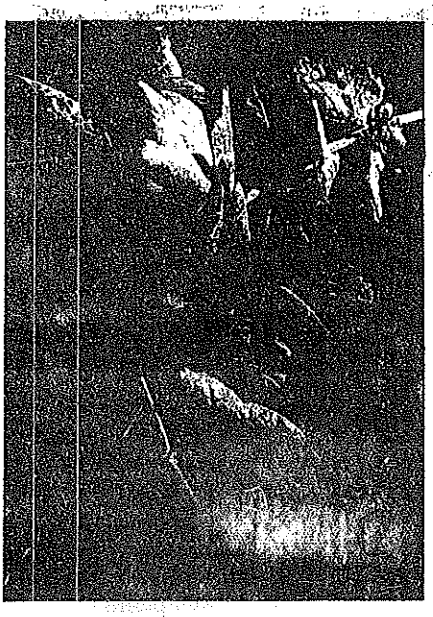
LN33 - 18 months (sensitive to Corky Bark)
Cab. Franc roots - for leafroll

LEAFROLL disease of grapevines



By Ed Weber, Deborah Golino, and Adib Rowhani

Introduction

Leafroll, a virus-like disease of grapevines, is worldwide in distribution. In some viticultural regions, nearly 100% of vines are infected.[16] Despite having been recognized for nearly 150 years, leafroll continues to be a serious problem in all major grape growing areas. Clean stock programs have been established in several countries in order to produce, maintain, and distribute grapevines which are free of leafroll and other diseases.

History

Leafroll likely originated in the Near East along with *Vitis vinifera*.[10] The disease has been spread throughout the world through the movement of infected plants and cuttings. Leafroll does not occur naturally in *Vitis* species native to North America.[10]

Reports of disorders which were likely caused by leafroll occurred as early as 1853 in France, where it was known as *rougeau*, and 1905 in California, where it was called red-leaf.[8] In 1936, it was estimated that 80% of the vines in Germany were infected with leafroll.[19]

Leafroll was first identified in California in 1958 by Goheen *et al.*[7] They showed that leafroll was the cause of poor color development and maturation in the red table grape variety Emperor, a disorder which had previously been called white Emperor disease. Since that time, considerable research has been conducted to explain the cause and effects of grape leafroll disease.

Symptoms and effects of leafroll

Symptoms of leafroll vary with the time of year, the variety of grape, and environmental factors. Symptoms may not be expressed on all infected vines.

Budbreak and shoot development are often delayed in leafroll-infected vines. This is a usually short-lived phenomenon, apparent only for a few weeks.

Leaf symptoms first become apparent in early to mid-summer, often appearing earlier on vines which are water-stressed. These symptoms increase in number and severity until the first frost. Leaves on the basal portion of shoots begin to roll downward at the margins. The interveinal areas change color and may become necrotic. Most rootstock varieties show no foliar symptoms of leafroll.

In most red varieties, leafroll causes a reddening of the interveinal areas while the veins remain green. The veins may or may nor retain their green color in advanced stages of the disease.[16] Leaves of some red varieties, particularly those with deeply pigmented fruit, develop uniform red color without green veins.

In white varieties, the interveinal area may become chlorotic. This symptom is often subtle and may not be recognized.

The extent of leaf-rolling varies considerably among infected varieties. Some, like Chardonnay, show pronounced leaf-rolling by harvest. Others, such as Thompson Seedless and Sauvignon blanc, show little or no leaf-rolling at all.[9] In these white varieties, leafroll is nearly impossible to detect on a visual basis.

As the growing season progresses, more and more leaves display leafroll symptoms, progressing from the base of the shoot to the tip. The bright red leaves that delight autumn tourists are simply an expression of grape leafroll disease.

Leafroll causes a degeneration of primary phloem tissue in young shoots, leaves, and fruit pedicels.[14] Starch accumulates in infected leaves coincident with this phloem disruption.

Leaf symptoms similar to leafroll can be caused by injuries, or other diseases, which disrupt the phloem. Mechanical injury to the trunk, bending or cracking of canes, poor graft unions, and girdling can all damage the phloem and lead to leaf-rolling and coloration similar to that caused by leafroll. Feeding by certain insects and mites, as well as some other disease agents (most notably *Phytophthora*), can cause similar symptoms.[9]

Leafroll reduces overall vine growth and yield. Annual pruning weights, cluster number and cluster size are all reduced in infected vines. The range in crop loss varies considerably, however, a 20% reduction is not uncommon.[10]

Fruit quality from leafroll-infected vines is also adversely affected. Sugar accumulation is delayed and anthocyanin production in red varieties is greatly reduced. The resultant fruit is low in sugar, poorly colored, and often is several weeks delayed in ripening, compared to fruit from healthy vines. In some seasons, fruit on infected vines never reaches desired sugar levels.

Whether there is a reduction in wine quality as a direct result of leafroll, independent of its effect on grape maturity, is unclear. However, delaying harvest by several weeks can be catastrophic if it subjects the fruit to fall rains.

Leafroll and potassium deficiency

Leaf symptoms due to leafroll have often been confused with those of potassium deficiency. As a result, many vineyards have been diagnosed as potassium-deficient, when in fact they were infected with leafroll.

Leafroll infection affects vines such that leaf blades from infected vines are lower in potassium, and other mineral nutrients, than from healthy vines.[3] Applying high doses of potassium can reduce the severity of leaf symptoms, but does not prevent their development. Under conditions where the supply of potassium is limited, leafroll can accen-

tuate potassium deficiency symptoms.

True potassium deficiency can be distinguished from leafroll in that potassium deficiency symptoms tend to appear earlier in the season, and first on leaves in the middle region of the shoot, rather than on basal leaves, as is the case for leafroll.[3]

Causal agents

The viral nature of leafroll was first suggested by G. Scheu in 1936.[19] Early evidence for this was that the disease could readily be transmitted by grafting, and no macroscopic causal agent could be found. Different strains or types of leafroll were recognized, based on differences in the severity of symptoms on infected vines. Early attempts to identify the causal agent of leafroll were unsuccessful.

Proving that a disease is caused by a virus can be difficult. Until certain research results are obtained, the disease is referred to as virus-like, and the virus is said to be associated with the disease. These terms usually suggest that the associated virus can be graft-transmitted, and can be eliminated from diseased vines through heat therapy or by shoot-tip culture.

Recent research has established that several different viruses are associated with leafroll disease.[12,15,21,23] Leafroll, as we know it, likely represents a group of diseases with similar symptoms which are caused by several different viruses, either alone or in combination.

Six serologically distinct closteroviruses associated with leafroll have been described.[12,13,23] Five are referred to as grapevine leafroll-associated viruses (GLRaV types I, II, III, IV, V). The other is known as grapevine virus-A (GVA).[4,17]

In addition, two other types of virus particles have been implicated in leafroll disease: a potyvirus-like particle,[20] and an isometric-like particle.[2]

The actual cause of leafroll disease remains unclear. A vine displaying symptoms of leafroll could be infected with any one of these viruses, or by a combination of more than one. Furthermore, there may be additional viruses associated with leafroll still to be discovered.

Leafroll spread

Leafroll is readily transmitted from infected vines to healthy vines through graft inoculation. Normal viticultural practices of bench-grafting, budding, and varietal conversion provide many opportunities for the introduction of leaf-roll into vineyards. Leafroll spread via pruning shears or saws is highly unlikely; a graft union must be present.

The use of certified, virus-tested grape materials for propagation and establishment of vineyards is the best insurance against introducing leafroll to new vineyards.

Natural spread of leafroll from infected vines to healthy ones growing nearby has been observed in California, but usually at a very low rate of spread.[11] Higher rates of spread have been observed in South Africa and New Zealand. Several mealybug species have been shown experimentally to transmit leafroll.

In South Africa, the vine mealybug, *Planococcus ficus*, is able to transmit GVA and GLRaV type-III from infected vines to healthy ones.[5,6] Work in Israel has shown that *Pseudococcus longispinus*, a mealybug known to occur there, can transmit GLRaV type-III from vine to vine.[21] Another mealybug, *Planococcus citri*, has been shown to transmit GVA to an herbaceous indicator plant.[18]

Recently, evidence of significant spread of leafroll in a California vineyard has renewed interest in identifying a vector for natural spread.

Leafroll detection and diagnostic methods

Visual observation of vines is not a useful method for diagnosis of leafroll. Symptoms of leafroll are highly variable depending on variety, time of year, the strain of leafroll present, and the environment. Some infected vines may show no visual symptoms of leafroll. Physical damage, insects, mites, other diseases, and nutritional disorders can all cause leaf symptoms which mimic those caused by leafroll.

Biological indexing tests can reliably detect the different viruses associated with leafroll. Indexing tests utilize grape selections which are especially sensitive to particular virus diseases. Such 'indicator' varieties have been used in grapevine certification programs for many years.

In an indexing test for leafroll, buds from the grape selection being tested are grafted to potted vines of the indicator variety. The grafted plants are then grown in a field plot for about 18 months. If the tested selection had leafroll dis-ease, the indicator vines will develop red leaves in the fall.

Indexing is considered a very reliable test as the indicator plant amplifies any virus present in the grafted bud. However, recent research suggests that in some cases, leafroll disease may escape detection by indexing tests. Indexing is not a practical diagnostic tool on a commercial basis because of the length of time required for disease expression.

Several indicators have been used to test for leafroll, including Emperor, Mission, Gamay, Cabernet Sauvignon, Pinot noir, Cabernet franc, and Barbera.[1] The sensitivity of these indicator varieties varies under different environmental conditions. At the University of California, Davis, Cabernet franc has proven to be the most reliable indicator for leafroll.

There are rapid tests available for leafroll. The most widely used is a serological test called Enzyme Linked Immunosorbent Assay (ELISA). This is a 2-day laboratory test which relies on antibodies specific to the agent being tested for. ELISA is routinely used in many crops to test for certain viruses, bacteria and fungi. It can also be used to test for pesticides or other contaminants of soil and water.

ELISA testing was recently developed for leafroll, but it is more complicated than for many other diseases. Because the viruses associated with leafroll are serologically distinct, separate antisera must be prepared against each one. In preparing antisera, purified virus samples are required. For some virus diseases, a plant other than grapevine is inoculated and virus is then purified from it. This minimizes interference in ELISA from reaction of the antisera to grape proteins or similar viruses which could be present.

This inoculation technique does not work with leafroll. Therefore, infected grape tissue is used for virus purification. A possibility exists that viruses not involved in leafroll disease co-purify with the grapevine leafroll-associated viruses. The result would be an antisera which reacts to these additional viruses, leading to false-positive test results for leafroll.

To thoroughly ELISA-test a selection for all the viruses currently associated with leafroll, at least eight separate tests would be required, one for each virus. In the U.S., antisera have been purified and are available only for GLRaV types I, II, III, and IV.

ELISA results can be affected by the type of tissue being tested (leaf, petiole,

root, etc.) and by the time of year collections are made. Leaf blades and petioles are commonly collected for ELISA testing. Because leafroll agents are phloem-limited, petioles and/or major veins in the blades are used.

Leaves should be collected after bloom. Earlier in the season the virus concentration (titer) in leaves is very low.[22] Basal leaves should be sampled as these will have the highest titer. This differs from other virus diseases, such as fanleaf, in which the highest titer occurs in young leaves near the shoot tip.

Bark scrapings from dormant canes also provide good tissue for leafroll ELISA testing. Dormant cuttings can be collected and refrigerated for later analysis. The ability to utilize stored wood makes ELISA a useful technique for testing propagating materials.

Test results from ELISA must be properly interpreted. A positive ELISA test for leafroll usually indicates that the particular leafroll-associated virus is present in the tested sample. Occasional false-positive and false-negative results occur using ELISA. Research is underway to determine why this happens.

A negative ELISA test does not mean that the selection is free of leafroll — only that the particular virus being tested for was not detected. Leafroll-associated viruses can be present at very low levels which escape detection by ELISA.

Additionally, recent studies indicate that leafroll-associated viruses may not be uniformly distributed within a vine. Tissue collected from one portion of the vine could test negative, while another portion tests positive.

ELISA testing for GLRaV types I-IV is available through commercial laboratories. Results from one laboratory suggest that GLRaV type-III is the most common in California selections (see sidebar).

Other rapid tests are being developed or are in use experimentally for leafroll detection. Again, because more than one virus is involved, applying these new tests to leafroll will be more difficult than for other diseases.

Leafroll at FPMS

Foundation Plant Materials Service (FPMS) at UC-Davis, is a repository for virus-tested, true-to-variety grape selections. FPMS provides cuttings to commercial nurseries participating in the California Grapevine Registration and Certification Program. Certified selections from FPMS have undergone several indexing tests before release to nurseries for propagation.

In 1992, leafroll disease was detected in numerous certified selections at FPMS. These selections had previously tested free of the disease by indexing. The discovery of leafroll came as a result of the first extensive ELISA-testing of FPMS vines. Approximately 5% of the certified mother vines at FPMS were initially tested; about 20% of these tested positive for leafroll. Further testing of FPMS mother vines is underway.

Four FPMS certified mother vines with leafroll-like symptoms tested positive for leafroll in 1991 using ELISA. Leafroll in these vines was confirmed in 1992 by indexing.

It is believed that leafroll spread from diseased to healthy vines which were growing adjacent to each other in the FPMS vineyards. How this happened has not yet been determined.

FPMS, the California Dept. of Food & Agriculture, University of California researchers, and industry members are cooperating in updating the California Certification Program to incorporate new testing methodology, and to minimize the chances of future spread of diseases to certified vines. The status of the infected materials at FPMS, and the implications for participating nurseries, is under review.

Summary

Grapevine leafroll disease is a serious problem which should not be considered lightly. Using certified rootstocks and scions continues to be the best insurance against introducing leafroll, or other damaging diseases, into new vineyards. Further understanding of grapevine leafroll disease, advances in disease testing methodology, and stricter standards in certification programs will all serve to improve the quality of grapevine planting materials. ■

Ed Weber is Napa County Farm Advisor, University of California Cooperative Extension. Deborah Golino is Research Plant Pathologist, USDA-ARS at UC-Davis. Adib Rowhani is Specialist, Dept. of Plant Pathology, UC-Davis.

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Leafroll ELISA test results

By Glenn Friebertshauser, Agri-Analysis Associates,

Agri-Analysis is a commercial laboratory in Davis, CA which specializes in the use of enzyme-linked immunosorbent assays (ELISA) for the detection of plant disease agents and pesticides in soil and water. Presently, leafroll ELISA tests are available for GLRaV types I, II, III, and IV.

After assaying several thousand samples over the past 18 months, several interesting observations have been made.

Positive tests for leafroll have occurred in 21% of the total samples submitted. Different sets of samples may have widely varying incidences of positive samples, depending upon whether samples are from visually symptomatic plants submitted for confirmation of diagnosis, or are from a random plant nursery survey.

In the samples which were leafroll positive, type-III was the most commonly encountered, followed by types II and I. Type-IV has not been confirmed as occurring in any of the wine grape varieties, although it has been widely found in table grape selections. The percentage breakdown of positive ELISA leafroll tests is shown below.

Breakdown of positive ELISA leafroll tests

Type I	17%
Type II	25%
Type III	31%
Types I, II	5%
Types I, III	1%
Types II, III	2%
Types I, II, III	19%

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