

1. Project Title: Integrative studies of vector-related field epidemiology for grapevine red blotch-associated virus and grapevine leafroll-associated virus in Oregon.

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Introduction:

There is at present little information regarding current infection patterns of virus in Oregon winegrape vineyards. Information on such patterns of infection will provide clearer indication of the epidemiology of both grape leafroll-associated virus (GLRaV) and grape red blotch-associated virus (GRBaV). Newly formulated PCR primers are available to determine the genomic status and spatial distribution of vines infected with GRBaV and GLRaV in Oregon vineyards (Krentz et al. 2012, Rwahnih et al. 2013). The temporal and spatial distributions of insects that are vectors of GLRaV strains have been recently determined (Walton et al. 2013, Dalton et al. 2014), but this information is currently unknown for GRBaV.

Work investigating grapevine viruses of economic importance is coordinated with other research dealing with vector transmission biology (Kent Daane), virology and non-crop hosts (Sudarshana Mysore, Frank Zalom), and viticulture (Rhonda Smith). These studies aim to create a better understanding of the regional epidemiology and importance of GLRaV, GRBaV, and their respective potential insect vectors in Oregon. Our California collaborators are doing important work on the most probable insect species that transmit the Geminiviridae (Ghanim et al. 2007, Chen and Gilbertson 2009, Cilia et al. 2012) including leafhoppers that may vector GRBaV (Poojari et al. 2013). Our goal is to build on the current information available on the potential

insect vectors, with focus on their seasonal presence and spatial distribution in Oregon vineyards. In addition, we will supply our California collaborators with preserved insects during 2014-2015.

A significant goal of this work is to direct a future control program for GLRaV and GRBaV, and is specifically aimed to provide timely updates and management tools for wine grape growers regarding management of these two viruses. At this time, there is no accurate information on the epidemiology of GRBaV in vineyards. It is not clear which insects may spread this virus, whether it originated from infected plant materials or from non-crop host plants. Materials collected in Oregon will accelerate ongoing studies in California, which are aimed at testing the importance of possible insect vectors driving virus epidemiology.

Researchers at UC Davis and Cornell are studying the disease's impact on fruit quality and yield. We will coordinate and complement the work by the virologists and entomologists on these teams. This project will focus only on the historical and future field distribution of insect vectors, GLRaV and GRBaV viruses. We will continue our field collections of potential vector insects and supply information from Oregon to the entomology groups in California who are conducting the transmission studies of insect vectors.

Objectives:

- 1. Follow insect vector distribution and disease progression in established vineyard plots for grape red blotch-associated virus (GRBaV) and grapevine leafroll-associated virus (GLRaV) in Oregon.**
- 2. Extension of information on the importance of vectors, GLRaV and GRBaV in Oregon vineyards.**

Materials and methods

Samples were taken from two study blocks in Southern Oregon and were analyzed for both viruses in 2014. Due to high virus incidence, one study block was taken out of production (Vineyard 1). A separate vineyard in Southern Oregon was selected as a replacement site for the 2015 season. In the Willamette Valley, one block was analyzed during 2014 and an additional block was included for analysis during 2015. Two sites in the Columbia Basin were included in this study. The five vineyards that represent Southern Oregon, the Willamette Valley, and Eastern Oregon were surveyed for both insects and virus status during 2014.

i. Insect vector distribution and species composition

Potential insect species that may vector viruses were surveyed by using yellow sticky traps placed along the border (4 border traps/vineyard) and center (2 center traps/vineyard) of selected blocks. Trap collections were conducted once per month starting in May and continuing until September (5 sampling dates), typically the period when most of the candidate insect vector species are present at higher population levels. Potential insect vector sampling was additionally conducted using sweep-netting techniques during July of 2015. Here we used a systematic sampling pattern in a minimum of 40 locations of each study vineyard. Sweep net-collected insects for each sampling location and date was directly placed into separate vials containing

95% ethyl alcohol. These samples were investigated under a stereomicroscope to determine species composition using morphological characters as highlighted in Saguez et al. (2014). A subsample of vector insects was shipped to California entomologists for viral detection. All sampled vineyards contained at least 32 rows and 22 pole-to-pole “bays” (Charles et al. 2009) and 3-6 vines per bay. Data collected during 2014 will be combined with 2015 data of all potential insect vectors and analyzed using standard ANOVA in order to describe the presence of insects similar to the methodology described in Walton et al. (2013).

ii. Disease progression in established vineyard plots for grape red blotch-associated virus (GRBaV) and grapevine leafroll-associated virus (GLRaV) in Oregon.

Newly formulated PCR primers were used to determine the genomic status and spatial distribution of GRBaV together with that of GLRaV in Oregon vineyards (Krentz et al. 2012, Rwahni et al. 2013). Two hundred vines per vineyard were systematically sampled and plant materials placed in an -80°C freezer within 24 hours of collection. Leaf petiole samples from each of the sampled vines were annotated and shipped to California virologists for genomic verification of virus status. The spatial distribution from analyzed plant materials was assessed using SADIE (Spatial Analysis by Distance Indices, Perry 1995, Perry 1996, Perry & Dixon 2002) in order to document any changes in distribution over time. Non-parametric spatial analysis was conducted in order to describe general spatial trends of virus infestation levels as indicated by the collected plant materials. The SADIE analytical procedure (Perry 1995) was used to determine the overall index of aggregation, I_a , of each type of virus. When I_a is near to unity, random arrangement of virus is observed. Values larger than unity indicated aggregated arrangement. Regular arrangement is indicated by values smaller than unity (Perry 1996; Maestre and Cortina 2002; De Villiers 2006). The dimensionless index of clustering, v_{ij} , measures the degree of clustering in areas with above-average density of virus, i.e., patches (v_i are values at or above 1.5; light colored areas in Figures 1-4) or areas with below-average density, i.e., gaps (v_j are values below -1.5; dark colored areas in Figures 1-4) (Winder et al. 2001, 2012; Maestre and Cortina 2002; De Villiers 2006). Values of v_{ij} between -1.5 and 1.5 indicate randomness (Winder et al. 2001; Perry and Dixon 2002; De Villiers 2006). To test for non-randomness, the mean values of the clustering indices, \bar{v}_i and \bar{v}_j were used. Patches of relatively high virus density are indicated by SADIE coordinate output values larger than 1.5, randomness indicated by coordinate values between 1.5 and -1.5, and gaps are indicated by coordinate values less than -1.5. Significant clustering, random association, and gaps were visually illustrated by inputting SADIE cluster analysis coordinated into Surfer® v12 (Golden Software, Inc., Golden, CO) and using the inverse distance weighted method.

Results

i. Insect vector distribution and species composition

The sweep-net sampling and sticky card monitoring in Oregon vineyard study blocks in 2014 displayed the presence of several species of insects that may potentially vector GRBaV (Table 1). These leafhoppers include the grape leafhopper, Western grape leafhopper and potato leafhopper. Separate genomic tests of potential GRBaV insect vectors from California showed the presence of the virus in at least one of the suspected insect vector species present in Oregon collections

(Zalom, pers. comm.). Currently no information is forthcoming from insect virus status from the collected samples. Subsamples of insects collected in Oregon are being analyzed using PCR to determine whether they contain GRBaV. By coordinating with our California collaborators, we have identified potential insect vector species (Almeida and Purcell 2003a, b) that commonly occur in Oregon vineyards by means of surveys and trap counts (Table 1). *Empoasca spp.* (grape leafhopper, 20%) and *Erythroneura elegantula* (Western grape leafhopper 18%) are leafhopper species that were consistently collected in all of the grape-growing regions. Also of note is the presence of aphids in all regions. Past work on viruses similar to GRBaV (family Geminiviridae) have been recorded as being vectored by leafhoppers (Watanabe and Bressan 2013). Such viruses rely on hemipteran vectors and can be examined for translocation using real-time PCR and immunofluorescence assays on dissected tissues. Time-course experiments and transmission assays coupled with these genomic tests have proven useful for assessing aphid vectors such as the aphid *Pentalonia nigronervosa* known to transmit banana bunchy top virus (Watanabe and Bressan 2013).

Regarding spatial distribution of leafhoppers, it is impossible to show clustering or gaps of insects due to their high levels of mobility. Leafhoppers are known to disperse significantly in all vineyards.

ii. Disease progression in established vineyard plots for grape red blotch-associated virus (GRBaV) and grapevine leafroll-associated virus (GLRaV) in Oregon.

Virus incidence (both types) of the five tested vineyards in 2014 were inconsistent. In Southern Oregon the GRBaV and GLRaV virus incidence were 43% and 3.5% (Vineyard 1), 11.5% and 0% (Vineyard 2) respectively. At the tested Willamette Valley site, GRBaV and GLRaV virus incidences were 32.16% and 3%. In the Columbia Basin GRBaV and GLRaV virus incidence were 0% and 0.5% (Vineyard 1), 0% and 8% (Vineyard 2) respectively.

In Southern Oregon vineyard 1 (Figure 1) GLRaV was concentrated in the southwestern corner, and red blotch was predominantly in the northeastern corner of the study vineyard block. This vineyard was subsequently removed because of high virus incidence. In Southern Oregon vineyard 2 (Figure 2) no GLRaV was detected, but GRBaV was found mainly in the southeast and northeast corners of the sampled area. In the Willamette Valley vineyard 3 both GLRaV and GRBaV were found. GRBaV was found in the southwest to middle section of the vineyard. GLRaV was found in a comparatively smaller area in the western portion of the block.

In the two vineyards sampled in the Columbia Basin (Figure 3), only GLRaV was found in both blocks, with no GRBaV. GLRaV in vineyard 4 was situated in the southeastern corner of that block. In vineyard 5 GLRaV was found in two large portions towards the middle northern and middle southern portions of the vineyard. There were slightly smaller portions of virus towards the western portion of that block.

Our collaborating growers in all regions have noticed that vines symptomatic of one virus or the other consistently had longer ripening times and lower °Brix as harvest approached. Currently,

studies of crop quality are being conducted in California and Washington state. In Oregon, preliminary evidence shows a negative impact of virus presence associated with vine photosynthesis (Pagay pers. comm.).

Objective 2. Extension of information on the importance of vectors, GLRaV and GRBaV in Oregon vineyards.

Results will be provided to growers, grape industry representatives and OSU Cooperative Extension personnel through grower reports and seminars. In addition, information from this work was held at a regional vineyard workshop in the Willamette Valley during 2014. Additional winter meetings were held in Medford (organized by Rick Hilton) and Milton-Freewater (organized by Clive Kaiser) as well as in Roseburg (organized by Steve Renquist). Similar meetings are planned at the annual Scouting workshop to be held in Medford during July 2015. Monthly conference calls organized by Dr. Patricia Skinkis are being used in order to share new information on virus epidemiology and vector status. Results will be published in popular and scientific journals during winter 2015.

Discussion

It is clear from the vector insect collections that leafhopper species studied in California are similar to those found in Oregon vineyards. Current information coming from insect collections is limited. This may be due to poor virus acquisition ability by insect vectors, and also because of the current limited funding provided to work on possible insect vectors. It is possible, however, from the spatial analysis of vine-virus status that distribution patterns (e.g. Figure 1 a, b) are not regularly distributed within vineyard blocks and that clusters of virus-infected plants can be found in nearly all vineyards that were sampled in Oregon. For these reasons we believe that the distribution of both viruses suggests vector dispersal, but the differing distribution patterns found within vineyards suggest different vector insects are associated with each of the viruses. This spatial distribution describes statistically significant patching and gaps of both virus types (Fig. 1; Perry & Dixon 2002) in the affected Oregon vineyards. The mealybug vectors of GLRaV, their seasonal phenology and Oregon distribution are known (Walton et al. 2013). Currently the relationship between GLRaV and mealybug infestation is emerging. However, no such clarity currently exists for potential vectors of GRBaV, and as of yet detailed knowledge of field epidemiology of this disease is lacking. Specific to this topic, our data additionally indicate that GRBaV-infected plants are concentrated toward the edge of the sampled vineyard blocks. In some cases it is clear that infected plants may have served as the source of initial infection. Radiation of virus-infected plants may have occurred over time. However, the link between specific insect vectors and spread of GRBaV is not clear. Additional genomic analysis of aphids, white flies and leafhoppers will hopefully provide more clarity on this aspect of virus epidemiology. The possible impacts of alternative host plants and their role on spread of GRBaV need to be further investigated.

The new genomic diagnostic tools allowed us to determine spatial distribution of both virus types in all surveyed vineyards. Current data and virus distribution patterns lead us to believe that insects are the responsible vectors of both viruses (Cabaleiro et al 2006, Sharma et al 2011). Continued work and subsequent surveys of red blotch distribution of virus-infected plants is essential in order to better understand the epidemiology related to the importance of insect vectors or alternate host plants in Oregon vineyards. Our analysis can additionally describe the association or dissociation of symptomatic vines with populations of potential virus vectors. We believe that this information will support a better understanding of the role of insects as possible vectors of both GLRaV and GRBaV.

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Table 1. Potential grapevine red blotch-associated virus insect vector species found in Oregon vineyards during 2014.

<u>Species (common name)</u>	<u>% composition</u>
<i>Deltocephalus grex</i>	26.8
<i>Empoasca spp.</i> (grape leafhopper)	20.0
<i>Erythroneura elegantula</i> (Western grape leafhopper)	18.0
<i>Exitianus exitiosus</i>	18.0
Cercopoidea (froghoppers)	8.2
Aphids	2.8
<i>Fieberiella florii</i>	2.6
<i>Helochara deltoides</i>	2.1
<i>Athysanus argentarius</i>	0.7
<i>Deltocephalus spp</i>	0.2
<i>Empoasca fabae</i> (potato leafhopper)	0.2
<i>Gyponana praelonga</i>	0.1

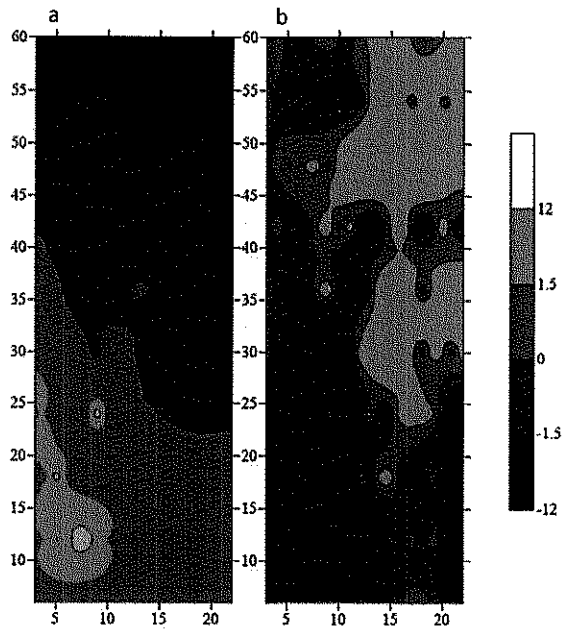


Figure 1. Vineyard 1 in Southern Oregon (subsequently removed). Grape Leafroll-associated Virus (a) and Grape Red Blotch-associated Virus distribution (b) as recorded during 2014. Dark areas with levels below -1.5 indicate significant gaps of virus-infected vines. Light areas with levels above 1.5 indicate clusters of virus-infected vines.

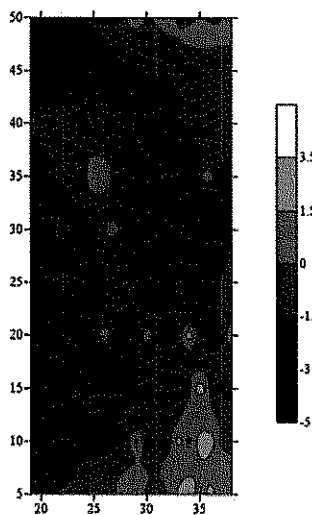


Figure 2. Vineyard 2 in Southern Oregon. No Grape Leafroll-associated Virus was found in this block, Grape Red Blotch-associated Virus distribution is shown as recorded during 2014. Dark areas with levels below -1.5 indicate significant gaps of virus-infected vines. Light areas with levels above 1.5 indicate clusters of virus-infected vines.

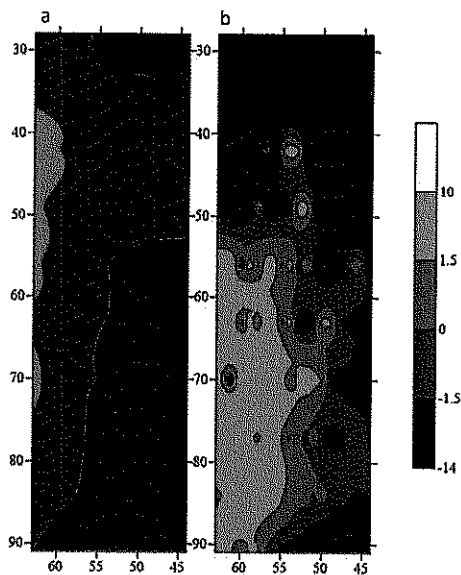


Figure 3. Vineyard 3 in Willamette Valley, Oregon. Both Grape Leafroll-associated Virus (a) and Grape Red Blotch-associated Virus (b) was found in this block, distribution is shown as recorded during 2014. Dark areas with levels below -1.5 indicate significant gaps of virus-infected vines. Light areas with levels above 1.5 indicate clusters of virus-infected vines.

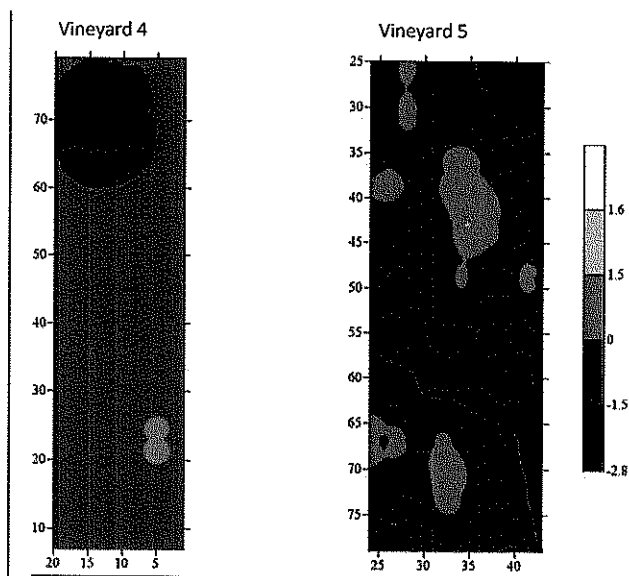


Figure 4. Two vineyards in the Columbia Basin, Oregon. Only Grape Leafroll-associated Virus was found in these vineyard blocks, and virus distribution is shown as recorded during 2014. Dark areas with levels below -1.5 indicate significant gaps of virus-infected vines. Light areas with levels above 1.5 indicate clusters of virus-infected vines.