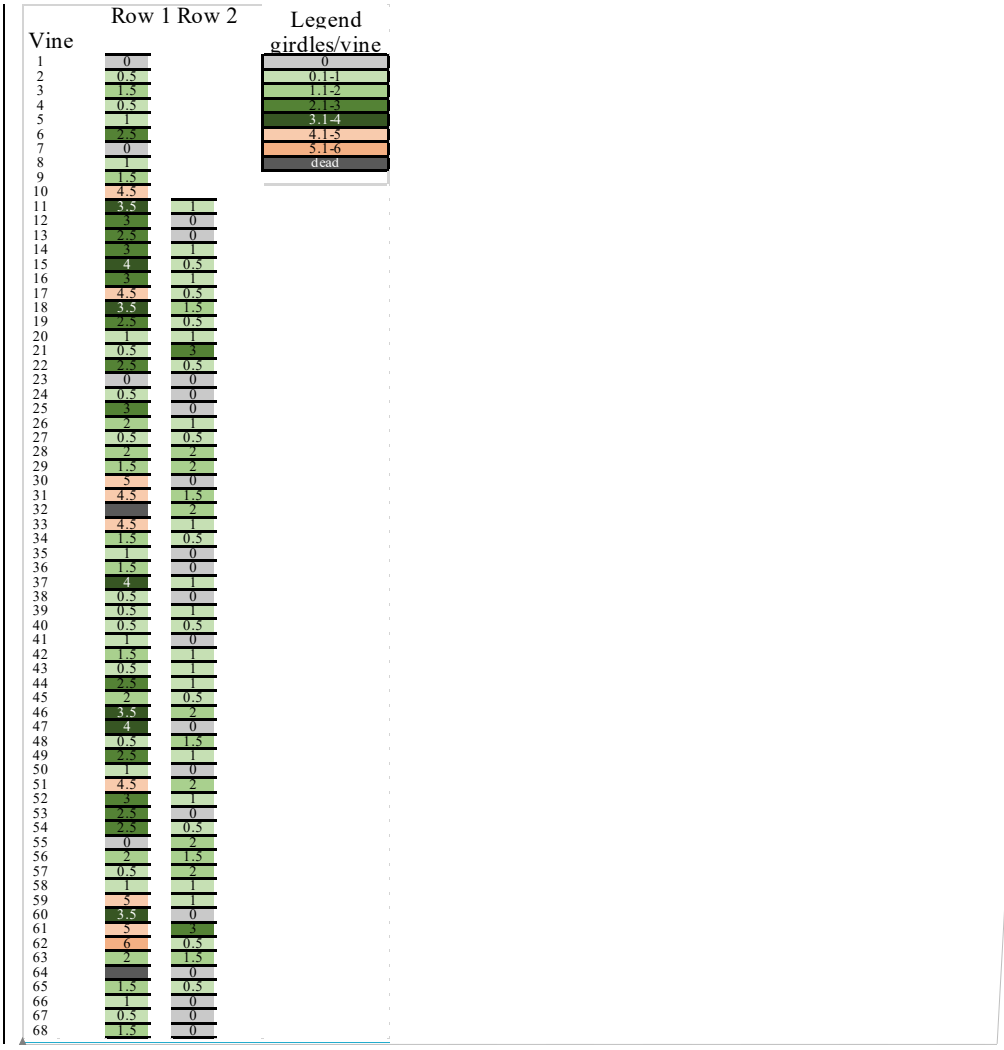


Figure 3. Caliper of treehopper-damaged leaf and stem tissue damaged by *Tortistilus albidosparsus* from YV (Willamette Valley) and *T. wickhami* from CJV (Southern Oregon), as measured above the girdling point. Data from 2017 field season.

In 2018, the same two outer-most rows at YV were surveyed two times. The average number of girdles per vine is depicted across seasons in Fig. 4. Most vines (91.2%) in Row 1 nearest the field edge had at least one girdle, whereas 69.0% of the vines in Row 2 were affected. The highest number of girdles per vine, averaged over the two seasons, was 6. Girdling consistent with treehopper feeding damage was also observed on hawthorn, apple, wild carrot, and vetch.



Unknown
Field Code Changed

Figure 4. Average number of girdles observed on vines along edge rows 1 and 2 at Yamhill from 2017-2018.

A survey to document symptomatic vines was conducted in a separate block at YV in 2016 and in 2018 (Table 5). Between the two seasons, visual surveys produced consistent findings in 774/958 vines (80.8%). In total, 37.5% of the surveyed vines appeared to have symptoms of GRBV, whereas 43.3% of surveyed vines appeared to be asymptomatic. Several vines showed questionable symptoms. In 2016 a subset of asymptomatic vines was sampled for GRBV analysis using qPCR. Collections were made from the same vines in 2018, but the samples have not been analyzed to date.

Table 5. Results of visual survey for symptoms of GRBV infection in a block of Pinot noir grapes at YV. Survey occurred in Fall 2016 and was repeated in 2018.

Row	Total vines	2016 & 2018 % asymptomatic	2016 & 2018 % symptomatic	2016 & 2018 % differential
0	32	15.6%	21.9%	62.5%
1	33	6.1%	12.1%	81.8%
2	33	54.5%	0.0%	45.5%
3	71	33.8%	39.4%	26.8%
4	73	61.6%	28.8%	9.6%
5	74	67.6%	20.3%	12.2%
6	76	68.4%	19.7%	11.8%
7	79	65.8%	21.5%	12.7%
8	80	71.3%	21.3%	7.5%
9	81	49.4%	39.5%	11.1%
10	64	15.6%	57.8%	26.6%
16	55	27.3%	61.8%	10.9%
17	50	22.0%	58.0%	20.0%
18	45	8.9%	62.2%	28.9%
19	42	21.4%	76.2%	2.4%
20	37	27.0%	67.6%	5.4%
21	33	33.3%	54.5%	12.1%
Total Vines	**958**	**415**	**359**	**184**

Seasonal observations of treehoppers in Southern Oregon. In total, 19 *S. festinus* adults were found in Southern Oregon in 2017 and were either associated with vineyards or from sampling in alfalfa fields. Most of the *S. festinus* were collected by sweeping the groundcover vegetation. Sampling in alfalfa fields resulted in *S. festinus* collected on a single date in two adjacent alfalfa fields. In 2018, *S. festinus* was, with a sole exception, found in sweep net samples in one vineyard and comprised 6.4% of the total treehoppers found (see Table 1). 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 were farmed organically. In 2018 *T. albidosparsus* was detected in five of the nine vineyards and made up 21% of the sampled

treehoppers. This species was found primarily in visual searches (see Table 2). The one location where it was found in sweep net sampling was the organic vineyard/orchard where it was often found in the orchard floor vegetation.
The most abundant treehopper collected (n=804) in southern Oregon in 2017 and 2018 was *T. wickhami* (see Table 1). Most specimens were collected at CJV, and collections indicated a strong edge effect of treehopper distribution (Fig. 5). Most of the *T. wickhami* were found by visual searching; however, in 2018, 17% of the total *T. wickhami* were trapped in sticky cards and about 6% with the sweep net. *Tortistilus wickhami* was the only treehopper trapped in the yellow sticky cards. The visual searching and sweep netting were not done in a systematic fashion so those results should be considered qualitative. However, in 2018 the sticky traps were deployed and checked on a fairly uniform and regular basis beginning at the end of June and extending through September. At the end of the season each trap location was examined, and the degree of treehopper girdling activity was assessed on the vine where the sticky trap was placed, along with the two neighboring vines. Girdling was observed both on leaf petioles and on shoots. In very rare instances girdling was observed on the fruit rachis. The results of the 2018 girdle assessment and the number of *T. wickhami* per sticky card are shown in Table 6.

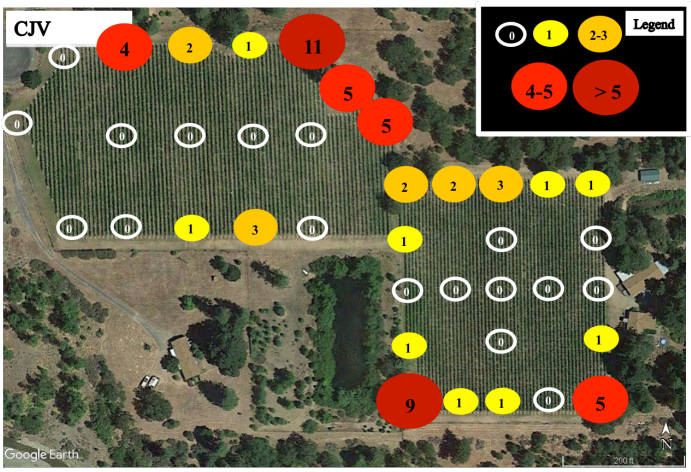


Figure 5. *Tortistilus wickhami* collections in CJV (Southern Oregon) during 2017. Numbers show cumulative number of adults collected from yellow sticky card placement sites.

Two trials were conducted to test the effects of imidacloprid systemic insecticide application and organic deterrent sprays on treehopper distribution within a vineyard. On August 1, 2018, a grower made a foliar treatment of imidacloprid. *Spissistilus festinus* were collected and caged on the treated foliage the day after treatment and a comparable number of individuals were placed on untreated grape vines located at the SOREC research station. *Tortistilus albidosparsus* were collected the following day from the same alfalfa field primarily from one edge that was bordered by a hedgerow, and again comparable numbers of *T. albidosparsus* were caged on

treated and untreated foliage. Four sleeve cages were used for *S. festinus* and three cages for *T. albidosparsus* in each of the treated and untreated areas with four to six treehoppers being placed in all of the cages. An initial assessment of mortality was made in the field on August 6, 2018. The cages were removed on August 10, and a final determination of mortality was made in the lab. Mortality of *S. festinus* was 94.7% on the initial evaluation date but decreased to 84.2% after the insects had been exposed to the treated foliage for eight days. The degree of intoxication was variable, and observation of some moribund individuals complicated the final evaluation. The mortality of *T. albidosparsus* was 30.8% on the initial sample date but increased to 100% after seven days exposure to the treated foliage. Mortality in untreated vines was appreciably higher for *S. festinus* than for *T. albidosparsus*. In this small-scale study, exposure to grapevines freshly treated with imidacloprid resulted in treehopper mortality but the effect on *S. festinus* was not as clear-cut as the effect on *T. albidosparsus* (Table 7).

Table 6. Treehopper activity in nine southern Oregon vineyards as evidenced by trap catch and associated girdling activity.

Vineyard	Mean petiole girdles vine^{-1}	Mean shoot girdles vine^{-1}	Total girdles vine^{-1}	Mean number *T. wickhami* yellow sticky card^{-1}
SO1	0.24	0.43	0.67	0
SO2	0.46	1.26	1.72	0.31
SO3	0	0	0	0
SO4	0.64	2.64	3.28	0
SO5	1.61	3.06	4.67	0.75
SO6	0.15	0.08	0.23	0
SO7	0.38	0.63	1.01	0.24
SO8	1.06	1.24	2.30	1.62
SO9	0.28	1.14	1.42	0

Table 7. Results of caging *S. festinus* and *T. albidosparsus* on vineyard foliage treated with imidacloprid on 8/1/2018 in comparison to caging on untreated vines. Vines were infested with *S. festinus* on 8/2 or with *T. albidosparsus* on 8/3. Values indicate the percentage of treehopper mortality determined on respective evaluation dates.

Evaluation Date	*S. festinus* treated vines (n=19)	*S. festinus* control untreated vines (n=21)	*T. albidosparsus* treated vines (n=13)	*T. albidosparsus* control untreated vines (n=14)
8/6	94.7	19	30.8	0
8/10	84.2	28.6	100	6.7

The organic crop protectant Surround®, a sprayable formulation of kaolin clay, was applied in 2018 to vines in a certified organic vineyard where considerable treehopper activity and girdling damage to vines had been observed in 2017. Treatments were applied to the outside row of the vineyard where the girdling damage had been most evident. The product was applied according to label instructions, and plants were treated at either a two-week interval (sprays applied on 6/27, 7/11, 7/26, and 8/10), or at a four-week interval (sprays applied on 6/27 and 7/26). Both

treatments were compared to an untreated control. The treatments were replicated five times and each replicate consisted of three vines with the middle vine being evaluated for treehopper activity. The vines were inspected on two dates, 8/16 and 9/27. The results of the vine inspections yielded fairly consistent evidence of treehopper activity in the untreated vines, while there was no treehopper activity in the vines that had been treated with Surround® every two weeks (Table 8). Just two leaf petioles with girdling were observed on the first evaluation date in the treatment where the vines had been treated twice with kaolin, but no girdling damage was seen on the subsequent evaluation. Most of the girdling damage in the untreated vines was on the shoots, while no shoot girdling was seen in any of the treated vines. One treehopper was observed on an untreated vine during the second evaluation. The data were analyzed using a randomized complete block ANOVA and the difference in degree of girdling among treatments was not statistically significant on the first evaluation date but was on the second date with the untreated vines having a significantly higher level of damage than either of the Surround treatments. This small-scale study showed that treehoppers were effectively deterred by repeated applications of Surround®, which is OMRI certified for use in certified organic production. This trial was essentially a choice test, and follow-up testing on a large scale is needed to determine if the repellent effect of the compound would still occur when entire blocks are treated.

Table 8. Mean number of girdles per vine and treehopper capture following treatment of vines in an organic vineyard with Surround® sprayable kaolin clay.

Interval	Petiole girdles on 8/16	Petiole girdles on 9/27	Shoot girdles on 8/16	Shoot girdles on 9/27	Total girdles on 8/16	Total girdles on 9/27	Treehoppers on 8/16	Treehoppers on 9/27
Untreated check	0.2	0.6	2.8	2.2	3.0	2.8	0	0.2
14-day interval	0.6	0	0	0	0	0	0	0
28-day interval	0	0	0	0	0	0	0	0

Objective 2: Conduct controlled transmission biology experiments.

Greenhouse GRBV transmission bioassays. 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 period (AAP) and single insects were then transferred to GRBV-free plants for a 48-hour inoculation access period (IAP). 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 with qPCR at the end of 2017 and 2018, and no plants from the 2016 greenhouse transmission bioassay tested positive for GRBV.

The greenhouse bioassay was repeated in 2017 with modified methodology. In August 2017, rooted cuttings of GRBV-infected plants were infested with *T. wickhami* and *T. albidosparsus* adults. Following a six-day AAP, cohorts of five insects of the same species were put onto disease-free vines. The IAP was seven days, after which all insects of each cohort were placed onto previously uninfested GRBV-negative vines. At the end of each IAP, one cohort of each species was collected directly from a randomly selected infested vine and stored in 95% EtOH at -9 ºF (-23 ºC) 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. All vines used in the 2017 greenhouse bioassay were tested in Fall 2018 for presence of GRBV, and no vines tested positive for GRBV.

Cohorts of insects used in the 2017 greenhouse bioassay were tested using qPCR for presence of GRBV to evaluate whether virus particles can be uptaken by the treehopper species under examination, as well as to determine the persistence of the virus in the insect body. Insects of *S. festinus*, a confirmed treehopper vector of GRBV (Bahder et al. 2016), were provided a 6-day AAP on GRBV-infected vines at University of California – Davis, then immediately frozen, and then shipped on dry ice to OSU to serve as a positive control. Insects were prepared for genetic analysis as per Bahder et al. (2015) and then tested by qPCR for presence of GRBV using primers F1580 and 1693R to amplify virus DNA. The results showed that treehoppers provided an AAP of 6 days will uptake GRBV particles. The virus persisted within the tested treehopper species for the entire 6-week period in both *T. albidosparsus* and *T. wickhami* (Table 9).

Table 9. 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 IAP of the 2017 greenhouse infestation trial. Date indicates when insects were removed from host vines.

Species	Date	GRBV-Positive	Total	Percent infected
S. festinus	10/26/17	7	9	78%
	Subtotal	**7**	**9**	**78%**
T. albidosparsus	8/29/17	2	5	40%
	9/5/17	5	5	100%
	9/12/17	4	5	80%
	9/19/17	3	5	60%
	9/28/17	4	4	100%
	Subtotal	**18**	**24**	**75%**
T. wickhami	8/29/17	2	4	50%
	9/5/17	5	5	100%
	9/12/17	4	5	80%
	9/19/17	0	5	0%
	9/28/17	2	3	67%
	Subtotal	**13**	**22**	**59%**
	Total	**38**	**55**	**69%**

Because greenhouse infestation trials in 2016 and 2017 used insects in the adult stage, a study took place in Summer 2018 to test transmission of GRBV by immature treehoppers. *Tortistilus albidosparsus* nymphs were collected from the field on June 26 and were placed onto GRBV-positive rooted grapevine cuttings in the greenhouse on June 28. Following a 6-day AAP, the instar stages of all nymphs were estimated, and cohorts of 5 nymphs were transferred onto GRBV-negative grapevines to provide a 7-day IAP. Surviving nymphs were removed from grapevines on July 11, and their instar stages were estimated. Fifth-instar nymphs were then placed onto cover crop species maintained in small containers in a growth chamber.

Cover crop trials. Surviving 5th instar nymphs from the greenhouse bioassay were selected for placement on cover crops. Cover crops included frosty berseem clover (*Trifolium alexandrinum*), 'GO-MOB' red clover (*Trifolium pratense*), 'Oregon Trail' snap pea (*Pisum sativum*), 'Lonestar' annual ryegrass (*Lolium multiflorum)*, and 'Carlinda' turnip rape (*Brassica rapa*). Three replicates of each cover crop were used. Five nymphs were placed on the vegetation in each pot on July 11. Each pot was secured within an organza mesh bag that was tied closed at the top to prevent escape. Every 2 days the cover crops were examined for surviving insects. The majority of nymphs had died on turnip rape by July 17, and all specimens on this cover crop species had died by July 29 without a single individual molting into the adult stage. At the end of the observation period, most nymphs had emerged as adults in all other cover crop species (Fig. 6). By 6 August, only adults were remaining.

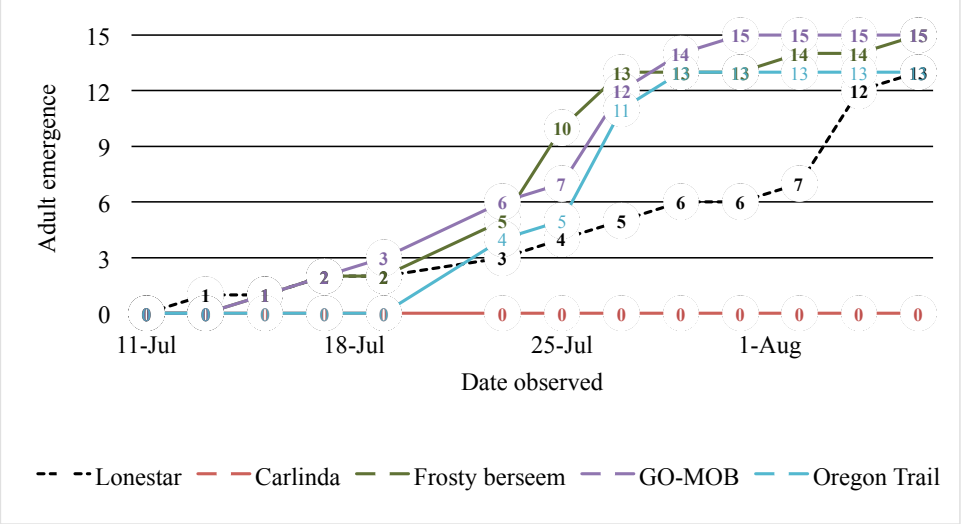


Figure 6. Emergence of adult *Tortistilus albidosparsus* in 2018 following placement on cover crop species *Lolium multiflorum* 'Lonestar'; *Brassica rapa* 'Carlinda'; *Trifolium pratense* 'GO-MOB', *Trifolium alexandrinum* 'Frosty berseem'; and *Pisum sativum* 'Oregon Trail'. Numbers indicate how many adults had emerged by corresponding date.

Virus movement within vine
A trial testing migration of virus particles within the grapevine was conducted beginning in September 2018. Adults of *T. albidosparsus* were placed onto field-grown GRBV-positive grapevines in small clip cages to provide a 6-day AAP. Insects were then individually caged onto petioles of GRBV-negative grapevines for a 24h IAFP or a 72h IAP. Representative insects were collected directly into 70% EtOH for further genetic analysis for presence of GRBV. The area of petiole tissue that was directly exposed to the insect within a clip cage was collected, and five additional samples from different areas of the infested grapevine were also collected. The procedure was repeated in October 2018 using *S. festinus* collected from southern Oregon. The samples were assessed for presence of GRBV particles to determine the dynamics of within-plant movement of GRBV, but results using qPCR were ambiguous. On one vine, the leaf below a *S. festinus*-infested leaf appeared positive following a droplet digital PCR (ddPCR). No other questionable samples from the qPCR assay tested positive using ddPCR.

Results to date have not shown evidence of GRBV infection mediated by *Tortistilus* species in the tested grapevines. Genetic analyses of insect samples and previously infested grapevine materials revealed that only the adult stage hosted GRBV particles. Associations with feeding damage in the field and persistence of GRBV within the insects continue to provide indirect support that *Tortistilus* treehoppers may be able to transmit the virus.

Objective 3. Obtain baseline information on current levels and extent of Red Blotch.
In 2018 we collected grapevine samples from plants at YV that had previously tested negative for GRBV in 2016. While visual symptoms were largely unchanged from the 2016 field survey, a small number of grapevines had become symptomatic by 2018 (see Table 5). The samples originated from a separate block at the same vineyard than had been previously assessed for spread of GRBV (Dalton et al. 2019).

Limited samples were received in 2017 from a grower in eastern Oregon. These samples tested negative for GRBV. Additional samples were received in October 2018 from a different vineyard in eastern Oregon and also tested negative for GRBV.

Objective 4: Extension of information of grapevine red blotch-associated virus, and insect vectors.
In 2017, 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. In 2018 outreach activities continued, primarily targeting local grower groups. 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. An article documenting the spread of virus over successive years of genomic analysis was recently accepted for publication in the journal Plant Disease (Dalton et al. 2019) and is currently *in press*. Several extension outreach activities were conducted during 2017. Additional results will 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 produced and pending, and presentations made that relate to the funded research

Publications

1. 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. 2019. Spatial associations of vines infected with grapevine red blotch virus in Oregon vineyards. Plant Disease 103(7):1507-1514. DOI: 10.1094/PDIS-08-18-1306-RE.
2. Dalton, Daniel T. and Vaughn M. Walton. Field phenology of *Tortistilus albidosparsus* in the periphery of a vineyard in the Willamette Valley. *In preparation*.

Presentations

- Nieri, Rachele, Daniel T. Dalton, Jessica Z. Buser, Samantha M. Nizich, Nik G. Wiman, and Vaughn Walton. 2019. A vibrational pest management strategy to control treehopper pests. OWRI Grape Day, Corvallis, OR. (April 2019)
- Daniel T. Dalton, Vaughn M. Walton, Richard J. Hilton, Rachele Nieri. 2019. In-field spread of *Grapevine red blotch virus* and associations with treehopper feeding damage. OWRI Grape Day, Corvallis, OR. (April 2019)
- Nieri, Rachele, Daniel Dalton, Jessica Buser, Samantha Nizich, Nik G. Wiman, and Vaughn Walton. 2019. The vibrational mating duet and the potential for a vibrational pest management strategy of treehopper pests. 103rd Annual Pacific Branch Entomological Society of America Annual Meeting, San Diego, CA. (April 2019)
- Dalton, Daniel, Richard Hilton, Rachele Nieri, and Vaughn Walton. 2018. Exploring insect vectors for red blotch disease. Oregon State University Vineyard Red Blotch Workshop, Salem, OR. (November 2018)
- Hilton, R. J. Vineyard and Orchard Integrated Pest Management. OVS Meeting, Medford, Oregon. Local, Invited. (February 2018)
- Hilton, R. J. Technical Meeting. Jackson County, Oregon. (April 2018)
- Hilton, R. J. Vineyard Pest Workshop. Jackson County, Oregon. (July 2018)
- Hilton, R. J. 2nd Annual Middle Rogue Integrated Pest Management Festival. Central Point, Oregon. (September 2018)
- Dalton, Daniel T., Richard J. Hilton, and Vaughn M. Walton. 2018. Field observations of *Tortistilus* species associated with Oregon vineyards. Poster presentation, OWRI Grape Day, Corvallis, OR. (April 2018)
- 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. Red blotch in vineyards, Plant Improvement Committee, The Dalles, Oregon. (January 2018)

- Daniel Dalton, Betsey Miller, Cherre Bezerra Da Silva, Dalila Rendon, Kyoo Park, Serhan Mermer, Gabriella Tait, Valerio Rossi Stacconi, Linda Brewer, and Vaughn Walton. 2018. Toward understanding of spotted wing drosophila and other insect pests in horticultural systems of Oregon, USA. Presentation, Justus Liebig University Weekly Seminar, Giessen, HE, Germany. (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.
- 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.
- 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.
- 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.
- Buser, Jessica Z., Daniel T. Dalton, and Vaughn M. Walton. 2017. Identification of grapevine red blotch virus in grape petioles using quantitive real time polymerase chain reaction. Poster presentation, Oregon State University Summer Undergraduate Research Symposium, Corvallis, OR. September 14, 2017.
- Dalton, Daniel T., Vaughn M. Walton, Richard J. Hilton, and Mysore R. Sudarshana. 2017. Implicated Vectors and Spread of Grapevine Red Blotch-associated Virus in Oregon Vineyards. Poster presentation, OWRI Grape Day, Corvallis, OR. April 6, 2017.
- Dalton, Daniel T., Richard J. Hilton, and Vaughn M. Walton. 2017. Implicated Vectors and Spread of Grapevine Red Blotch-associated Virus in Oregon Vineyards. Presentation, 101st Annual Pacific Branch Entomological Society of America Annual Meeting, Portland, OR. April 4, 2017.
- Vaughn Walton, Rick Hilton, Daniel T. Dalton, C. Kaiser, Brian Bahder, Kent M. Daane, Frank Zalom, and Mysore Sudarshana. 2017. Integrative Studies of Vector-Related Virus Epidemiology. Presentation, Oregon Wine Industry Symposium, Portland, OR. February 21, 2017.

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 spread of GRBV. 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 and Results by Objective

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

Distribution, non-crop host plants, and seasonal phenology of candidate vector insects of GRBV was determined in 2017 and confirmed in 2018. The complete lifecycle of *T. albidosparsus* was identified. The insects overwinter as eggs in perennial host plants. Nymphs hatch from eggs, move to plants including vetch and wild carrot, and develop to adults. Adults use woody hosts such as oak, apple, and grapevine for reproduction in the fall. Insects were captured in 2017 by a combination of collection techniques including vacuum sampling, sweep netting, sticky trap monitoring, and observing feeding symptoms on vines. In 2018, collections were best carried out by visual searches for nymphs and adults. GRBV was found to be spreading in southern Oregon and Willamette Valley winegrape production sites (Dalton et al. 2019 and unpublished data).

2. Conduct controlled transmission biology experiments.

Greenhouse transmission trials showed persistence of GRBV in the candidate insect vector species for at least five weeks after acquisition. Additional transmission biology experiments were conducted in the greenhouse in 2017 and in 2018. Testing of greenhouse materials is ongoing. It is anticipated that grapevines infested in 2018 with virus-containing treehoppers will be tested in summer 2019 for presence of GRBV. To date, successful transmission of GRBV by *Tortistilus* treehoppers remains elusive.

3. Obtain baseline information on current levels and extent of Red Blotch.

Vineyards in southern Oregon were surveyed for symptoms of GRBV and GLRaV. Genetic testing of symptomatic vines is ongoing to determine degree of co-infection of the two viruses. Plant samples from Eastern Oregon tested negative for GRBV. Field materials from Southern Oregon and the Willamette Valley are currently being retested.

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

An article was recently accepted documenting the spread of virus over successive years of genomic analysis (Dalton et al. 2019) and is currently *in press*. Several extension outreach activities were conducted during 2017 and 2018. Several presentations have been given at scientific meetings and public research expositions.

Funds Status:
All funds were spent based on the project budget.

Summary and status of intellectual property associated with the project.

None

Literature cited

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