



# VITICULTURE & ENOLOGY

## TECHNICAL NEWSLETTER

FALL 2019

### Welcome to the Fall 2019 Newsletter

This edition contains research updates and a comprehensive list of publications summarizing research conducted by faculty of the Oregon Wine Research Institute at Oregon State University. Dr. Bob Martin, Research Plant Pathologist (Virology), USDA, opens the newsletter with a research update on grapevine red blotch disease that provides growers with management suggestions. Dr. Vaughn Walton, OSU horticultural entomologist, writes about a novel tool for the management of spotted-wing drosophila in vineyards. We wrap up the newsletter with a list of new publications authored by Oregon Wine Research Institute researchers.

This issue is posted online at the OWRI website <https://owri.oregonstate.edu/owri/extension-resources/owri-newsletters>. Learn more about our research and outreach programs as well as core faculty contact details [here](#).

Cheers,  
The OWRI Team

### Grapevine Red Blotch Disease - New Detection Method and Management Suggestions

*Dr. Robert R. Martin, Research Plant Pathologist/Virology, USDA-ARS Horticultural Crops Research Unit, Corvallis, OR*

**The Virus:** Grapevine red blotch virus (GRBV) is the causal agent of grapevine red blotch disease (GRBD). GRBD was confused with grapevine leafroll disease for many years, but was recognized as a distinct disease in Cabernet Sauvignon in the Napa Valley in 2008. GRBV was described in 2012 initially from New York and then from California. It was shown to be the causal agent of GRBD in 2015 by Dr. Marc Fuchs' lab at Cornell University (Yepes et al. 2018) when they completed Koch's postulates, isolated GRBV from infected vines, propagated it in culture, reinoculated the GRBV into healthy vines, recreated the disease, and then isolated the same virus from these inoculated vines. GRBV is now known to occur in all grape production regions in Oregon and has been found widespread in the US (Sudarshana et al. 2015). It has also been reported from Canada, India, Mexico, and South Korea. The virus has features similar to members of the *Geminiviridae* family of plant viruses, all of which contain small circular DNA genomes.

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GRBV is the member of a new genus, *Grablovirus* in the *Geminiviridae* family. In 2017, a second virus in this genus, named Wild Vitis Virus-1 (WVV-1), was characterized from wild grapevines in Napa County, California. At this time, the WVV-1 has been detected only in wild grapevines and not in nearby commercial vineyards. However, GRBV has been detected in wild grapevines, though it is not known if it has moved from commercial vineyards to wild grapevines or vice versa. The importance of wild grapevines as a reservoir for GRBV transmission to commercial vineyards is unclear.

**Symptoms:** The symptoms of GRBD are similar to those caused by Grapevine leafroll associated viruses in red-fruited cultivars, but there are several subtle differences. Leaves of grapevines infected with GRBD often lack downward curling and the veins often turn reddish rather than staying green as observed with grapevine leafroll disease. Growers should be aware that these differences in symptoms are generalities, and the two diseases can easily be confused based on symptoms alone. In white-fruited cultivars, symptoms of GRBV infection is much more subtle; there can be interveinal chlorosis later in the season, which may become necrotic, especially in Chardonnay. Symptom severity can vary greatly depending on other stresses the plants are under. Water stress combined with GRBV results in more severe symptoms and a greater impact on fruit quality compared to GRBV infected plants without water stress (Levin and KC 2018). Thus, symptoms can vary considerably from year to year. We recommend that grapevines be tested to confirm the cause of the symptoms before any management decisions are made, since reddening of the foliage can be caused by multiple factors including nutrient deficiencies, girdling, other viruses, trunk diseases, etc.

In many cases, the major impact of GRBV is on fruit quality rather than yield, though reduced yield has been reported in vines infected with GRBV. Fruit from vines infected with GRBV often have delayed and uneven ripening. In cool climate areas, the delayed ripening is a concern, as fruit will not have sufficient time to ripen before the threat of rains or frost late season. Fruit juice from GRBV-infected grapevines typically has reduced sugars (Brix) when

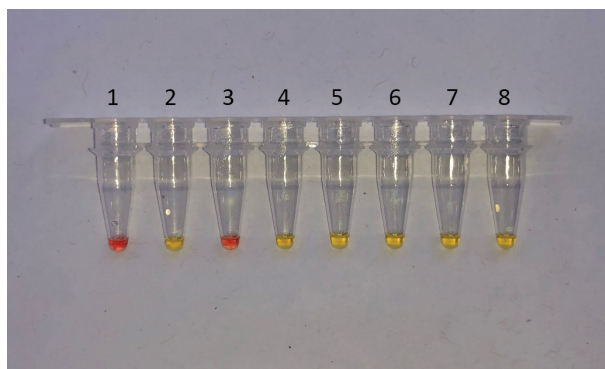
compared with uninfected grapevines. Reduction of up to 7 °Brix has been observed in some vineyards in California. In Oregon, differences from 0-2 °Brix have been observed. Additionally, increased acidity and reduced amounts of anthocyanins and phenolic compounds may lead to reduced fruit and wine quality. The reduction in wine quality can result in reduced value of a wine by greater than 50% (Franson 2014). Efforts to mitigate the effect of GRBV on fruit quality, such as the application of abscisic acid to clusters at véraison, have not been successful in enhancing fruit maturity or phenolic profiles (Skinkis, Levin, Osborne, and Qian, in progress).

**Detection:** GRBV detection by polymerase chain reaction (PCR) was documented in 2012, and since that time there are several commercial diagnostic laboratories that provide testing for GRBV as well as other viruses of grapevines. Sampling is critical for accurate virus detection since it has been shown that young tissues early in the season have lower virus titers that can lead to false negative results. Uneven distribution of the virus in grapevines can also lead to false negatives if sampling is not done appropriately. For optimal testing results, multiple older leaves from the base of canes on both sides of the trunk should be sampled, preferably late in the season (Setiono et al. 2018, KC in progress). With the LAMP assay for GRBV described below, we detected GRBV reliably in young leaves early in the season in Pinot noir and Pinot gris (mid-May).

In early 2019, a Loop-Mediated Isothermal Amplification (LAMP) method for GRBV was developed by Keith Perry's lab at Cornell University (Romero Romero et al. 2019). This technique is much more sensitive than PCR, has simple sample extraction methods that only requires water (not more sophisticated or toxic reagents), and requires basic lab equipment, including several pipettes and a heating block. This method is simple enough to set up in a winery laboratory or on a table top. The LAMP assay takes about 2 hours to complete and costs about \$2.50 per test. A positive reaction results in a color change from pink to yellow (Figure 1). The results of the assay in the figure shows a positive result for GRBV detection in composite samples of eight leaves when only one of the 8 leaves was positive (tubes 7 & 8 in Figure 1). I recommend composite



samples of 4 leaves with this assay as a reliable test, as we did have an example where 1 infected and 7 healthy leaves in a composite sample gave a negative result. I also recommend that the leaves be folded in half before sampling. The GRBV status of the plants used for the test shown in Figure 1 was confirmed by traditional PCR analysis in my lab.



**Figure 1.** End result of a LAMP assay for grapevine red blotch virus in Cabernet franc. Yellow color indicates virus presence (GRBV+). From left to right the microtubes reflect the following: 1. healthy leaves with no virus, 2. GRBV infected leaves, 3. water control, no leaf tissue, 4 and 5. one infected leaf and three healthy leaves mixed together in a sample, 6. one infected leaf and one healthy leaf, 7 and 8. one infected leaf and seven healthy leaves. All samples were confirmed as accurate by traditional PCR in the Martin Lab.

A major concern with the LAMP assay is the extreme sensitivity and potential for contamination. If doing this assay in your own facility, it is best to compare results with standard PCR testing by an external lab to get started. Also, it would be good to retest positives to avoid questions of contamination, or at least repeat the positive results to confirm. We have carried out three workshops where vineyard owners, vineyard managers, consultants and researchers have used this technique successfully. Several of these people plan to set up this assay in their own facilities with the goal of testing symptomatic vines before implementing a management plan.

**Vectors:** Transmission of GRBV by two vectors, the Virginia creeper leafhopper (*Erythroneura ziczac*) (Poojari et al. 2013) and the three-cornered alfalfa hopper (*Spissistilus festinus*) (Bahder et al. 2016) has been reported by researchers. At this time, only the three-cornered alfalfa hopper has been confirmed as a vector of the virus in a lab setting. Neither of these insects has been documented to transmit the virus in the field. Thus, the

importance of these insects in spread of the disease in vineyards is still uncertain. There was an association between the presence of viruliferous (virus carrying) three-cornered alfalfa hoppers and virus spread in the vineyard (Cieniewicz et al. 2018). In Oregon, trials using healthy potted trap plants that were placed in vineyards with high incidence of GRBV at monthly intervals over two growing seasons resulted in only one of 630 trap plants becoming infected. Thus, timing of transmission in the field could not be determined from these trials. Considerable effort has gone into examining several *Tortistilus* species as vectors of GRBV since they have been observed in Oregon and California vineyards (Walton in Oregon and Zalom in California). Jana Lee (USDA-ARS, Horticulture Crops Research Unit) has collected a broad range of insects from a vineyard with a high incidence of GRBV and using them in transmission trials. At this time these projects have not resulted in identification of additional vectors of GRBV. There are still many unknowns about the transmission of GRBV in vineyards, but apparent spread of the virus has been documented in Oregon and California.

In other virus-vector systems, such as luteoviruses and their aphid vectors, there can be dramatic differences in transmission efficiencies between biotypes of the same aphid species of the same virus (Brumfield et al. 1992). An area of future vector work for GRBV transmission may be to collect multiple sources of the three-cornered alfalfa hopper and the Virginia creeper leafhopper and carry out transmission trials to examine the possibility that there are populations of one or both of these insects that are efficient vectors of GRBV.

**Management and Economics:** The most important step in the management of GRBV is to start with clean planting material. Many young vines may not express symptoms, and rootstocks remain symptomless. It is essential that the rootstock and scion be free of GRBV. In a study led by Miguel Gómez at Cornell University (Ricketts et al. 2017), the economic cost of GRBD ranged from a low in eastern Washington (~\$900/acre) when disease incidence was low and there was a small price penalty, to a high (~\$28,000 per acre) when the incidence of the disease was high and there was a high price penalty, such as in California's



Napa Valley. It was suggested that roguing of symptomatic vines and replanting with clean vines maximized profits if GRBD incidence was less than 30%. If the incidence of GRBD was greater than 30% a full vineyard replacement was recommended. The management plan for GRBD vineyards will depend on the production economics of individual vineyards and regions, and will require complex consideration.

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## Assessing a novel tool for the management of spotted-wing drosophila (*Drosophila suzukii*) in Pinot noir grapes

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Spotted-wing drosophila (SWD, *Drosophila suzukii*) is an established insect pest species that warrants more sustainable control methods. SWD can lay eggs and feed on damaged grape berries during the harvest period. SWD feeding and oviposition can result in increased populations of spoilage bacteria in wine grapes, particularly when fruit integrity is impacted due to cracking, fungal diseases, hail injury, and bird damage (Barata et al. 2012, Ioriatti et al. 2017, Hall et al. 2018). This problem is exacerbated in production regions such as Oregon where rain and high humidity during the harvest period increases disease and spotted-wing





*Drosophila* egg-laying pressure (Ioriatti et al. 2015). Because of the potential wine spoilage issues associated with SWD damaged grapes, it is important to develop SWD management strategies to employ in the vineyard (Blood 2019, Kirkpatrick et al. 2017, Lee et al. 2011). This study evaluated the efficacy of a novel SWD management tool, a pesticide-free arrestant (Tait et al. 2018), under Oregon vineyard conditions. This tool can be used for a variety of fruit, but little information is available concerning its effectiveness on wine grapes.

**Results:** The arrestant was trialed under high-pressure laboratory and field conditions, as well as under larger open-field conditions. Fully intact and compromised (incised with a scalpel blade) Pinot noir berries were exposed to SWD either in the presence or absence of the arrestant.

*Laboratory experiments.* In laboratory trials, ventilated containers containing fruit in the present or absence of the arrestant were used. The laboratory trials resulted in a reduction compared to the untreated control in oviposition levels in intact fruit. Fruit infestation in treatments with the arrestant had a 43.4% reduction in oviposition per berry compared to berries where no arrestant was used. In compromised fruit there was a 62.5% reduction in oviposition when the arrestant was used. The treatment, presence or absence of the arrestant, was the only factor that resulted in significant differences in fruit oviposition (Figure 1).



**Figure 1.** The experimental setup of the laboratory trials. Ventilated arenas were constructed from plastic beakers that contained ventilation holes and a plastic tube connected to a vacuum pump to ensure airflow (Tait et al. 2018). 12 adult *Drosophila suzukii* (6 males and 6 females) were released into each arena for a 24-hour period. Each arena contained 3 Pinot noir grapes and either 5 mL of water (control treatment) or 5 g of the arrestant. There were 10 replicates per each treatment per trial for all three trials.

*Field experiments.* Field trials were conducted under controlled and open-field conditions. Overall, treatments using the arrestant resulted in a reduction in the number of eggs laid under commercial-standard conditions.

*Controlled field experiments* were conducted by exposing enclosed berry clusters to SWD both in the absence and presence of the arrestant (Figure 2).



**Figure 2.** The experimental setup of controlled field trials. Trials were conducted at the OSU Botany and Plant Pathology research farm on Pinot noir wine grapes during September 25 – 28 and October 8 – 11, 2018. Grape clusters consisting of 20 – 25 fruits were exposed to 12 adult *Drosophila suzukii* (6 males and 6 females) for a 72-hour period per replicate. There were 20 replicates per treatment.

In intact fruits, the presence of the arrestant resulted in a 30.8% reduction in oviposition. Likewise, in compromised fruits, the presence of the arrestant resulted in a 52.6% reduction in oviposition.

*Open field experiments* were conducted by placing arrestant dispensers at a rate of fifty per acre in ~4 acres over a 14 day period (Figure 3).



**Figure 3.** Open field experiments were conducted in a Pinot noir vineyard in Yamhill County, Oregon during September 20 to October 4, 2019. Dispensers were placed at the base of vines at the rate of 50 per acre during the experimental period.



Initial counts before the application of the dispensers showed a 7X higher level of eggs in plots that were destined for arrestant treatments compared to control plots. After 14 days, intact berries in the presence of the arrestant displayed a statistically significant 83.4% reduction in oviposition. In compromised fruits, the presence of the arrestant resulted in a non-significant numerical 25.6% reduction in oviposition. Larval infestation in treated fruit was 63% lower than in control fruit. For compromised fruit, there was no statistical difference in larval berry infestations between treatments.

In conclusion, the use of a arrestant resulted in a reduction of both oviposition and fruit infestation levels by SWD in wine grapes. This reduction was observed in the laboratory and in the field under all experimental conditions, suggesting that the arrestant is attractive enough to SWD to sequester them away from grapes. The arrestant was effective in field conditions for up to 14 days. Weather conditions did not appear to affect efficacy levels. A reduction in spotted-wing drosophila activity and oviposition on the fruit could potentially decrease the negative effects of larval feeding and the vectoring of spoilage bacteria in commercial vineyard settings by disrupting the normal activities of SWD.

These findings indicate that the arrestant has the potential to significantly reduce SWD feeding and oviposition activities in commercial vineyard settings. This could reduce the vectoring of spoilage bacteria by SWD to grape berries reducing the risk of wine spoilage. Additional work is needed to determine the impacts of a reduction of SWD field activity on fruit quality during the harvest period.

*This study has been funded by the Oregon Wine Brotherhood.*

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## Research publications

Results of research are published in peer-refereed academic journals, peer-reviewed reports, or books, which validates the scientific work of the authors. The following articles describe research conducted by members of the Oregon Wine Research Institute at Oregon State University.

### Plant pathology and entomology

Dalton DT, Hilton RJ, Kaiser C, Daane KM, Sudarshana MR, Vo J, Zalom FG, Buser JZ, Walton VM. 2019. [Spatial associations of vines infected with grapevine red blotch virus in Oregon vineyards](#). Plant Dis 103:1507-1514.

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