

UPDATE

Laboratory testing for grapevine diseases

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Laboratory testing for grapevine diseases is useful for diagnosing problems in existing vineyards. Identifying infected propagation wood can help growers avoid spreading diseases to new vineyards.

In the past, detection of viruses in grapevines was largely accomplished by laborious and slow biological tests. Today, many of the most important grapevine viruses can be detected using fast laboratory tests. Although this article will focus on viruses, similar testing is available for diseases caused by fungi or bacteria.

Field diagnosis is challenging

Laboratory testing is a valuable tool when investigating vineyard problems because field diagnosis of grapevine diseases can be difficult. Symptoms displayed in the field are rarely unique to a particular disease. In addition, some infected vines may not show any symptoms indicative of their disease status.

In some cases, grapevine diseases produce distinct symptoms that make them easy to identify in the field. Late spring foliage symptoms of *Eutypa*, for example, are not likely to be confused with other problems. In this case, laboratory testing would not be necessary to identify the cause of the problem. With considerable experience, several other diseases can be reliably diagnosed based on field symptoms.

More often, a particular set of symptoms could result from a number of causes. Red leaves, for example, could be an indication of a virus disease, a fungal root disease, a nutritional deficiency,

physical damage, or feeding by mites, insects, or rodents. Similarly, leaf scorch could be a symptom of Pierce's disease, water stress, sulfur burn, or spray damage. Disease testing is often used to help sort out the true nature of the problem leading to symptom expression.

With most grapevine virus diseases, diagnostic symptoms only occur during certain times of the year. Leafroll, for example, causes leaves to redden in red-fruited varieties in the late summer and fall. Examination of these vines in the spring would give no indication of their disease status.

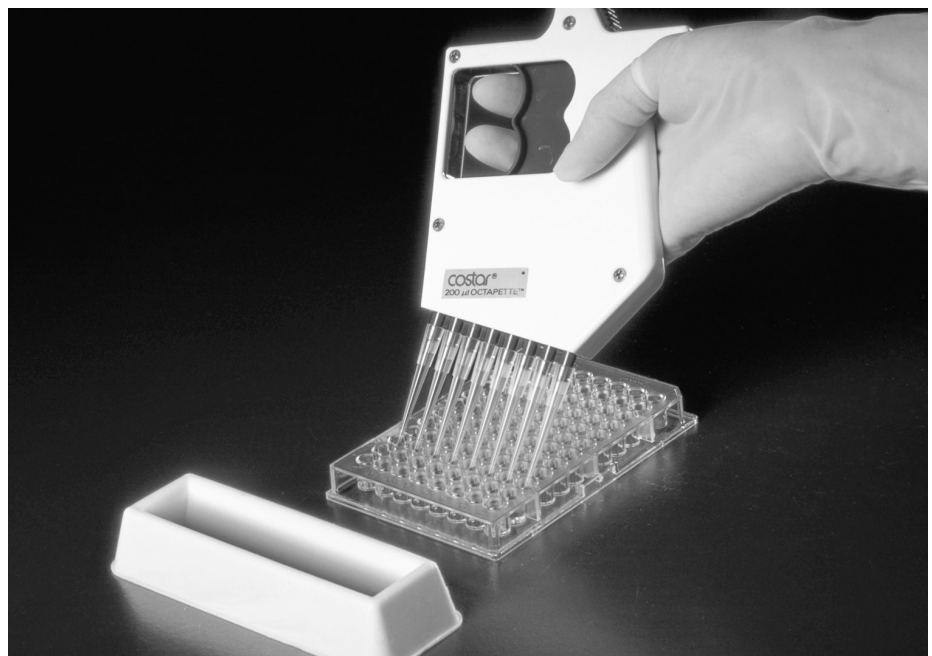
It is virtually impossible to diagnose grape virus diseases in the field during the dormant season, a time when many growers and nurseries cut wood for propaga-

In addition, grapevines infected with some disease agents (especially viruses) may *never* show any obvious symptoms! The concentration of disease-causing agents may be so low that no disease symptoms develop, or the infection may be in a grape variety that is tolerant to that particular disease.

Such latent infections can only be detected with reliable disease testing methods. This is an important consideration when collecting wood for propagating new vines, since virus diseases that may not be evident on one rootstock, or in certain growing conditions, may cause severe disease when infected wood is grafted to create new vines.

Testing methods

Several methods of disease testing are available from commercial plant pathology laboratories (see Table I). These include direct culture of disease agents; serological tests, such as ELISA; and molecular tests, such as Polymerase Chain Reaction (PCR). Other types of tests, such as indexing with biological indicators, are usually performed only at research institutions such as the University of California.



ELISA plate being loaded with virus antibody in preparation for grapevine sample analysis.

Direct culture of plant pathogens

Direct culture is the oldest form of laboratory testing and is still commonly used to help identify many fungal and bacterial disease agents. This method involves placing samples of diseased tissue onto selective culture media and observing what organisms grow. Certain types of selective media only allow fungi to grow, while others only allow bacteria to grow.

Several media recipes have been designed so that only certain fungi or bacteria will grow. The use of these selective media, along with microscopic observation (or other diagnostic testing) of the organisms that do grow, helps confirm the cause of many diseases.

In grapes, culture methods are commonly used for many fungal diseases, such as *Eutypa*, *Armillaria* (oak root fungus), *Phytophthora* (crown

rot), *Phomopsis*, and *Botrytis*, as well as bacterial diseases, such as crown gall and Pierce's disease. Viruses cannot be detected through culture methods.

In order to successfully culture a disease agent, it must be living in the sample provided to the lab. Ideally, samples should include active lesions or interfaces between live and dead tissue. Collecting dry leaves or dead stems will be of no value towards culturing.

Culturing is a slow, labor-intensive practice. Also, considerable expertise is needed to reliably identify organisms that grow on the plates. Check with a plant pathology laboratory to see what types of culture services they provide.

Serological tests — ELISA

Serological methods utilize antibody reactions with disease agents, usually viruses or bacteria. Antiserum is produced by first injecting an animal (typically a rabbit) with a purified preparation of a plant pathogen, such as a virus. The animal reacts to this foreign material by producing antibodies. These antibodies react specifically with the pathogen that was used to create them. The antibodies are then purified from blood serum and the resulting antiserum is used in diagnostic tests. The most commonly used serological test is Enzyme-Linked Immunosorbent Assay (ELISA).

Many laboratories offer ELISA tests for grapevine diseases caused by viruses and bacteria. ELISA tests are fairly simple to run and can provide results in just one or two days. For

Table I — Disease testing services

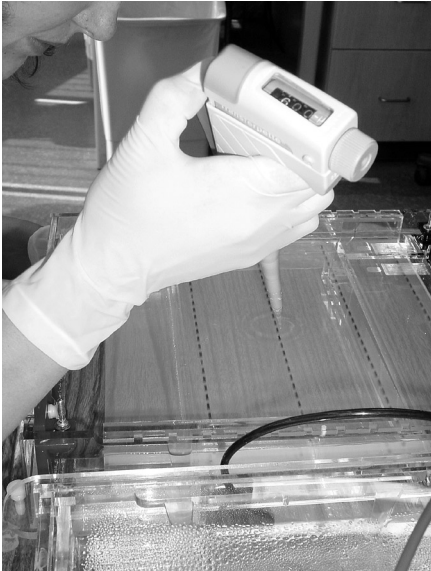
Lab	Indexing	ELISA	PCR	Phone	Website or Email
AgDia	No	Yes	Yes	800-622-4342	www.agdia.com
Agri-Analysis	No	Yes	Yes	530-757-4656	www.agri-analysis.com
California Seed & Plant Lab	No	Yes	Yes	916-655-1581	www.calspl.com
Foundation Plant Materials Service	Yes	Yes	Yes	530-752-3590	http://fpms.ucdavis.edu
STA Laboratories	No	Yes	No	408-846-9964	www.stalabs.com
Waite Diagnostics	No	No	Yes	618-8303-7426 Australia	http://planta.waite.adelaide.edu.au/waite_diag.hhtm

Table II — Guidelines for sampling and disease testing

Disease	When to test	Tissue to sample	ELISA	PCR
Fanleaf, yellow vein (and other nepoviruses)	Spring is best; fall and winter are okay	Active shoot tips in spring; shoots/canes for cambium scrapings in fall and winter	Reliable in spring	Reliable in spring, less reliable in fall and winter
Leafroll*	Late summer, fall, and winter	Petioles in late summer and fall; shoots/canes for cambium scrapings in fall and winter	Reliable in late summer and fall	Reliable in late summer, fall, and winter
Rupestris Stem Pitting	Year round	Petioles, leaves, or cambial scrapings	Not available	Reliable
Vitivirus	Spring, fall, and winter	Petioles, leaves, or cambial scrapings	GVA only; most reliable in spring	GVA, GVB, GVD
Fleck	Spring, fall, and winter	Shoot tips and young leaves in spring; shoots/canes for cambium scrapings in fall and winter	Reliable in spring	Reliable in spring, fall, and winter
Pierce's disease	Late summer and fall	Symptomatic leaves and shoots	Reliable in late summer and fall	Reliable in late summer and fall

*ELISA is available for GLRaVs 1 to 5 and PCR test for GLRaVs 1 to 5 and 7.

GRAPE GROWING



In the PCR test, grape pathogens are identified from characteristic banding patterns formed on an agarose gel.

ELISA to be successful, antiserum for each disease agent must be available in the lab, and grapevine test samples must come from the appropriate tissue, at the right time of year, and be in good physical condition.

One limitation to ELISA testing is that antisera have not been produced for all grapevine viruses. Therefore, a vine could be infected with a particular virus, but if no antiserum against that

virus exists, ELISA will be unable to detect it. This is the situation with some of the leafroll viruses (see sidebar).

Another issue with serological testing is the purity of the antiserum. In some cases, an antiserum may react against more than one virus, or against other components of plant sap that were present with the virus when the antiserum was produced. This could lead to confusing results, or potentially false positive results if the antiserum reacts to something other than the virus. A good lab should use proper internal controls in each test and advise clients when a particular ELISA test for a pathogen is prone to this problem.

Molecular tests — PCR

Recently, molecular tests have been developed that directly target the genetic material (genome) of plant pathogens. Rather than relying on antibody reactions, they specifically test for molecular sequences that are unique to a particular pathogen. One of the most sensitive molecular methods for pathogen detection currently available is PCR.

PCR involves the selective amplification of a small part of a pathogen's genome. If the pathogen is present in a sample, even in very low amounts, the amplification steps in PCR allow for its

detection. It is this amplification that makes PCR such a sensitive test.

PCR can be used for the detection of pathogens in grape because each virus, bacteria, or fungus has its own unique genetic code. In the past decade, molecular scientists have identified genetic markers for many of these pathogens. Commercial PCR testing is currently available for many viral and bacterial pathogens of grapevines. This is an active area of research and this list is sure to expand.

Biological indexing

Before reliable laboratory tests were available for grapevine viruses, biological indexing (testing) was used to detect these pathogens. Herbaceous indexing is performed in a greenhouse in the spring and involves rubbing an extract from the test vine onto leaves of sensitive indicator plants. If certain viruses were present in the test plant extract, the indicator plants will develop diagnostic symptoms in several weeks.

Woody or field indexing requires two years to complete. Indicator grapevine varieties that are especially sensitive to virus diseases are grafted with buds from the vine being tested. They are planted in the field and observed for two seasons for development of virus disease symptoms.

Although indexing tests are labor-intensive and time-consuming, they are still very useful if the vines being tested are valuable and a high level of confidence in the diagnosis is needed.

Reliability of laboratory testing

No diagnostic test is perfect. All the methods described above have the potential to produce false positive or false negative results.

False positives occur when a plant was actually free of a particular disease, but the test results indicate that it was present. False positives usually occur as a result of contamination or mislabeling of samples. These errors could occur in the field when samples are collected, or at the lab after the samples arrive.

Contamination is of particular concern with PCR because of the sensitivity of the test. Just a few bacteria or virus particles carried over from one sample to another could lead to false



To prepare a grapevine sample for testing, sample material and buffer solution are sealed in plastic bags and loaded onto a rack that holds them in place for grinding.

Grapevine Virus Diseases

NEPOVIRUS DISEASES

This virus group includes at least 13 different viruses that can cause disease in grapevines. They share in common transmission by nematodes and a polyhedral physical structure when purified and examined with an electron microscope. This is the source of the name "nepovirus": "ne" for nematode, "po" for polyhedral. Fortunately, only a few of these viruses are reported to be of importance in grapes in the U.S.

Fanleaf Degeneration — Grapevine fanleaf virus — GFLV

GFLV is perhaps the best characterized virus of grapevines, causing fanleaf degeneration in affected plants. It is widely distributed throughout the world. Fanleaf disease is a major viticultural problem in California, causing reduced yields due to poor berry set. The reduction in yield can be over 80% in some varieties. Symptoms include fan-like distortions of leaves and chlorotic yellowing as ringspots, vein banding, and mottling or mosaic patterns. The virus is transmitted by the nematode *Xiphinema index* and can infect all *Vitis* species.

Yellow Vein — Tomato ringspot virus — ToRSV

ToRSV causes yellow vein disease. A similar disease is caused by tobacco ringspot virus. These viruses are transmitted by several species of nematodes including *X. americanum*, *X. californicum* and *X. rivesi*. Symptoms of both diseases include shot berries, shoot stunting, and devigoration of the vine. These diseases are common in vineyards in the eastern U.S. and in fruit trees, but are rarely seen in California vineyards. The symptoms of yellow vein resemble those described for fanleaf, and they can be easily confused.

Arabis mosaic virus — ArMV

This virus is widespread in grapevines in Europe. Although not found in California vineyards, it has recently been reported as common in Missouri and some infections have also been reported in Canada. Infected grapevines show symptoms similar to those of fanleaf, and ArMV can be present in a mixed infection with GFLV. Several nematode species can transmit ArMV to grapevines, the most common being *Xiphinema diversicaudatum*.

LEAFROLL

There are at least seven distinct viruses reported to be associated with leafroll disease. These viruses are collectively referred to as grapevine leafroll-associated viruses (GLRaVs) and are designated GLRaV 1 through GLRaV 7. ELISA tests are currently only available in commercial labs in the U.S. for GLRaV 1-5.

Symptoms of leafroll disease may include downward rolling of leaves, leaf reddening in the fall of red-fruited varieties, poor fruit color development, and delayed fruit maturation. Yield losses of 10 to 20% may occur. In cases of mixed infections with more than one virus, vines may be severely weakened and vine death may occur.

RUGOSE WOOD COMPLEX

Diseases in the rugose wood complex are characterized by trunk and stem disorders (pitting and grooving). Foliar symptoms similar to leafroll may also occur. Diseases in this complex include corky bark, Kober stem grooving and rupestris stem pitting. Their effects on grapevines vary from mild to severe. Disease severity is compounded when multiple infections of the rugose wood complex occur, or by the presence of other viruses such as leafroll.

In recent years, individual viruses have been discovered and characterized which has made the detection of these disease agents much easier. There are still some rugose wood diseases for which the agent has not yet been described, making it necessary to perform laborious and slow biological tests.

Rupestris stem pitting-associated virus — RSPaV

RSPaV is associated with rupestris stem pitting of grapevines. This disease is usually of little consequence. Decline due to rupestris stem pitting has been reported, but is not well-documented. RSPaV is widely distributed and is not targeted for elimination in most certification programs.

Vitiviruses — GVA, GVB, GVC, GVD

The vitiviruses are a group of viruses associated with the rugose wood disease complex. Four vitiviruses have been discovered in grapevines: grapevine vitivirus A (GVA), grapevine vitivirus B (GVB), grapevine vitivirus C (GVC), and grapevine vitivirus D (GVD).

GVA is associated with Kober Stem Grooving. Affected vines may show swelling at the graft union and fail to thrive. Ungrafted vines may be infected, but usually do not show symptoms.

GVB is associated with corky bark disease. The disease affects only grafted vines. The severity of corky bark is more pronounced in vines infected with other rugose wood complex viruses.

Neither GVC nor GVD have been proven to cause disease in grapevine but their structure and genetic profiles have shown that they belong to the vitivirus group.

FLECK

Grapevine fleck virus — GFkV

GFkV is a graft-transmissible virus that causes symptoms of disease only in *V. rupestris*. Other *Vitis* species can be infected but remain asymptomatic. In infected *V. rupestris*, symptoms include localized clearings (flecks) in the veinlets of young leaves. In older leaves, the symptoms diffuse into a mosaic pattern and the leaves wrinkle and curl upward. Symptoms persist during mild weather and disappear with the onset of hot temperatures. Very little information is available about the economic importance of fleck virus.

OTHER VIRUSES

Many other graft transmissible diseases, likely caused by viruses, can infect grapevines. These include asteroid mosaic, enations, vein necrosis, and vein mosaic, among others. These diseases have been studied to varying degrees, but have never been demonstrated to be common or severe.

Occasionally, new diseases appear that are significant. Recently, a new stem lesion virus disease was discovered in California (see *California Agriculture*, July-August 2001). Also known as Redglobe virus, this disease can kill vines on certain rootstocks. Continuing research is necessary to identify important new diseases like this and to develop diagnostic tools to help minimize their future impact.

GRAPE GROWING

positive results. With ELISA, false positives can also occur if an antiserum reacts against plant constituents in addition to the targeted pathogen.

False negative results are much more common than false positives. False negatives occur when diseased vines are tested but the test results indicate that no disease agent was present. Most false negatives occur because the sample from the diseased vine did not have disease agents in it, or the sample was mishandled and was not in good condition when it arrived at the lab.

Virus infections are usually unevenly distributed in vineyards. Even within a single vine, viruses may be present in some parts but not others. If the tissue sent to the lab was from a part of a diseased vine that didn't contain virus, the result will be negative, albeit a false one.

False negatives can also occur due to a variety of problems at the laboratory that compromise the testing procedure. Good laboratories include controls in their tests in hopes of identifying these types of problems so that the test can be repeated.

Sample selection and handling

Sample selection is important for minimizing the chances for false negative results. Sampling particular parts of the vine at certain times of the year can greatly increase the reliability of disease testing.

For example, to test vines for Grapevine Fanleaf Virus, shoot tips should be collected for testing in the spring. This virus is heat-sensitive and during summer, its concentration in vines becomes very low, making disease testing more unreliable. See Table II for additional sampling recommendations.

Proper handling and shipping of samples is also important. In general, samples should be delivered to the testing lab as soon as possible. If the samples have been exposed to excessive heat or drying, or if they are stored for too long,

PCR testing is available for the following pathogens:

Arabis mosaic virus
 Grapevine fanleaf virus
 Grapevine fleck virus
 Grapevine leafroll-associated virus 1
 Grapevine leafroll-associated virus 2
 Grapevine leafroll-associated virus 3
 Grapevine leafroll-associated virus 4
 Grapevine leafroll-associated virus 5
 Grapevine rootstock stem lesion-associated virus
 Grapevine vitivirus A
 Grapevine vitivirus B
 Grapevine vitivirus D
 Phytoplasmas
 Pierce's disease (*Xylella fastidiosa*)
 Rupestris stem pitting-associated virus
 Tobacco mosaic virus
 Tomato ringspot virus

it will not be possible to get reliable results. Be sure to consult with a lab for recommended delivery instructions.

Interpretation of results

In general, the larger the number of samples sent from a vineyard for testing, the higher the confidence level in the results. Keep in mind that in a given vineyard, more than one virus can infect individual vines, and it is possible for vines in the same vineyard to be healthy, infected with only one virus, or infected with more than one virus.

Positive results from a laboratory can generally be counted upon to be accurate. False positives are not common unless there were significant problems at the lab. Most labs run internal controls to check for these types of errors. However, if you suspect a problem because every one of your samples comes back positive, you might consider running them again. Including a healthy sample along

with your diseased ones is usually a good idea.

Negative results, on the other hand, are of limited value. Because of the problems inherent in sampling, the uneven distribution of disease agents in vines and concentration changes during the year, false negative results occur with high frequency. Keep in mind that a negative test result does not mean the vine is free of the disease being tested for.

If you suspect that a vine is diseased but the test results come back negative, don't let this be the end of the story. Consider other evidence such as vine performance and symptom expression, then do further testing until you are satisfied.

If you are using laboratory tests to screen vines for propagation, extra care is needed. Foundation vines in the California Registration and Certification program are tested over a period of at least two years using a combination of biological indicators, ELISA, and PCR tests. This provides a very high level of confidence about the virus status of the selections.

Although practical considerations may require the use of wood from commercial vineyards for propagation, particularly for field budding, it is highly recommended that growers do everything possible to avoid spreading pathogens with the wood.

Disease tests cannot be used to determine the general "health" of a grapevine. Rather, they can help determine whether or not a vine is infected with the particular pathogens being tested for. Because tests are not available for all known grape diseases, no vine can ultimately be declared "disease-free." However, the new laboratory technology available today makes the job of diagnosing vineyard diseases far more reliable than it has ever been in the past. ■

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