WHY 'CLEAN' PLANTS?

The saying "you reap what you sow" holds true for the grape and wine industry. 'Clean', healthy grapevines produce quality grapes for making premium wines and juices. Grapevine viruses can cause serious problems affecting quality and quantity of fruit. Planting 'clean' plants is the first line of defense against viruses, associated diseases, and economic loss.

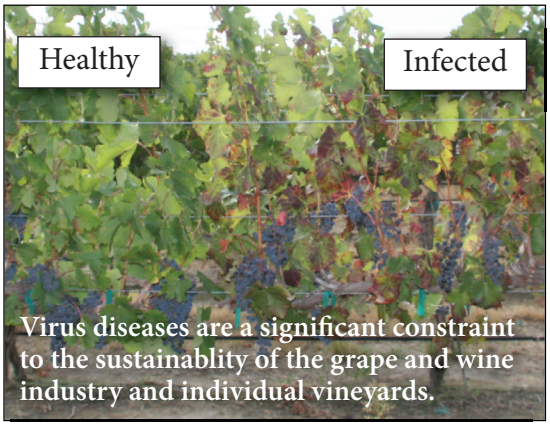
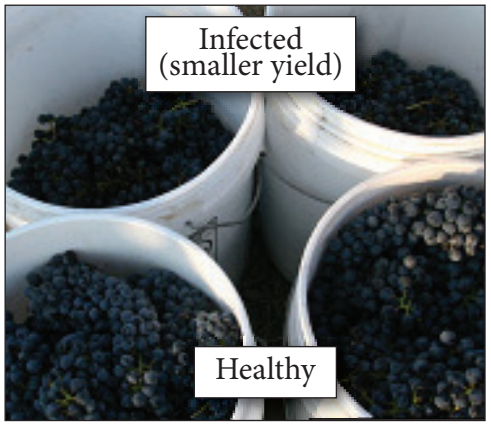


Fig. 1. **In Merlot, a grapevine affected by GLRD shows poor vigor compared to a neighboring healthy grapevine.**

Fig. 2. **Yield in a GLRD affected grapevine is significantly less when compared to yield from a healthy grapevine.**



IMPACTS

- Shorter vineyard life span and reduced yield. For example, cumulative yield per vine measured at harvest from 2008 to 2011 in a Merlot vineyard showed a 15-30% yield reduction from vines affected by Grapevine leafroll disease (GLRD) (*Fig. 1 and 2)*
- Clusters may be smaller and ripening can be uneven and delayed in vines infected by the Grapevine leafroll associated viruses (*Fig. 3*)
- Grapes produced on GLRD affected vines are of lesser quality due to reduced sugars, lower levels of pigments, and higher levels of acids
- Wine quality is reduced when grapes produced by infected vines are used for winemaking
- Results in loss of economic return to growers and wineries

Fig. 3. Viruses significantly impact fruit quality. Uneven ripening of berries is common in red-fruited varietals. Both red and white varietals commonly have smaller clusters due to infection with Grapevine leafroll associated viruses.

PREVENTION

Viruses are spread in nurseries and newly-established vineyards through the use of infected cuttings (*Fig. 4*). Planting 'clean' cuttings from reliable sources, like certified cuttings from nurseries, is the first step toward controlling diseases caused by viruses. If you plan to take cuttings from existing vineyards to plant new vineyards, it is important to have them tested for viruses before taking cuttings. Remember, planting a healthy vineyard is the foundation for advancing economic prosperity for growers and sustainable growth of Washington's grape and wine industry (*Fig. 4*).

Fig. 4. **A newly planted vineyard of Cabernet Sauvignon with one plant showing GLRD symptoms, indicative of disease spreading via infected planting materials.**

DIAGNOSIS

Never assume cuttings are virus-free based on a healthy appearance. Trying to identify viruses based on visual symptoms can be unreliable due to variation in the symptoms and the presence of multiple viruses (*Fig. 5*). Accurate diagnosis based on scientific methods is critical in determining the virus-infection status of grapevines.

Fig. 5. **A Pinot Noir leaf is showing symptoms of GLRD and fanleaf disease. Multiple disease symptoms that are a result of mixed virus infections are common, making specific disease diagnosis based on visual assessments challenging. Virus-specific tests are necessary to ensure cuttings are 'clean' prior to use; do not rely on visual inspection.**

TESTING

Two methods are currently used to detect viruses in grapevines:

ELISA (enzyme-linked immunosorbent assay, *Fig. 6*)
- convenient due to its simplicity and cost-effectiveness

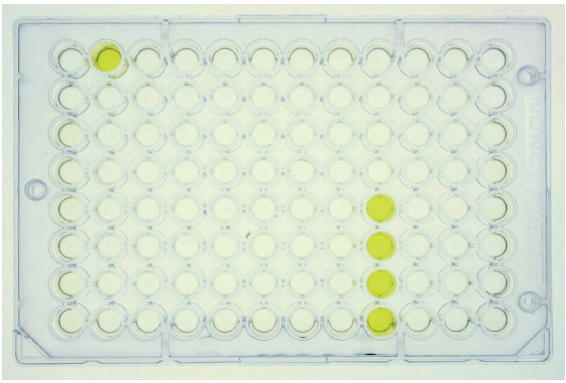


Fig. 6

Virus testing by ELISA. Yellow color in a well of the ELISA plate indicates presence of a virus and no color means absence of a virus. Appropriate controls are included in each assay to ensure validation of test results.

RT-PCR (reverse transcription-polymerase chain reaction, *Fig. 7)*
- more sensitive and more reliable in detecting viruses at much lower concentrations

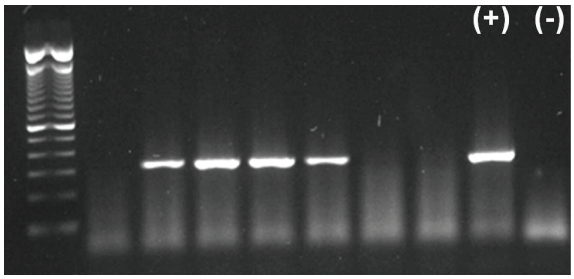


Fig. 7

RT-PCR assay for the detection of grapevine viruses. Samples from individual vines are tested separately. A defined portion of the virus genome amplified as DNA in RT-PCR is visualized as a white band. The size of a virus-specific DNA band is estimated relative to DNA bands of known size (shown in the lane on extreme left). The presence of a DNA band indicates that the sample is positive for a specific virus. Appropriate controls are included in each assay and the presence of a DNA band of expected size in positive (indicated by '+') but not in negative (indicated by '-') controls will ensure validation of RT-PCR results.

VIRUS DETECTION

ELISA (*Fig. 6*) and RT-PCR (*Fig. 7*) can be performed at Washington State University (Irrigated and Agriculture Research and Extension Center) and a limited number of other labs.

SAMPLING STEPS FOR GROWERS

Using appropriate sampling techniques is the first critical step in reliable diagnosis of viruses in grapevines. Depending on the time of the year, either leaf or cane samples can be used for virus testing. Leaf samples can be collected at any time during the growing season (usually from mid May to end of October, depending on the variety) and cane samples can be collected during the dormant period. The following instructions will offer guidelines for collecting appropriate samples for virus testing, but it is a good idea to check with your lab first.

Collecting leaf samples during growing season:

Step 1: Collect mature leaves with petioles (*Fig. 8*) from different parts of the grapevine to account for the possible uneven distribution of virus within a plant. Usually 6 to 8 leaves per plant are adequate for virus testing. Using a plastic sample bag as a glove, hold the base of the leaf petiole (the attachment point to the shoot), and pull downward to ensure the entire petiole stays with the leaf. Do not break the petiole. Drop individual leaves directly into a zip-top plastic bag and seal the bag (*Fig. 10*).

Step 2: Label individual bags. Document location of individual vines in a vineyard block with cultivar name and any other pertinent information (date, block, row, vine(s), variety). Be consistent!

Step 3: Keep in an insulated cool container (to protect from heating) until transportation to the lab. You can also store in a refrigerator until sent to the lab. Do not freeze the samples and do not place them directly against an ice pack.

Collecting cane samples during winter:

Step 1: Collect canes randomly from the grapevine to account for possible uneven distribution of virus within a plant. Insure that samples are collected from each cordon. Canes should be 2 to 3 internodes in length (*Fig. 9*).

Step 2: Place cane samples collected from each plant in a zip-top plastic bag (*Fig. 11*). Label individual bags. Document location of individual vines in a vineyard block with cultivar name and any other pertinent information (date, block, row, vine(s), variety). Be consistent!

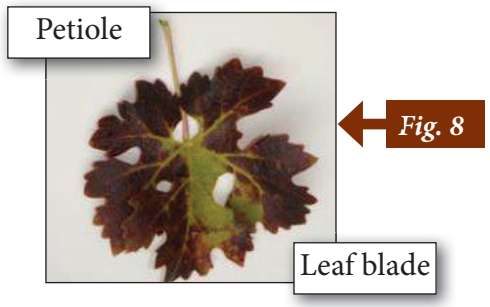


Fig. 9. **(Top) Appropriate size canes suitable for testing. (Bottom) Canes are not suitable for testing.**

Note: Leaf or cane samples collected from vines can be pooled for virus testing, but consult your lab for advice.

BEST MANAGEMENT PRACTICES FOR GROWERS

- When considering cuttings from existing blocks for new plantings (either your own or from others), have a representative number of samples tested for viruses before using them for planting.
- If you want to bring cuttings in from outside the state, talk to the State Department of Agriculture about quarantine rules and talk with your source about the status of certification. (http://agr.wa.gov/plantsinsects/plantquarantines/plantquarantines.aspx)

VIRUS TESTING

Where can a grower send samples for virus testing?

Samples can be sent to the following locations for testing on a cost basis. Each lab has different testing capabilities and fee structures.

Growers are advised to contact these service centers for pricing, shipping and other details:

Agri-Analysis LLC
930 Riverside Parkway, Suite #30
West Sacramento, CA 95605
info@agri-analysis.com
Phone: 800-506-9852

WSU ELISA Virus Testing Lab
24106 N. Bunn Rd
Prosser, WA 99350
Tina_Vasile@wsu.edu
Phone: 509-786-9382

Eurofins STA Laboratories
7240 Holsclaw Road
Gilroy, CA 95020
juditmonis@eurofinsus.com
Phone: 888-782-5220

Specific mention of a virus testing service does not indicate endorsement.

GLOSSARY

Assay: An investigative (analytic) procedure for qualitatively assessing or quantitatively measuring the presence or amount or the functional activity of a target entity.

Cane: A mature shoot after leaf fall.

Certified nursery: A nursery licensed by the state department of agriculture to sell horticulture plants and is in compliance with the nursery standards of that state.

Certified grapevine: A grapevine grown in compliance with the rules of a state certification program that includes originating from a foundation source that's tested and found free of harmful viruses, propagated for no more than 3 generations, and tested and inspected to assure phytosanitary cleanliness.

Cutting: A portion of a dormant cane usually 14-18 inches long used for vegetative propagation; *(cont.)* may also refer to a green or herbaceous shoot section to be propagated under mist in a greenhouse.

GLRD: Grapevine Leafroll Disease.

Leaf blade: Flat, thin part of the leaf. The shape and size of leaf blade varies with cultivar. Also known as the lamina.

Petiole: Stalk of a leaf blade.

Symptom: A sign or an indication of disorder or disease, especially when experienced as a change from normal function, sensation, or appearance.

Variety: Members of a species that differ from others of the same species in minor but heritable characteristics. Occurs naturally and may be perpetuated by humans as a cultivar. Examples include Cabernet Sauvignon, Pinot Noir, Merlot, Chardonnay, etc.

FOR MORE INFORMATION CONTACT
WSU-Irrigated Agriculture Research and Extension Center
Naidu Rayapati, PhD, Grape Virologist
Prosser, WA
naidu.rayapati@wsu.edu
Phone: 509-786-9215

FUNDED IN PART WITH
Specialty Crop Block Grant Funds through the Washington State Department of Agriculture
Olympia, WA
Phone: 360-902-1800

GRANT FACILITATED BY
Washington Wine Industry Foundation
Vicky Scharlau, Executive Director
Cashmere, WA
vicky@washingtonwinefoundation.org
Phone: 509-782-1108

Washington State University
Viticulture and Enology Program

WSDA 100 YEARS Specialty Crop Block Grant Program

Washington wine industry FOUNDATION