Pinpointing Grapevine Virus Diseases

Visual field diagnosis of grapevine diseases is important, but ultimately the grower should rely on laboratory tests to rule out infection or pinpoint the disease causal agent.

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GROWERS SHOULD BE on the lookout for disease-causing agents or pathogens that can decrease the quality of planting material. Planted vineyards can harbor different types of pathogens: fungal, bacterial and viral.

This is the first of a series of articles describing grapevine diseases. In this article, important grapevine diseases caused by viruses that are best detected in the fall by laboratory methods are reviewed. The most common laboratory methods for virus detection or diagnostics are described. The reader will be informed of certain characteristics (or symptoms) to look for and ways to prevent the spread of harmful viruses in the vineyard. Other articles will be published in subsequent issues covering the "spring season" viruses as well as economically important bacteria and fungi.

Establishing a healthy and productive vineyard requires a considerable investment of time and resources. This article will help growers, vineyard managers and nursery personnel to understand the consequences of virus infection in grapevine stock. Visual field diagnosis of grapevine diseases is important, but ultimately the grower should rely on laboratory tests to rule out infection or pinpoint the disease causal agent.

METHODOLOGIES

Plant viruses infecting grapevines are composed of a nucleic acid (RNA) and a protective protein coat (the virus' outer shell). The methods described are designed to either "bind to" or "copy" a small portion of the virus. Therefore they are specific to the virus we wish to detect. The detection/diagnostics is done by grinding and analyzing samples from plants. Two main methodologies, ELISA and PCR, are used routinely in the lab for grapevine virus detection. Biological indexing (used by certification programs), a method that relies on the ability of sensitive, healthy plants to show disease symptoms after being grafted with virus-infected vines, will not be described in this article.

ELISA

One of the most common and inexpensive diagnostic methods is ELISA. The assay (Enzyme Linked Immuno-Sorbent Assay) involves the binding of the virus' outer shell with a specific antibody. An antibody is a special kind of protein made by an animal's immune system in response to foreign invasion. Antibodies have specific binding sites that "recognize" a molecular shape. Because of the ability of antibody molecules to precisely recognize and bind to certain shapes on other molecules, antibody-binding activity is used to specifically identify viruses and other plant pathogens.

The binding of the virus to the antibody is unique, much like a jigsaw puzzle, fitting a unique piece into each place so there is no doubt about the accuracy of results. With the ELISA method, ground plant tissue extracts are placed in a test plate that has been coated with specific antibodies. If the virus is present in the sample, it will bind to the specific antibodies on the plate and be detected by an enzyme-substrate reaction that produces a color reaction.

The advantages of ELISA are that many samples can be tested at the same time, it is relatively inexpensive and it is fast (results can be obtained in about three days). However, it should only be used as a screening method because it can miss infections if the pathogen is found in low concentration. This is normally the case with viruses causing leafroll, graft incompatibility and rugose wood diseases. To assure absence of infection, a negative ELISA result should always be confirmed using PCR.

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Leafroll is found wherever grapevines are grown. Symptoms include downward rolling and interveinal reddening or yellowing of the leaves, depending on the color of the fruit.

PCR

The polymerase chain reaction (PCR) technique allows the amplification (i.e., multiple copies) of viral RNA, which might be present in the vine in low amounts. All viruses known to infect grapevines are made up of RNA. Prior to PCR the RNA must be converted into DNA. This technique is called RT-PCR (reverse transcriptase PCR) and

requires a piece of viral RNA and primers to get the copying process started.

The primers are short pieces of DNA that jumpstart the genetic copying process. The polymerase (a molecule that facilitates the DNA copying process) helps produce duplicates of the initial viral RNA. The duplication is repeated many times with each copy making more copies; so after 40 cycles, over a billion copies are reproduced. Now, with the exponentially large number of key RNA pieces, the virus can be detected.

In recent years we have increased our understanding about the genome composition of grapevine viruses, making PCR a method of choice for the sensitive and specific detection of these viruses. PCR is laborious and expensive, and should be used for the detection of viruses found in low concentration and confirmation of absence of infection.

GRAPEVINE VIRAL DISEASES

Most viruses that infect grapevines are graft-transmissible diseases; that is, they are transmitted from vine to vine through the process of grafting. Mealybugs and nematodes transmit certain grapevine-infecting viruses. Therefore, overall sanitation and pest control are important to maintain a healthy vineyard.

Commonly, viral diseases are named based on the description of the symptoms observed in diseased plants (e.g., leafroll, corky bark, fanleaf, stem pitting). Studies have shown that grapevines may carry mixed infections, and often times the same symptoms could be caused by more than one virus. Furthermore symptoms can be influenced by seasonal and climatic conditions.

Many practices done in the vineyard favor the transmission and perpetration of different viruses. For example, growers use "top working" as a method for quickly changing the grape variety in the vineyard. By grafting a scion variety onto a field-established rootstock, the grower could be potentially introducing one or more viruses each time a new variety is grafted.

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Plants infected with leafroll disease are slightly smaller than healthy ones. Often times the main vein of the leaf remains green. Grape clusters are smaller than normal and have a lower content of sugar.

It is important to start a vineyard with disease-free, tested plants. Any subsequent top work should only be done with disease-tested varieties. By planning ahead the grower will be saving time and money (replanting a diseased vineyard is very expensive). Because of potential transmission of viruses through plant-feeding insects or nematodes, it is important to periodically test any plant material that will be used for propagation or grafting purposes.

LEAFROLL

This disease is found wherever grapevines are grown. Plants infected with leafroll disease are slightly smaller than healthy ones. Symptoms include downward rolling and interveinal reddening or yellowing of leaves, depending on the color of the fruit of the grape variety. Often times the main vein of the leaf remains green. Grape clusters are smaller than normal and have a lower content of sugar.

Several different viruses, called grapevine leafroll-associated viruses (GLRaV-1 through -9) have been reported to be associated with leafroll disease. These viruses fit within two closely related virus groups: Closteroviruses (from "clostero," Greek for thread-like) and Ampeloviruses (from "ampelos," Greek for grapevine). One of these viruses, GLRaV-2, has been implicated in graft incompatibility symptoms. Generally these symptoms are not seen unless an infected scion is grafted onto certain rootstock varieties. Furthermore, a virus that was previously named "grapevine rootstock stem lesion associated virus" was found to be highly related to GLRaV-2 and is now known as the RG strain of this virus.

Commercial sources of ELISA kits have been developed specific to GLRaV types -1 through -7. Experimental sources of ELISA are available for GLRaV-4, -5 and -8, and its commercial availability would greatly improve the quality of detection of these viruses. All these viruses (except GLRaV-6) can be detected using RT-PCR.

RUGOSE WOOD COMPLEX

Four different diseases can be detected by biological indexing, but only three of them have been associated with viral infection. Rupestris stem pitting is caused by the "Rupestris stem pitting associated virus" (RSPaV, a Foveavirus, from "fovea," Latin for small pits); Kober stem grooving is caused by grapevine virus A (GVA, a Vitivirus, from "Vitis," the Latin name for grape); corky bark is caused by grapevine virus B (GVB, a

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Vitivirus). LN33 stem grooving syndrome is distinguished by biological indexing, but no virus has been found associated with this syndrome.

The different rugose wood complex syndromes are difficult to distinguish in the field. Typical symptoms are related to grafted vines and include swelling above the graft union and wood with pits or grooves that can only be seen after the bark is removed. The severity of symptoms varies with the rootstock or scion variety and the virus type, ranging from delayed budburst to vine decline and death. Environmental stress and/or the combination of rugose wood syndrome with other diseases may intensify the disease symptoms.

A reliable ELISA is available for GVA while RSPaV, GVA and GVB can be detected using RT-PCR. It is interesting to note that recently PCR has allowed the discovery of an RSPaV strain that does not cause symptoms on the Rupestris St. George indicator host. The virus was found in the original indicator plant material, and it is not known where the virus might have originated as it does not show symptoms in the indicator host.

By grafting a scion variety onto a field-established rootstock, the grower could be potentially introducing one or more viruses each time a new variety is grafted. It is important to start a vineyard with disease-free, tested plants.

FLECK

This disease is characterized by chlorotic, translucent spots (flecks) in the third- and fourth-order veins of young and medium-aged leaves. Leaves with numerous flecks are twisted and distorted. An icosahedral virus, grapevine fleck virus (GFkV) has been associated with this disease. ELISA and PCR are available for the detection of fleck virus. Biological indexing is performed with *V. rupestris* Saint George.

FALL TESTING

The fall season is the perfect time to look for and detect certain disease characteristics in the vineyard. The disease manifestation or symptoms of leafroll disease are sometimes obvious: leaves roll inwards, turn red or yellow, depending on the variety's fruit color. The coloration of leaves is more intense at the bottom of the canopy and works its way up as the season progresses.

Less obvious or no symptoms are produced by the viruses associated with wood rugose complex. While mother plants might show no symptoms of virus infection, symptoms of graft incompatibility show up a year or two after planting, which can be devastating to the grower. The cost of replanting a new vineyard might be prohibitive. Furthermore, replanting will add a diversity of different size vines, negatively affecting the cultural practices in the vineyard.

It is important to pay attention to the vine aspect of the mother vines and depend on laboratory tests when propagating or grafting vines from an established vineyard. This practice will assure freedom of disease in the future plantings. Using the correct sample methodology for testing is very important as the concentration of virus varies with the location in the vine. Growers should seek professional advice and take advantage of testing in the correct season to rule out plant infection prior to planting. Don't forget that a healthy vineyard is expected to live over 100 years. **wbm**