Sampling in the Vineyard

Guidelines for optimal virus detection

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Clonal grapevine propagation, the process of making cuttings from an original vine, has advantages and disadvantages as it relates to plant health. Depending on soil conditions and the presence of root-feeding insects or nematodes, the grape-producing varieties (scion) are grafted onto rootstocks. If many cuttings are produced from a virus-infected vine, the outcome will be the production of many infected plants and, when planted, disease infection in the vineyard.

On the other hand, if the siblings were produced from a healthy mother vine, the outcome will be uninfected vines that, when planted, will result in healthy and productive vineyards. In practice, cuttings from infected and non-infected mother vines are mixed unknowingly prior to grafting. One should expect the vineyard to be represented by vines with a mixed infection status (some vines would be infected with viruses and others would not).

Following are guidelines for the collection of representative samples to be submitted to a laboratory for viral detection.The sample size and type will depend on the reason for testing, size of the vineyard and virus species to be detected.

SAMPLING TO DETERMINE CAUSE OF DISEASE

A vineyard may have been planted with material initially infected with viruses or become infected after planting (for example, the previous vineyard was infected with a virus vectored by soil-invading insects or nematodes). Different patterns of infection may be observed depending on the situation: clusters, which are confined within a specific area (example: grapevine fanleaf virus transmitted by nematodes); gradients, which spread from initial site of infection (grapevine leafroll-associated virus-3 transmitted by mealybugs); or total infection (grapevine leafroll-associated virus-2 – no known transmission other than grafting), where the entire vineyard is affected.

Testing vines prior to planting is required due to the large initial investment in vineyard development.

In this highly infected vineyard, every vine displays virus-specific symptoms. The collection of samples from one or two vines will suffice to determine the cause of disease at the laboratory.

The chances of pinpointing the virus infection in a specific vineyard will depend on the grower collecting the right type and number of samples, as well as the laboratory applying the best testing methodology of detection. If a grower tests a vineyard due to the presence of suspicious symptoms, the sampling will be done by collecting portions of vines displaying symptoms. Generally, if similar disease symptoms are present throughout the vineyard, fewer samples are required for virus detection.

However, if the symptoms are diverse, multiple samples representing different symptoms may be required. It is recommended to work with a knowledgeable plant pathologist who can suggest the type of tests and samples to collect to determine the disease-causing agent. If the results show no evidence of infection, the pathologist may recommend increasing the number of samples or testing for other disease-causing agents such as fungi and bacteria, which may cause similar symptoms in the vineyard.

AT A GLANCE

- Clonal grapevine propagation can severely impact the health of a vineyard.
- Choosing the correct sample for virus diagnostics is essential to obtaining reliable results in the laboratory.
- In theory, 100% assurance that the planting material is virus-free requires 100% testing.
- Plan ahead to develop a testing plan that fits the vineyard's budget and quality goals.

SAMPLING FOR PRODUCTION OF CLEAN PLANTING STOCK

When the purpose of testing is the production of clean planting stock, a larger number of samples will be required for laboratory testing. Mother vines may have been selected initially due to a "healthy appearance" and consequently no symptoms are present in the vineyard block. While vines can appear healthy, they still can harbor pathogens. In theory, 100% assurance that the planting material is virus-free requires 100% testing. Testing all plants at once may not be economically feasible. One could possibly develop a testing plan in which all mother vines are tested on a rotation basis.

For example, one-fifth of the vines are tested each year in the course of five years (frequency of testing can be adjusted depending on the number of vines available and budget). Another option is to apply statistical sampling and analysis that minimize the number of vines needed to be tested to assure a level of confidence (or certainty) that less than a predetermined percent of the block is infected (100% clean is not possible to guarantee).

Either way, preventative testing (the annual testing of a number of healthy-looking vines) is required to monitor the presence of viruses and other pathogens in the nursery. For economic reasons, the most intense sampling should be done at the mother-block level, as the testing of each mother vine will provide information about hundreds of progeny vines. Because a grafted vine consists of the union of a rootstock and a scion variety, it is important that both varieties are tested (especially because root-

TABLE 1

Infection Rate	10%	5%	1%	1%	1%
Confidence Level*	90%	90%	90%	95%	99%
Number of vines in vineyard	**NUMBER**	**REQUIRED**	**TO**	**BE**	**TESTED**
10	All 10	All 10	All 10	All 10	All 10
100	20	37	91	96	All 100
1000	22	44	205	258	368
infinite	22	45	229	298	458

Number of samples to be tested based on vineyard size, desired confidence level and virus infection rate.

stocks do not display virus symptoms in the vineyard).

APPLICATION OF STATISTICS AND SAMPLING

In settings such as commercial vineyards and nurseries where the number of grapevines is large and some may harbor disease but have not yet exhibited symptoms, statistical sampling and analysis is ideal because relatively fewer vines can be tested to determine the disease incidence of the vineyard or block. When initiating a statistical approach, one assumes there is some level of infection (even if it is one vine per million plants) but lacks initial infection data (infection rate, distribution in the field, clustering, etc.). The initial use of statistical sampling requires the assumption of a probability, called the confidence level, that a certain percentage of the group of plants are disease-free to some level and that the infected vines are randomly located throughout the group of vines.

Depending on the number vines in the group, the confidence level and the initially assumed infection rate, one can use a formula to determine how many vines should be tested for disease (see Table 1 for select situations). For example, if a vineyard has 1,000 vines with a suspected infection rate of 1%, one must test 229 vines (with no more than one testing positive) in order to be 90% confident that the vineyard infection rate is at or below 1%. If more than one vine were to show infection, say three, one would use the formula that generated Table 1 to find appropriate values of infection rate and confidence levels that more realistically match what was found during the first statistical test run.

**Confidence Level represents the probability that a specific infection rate will occur for each new set of trials. For example, a confidence level of 95% means that if the situation is repeated 100 times, 95 of the repeated situations would have 10% or less infection rates.*

Once the disease infection level status has been established, an ongoing monitoring process in which the results of the tested vines are fed back into the previous results can be implemented using more advanced statistical methods.

PRACTICAL CONSIDERATIONS

This article shows the complexity of testing grapevines for the presence of viruses. Probabilistic statistics are important tools to be applied to grapevine virus testing. Once the infection rate in the vineyard is better understood, these tools can be refined to meet the zero-infection tolerance goal. The sampling will be different depending on the purpose of testing (clean stock versus determining a problem in the vineyard), as well as the type of virus we aim to detect, as the biology of each virus is different.

Ultimately, the number of samples submitted for testing is budget-driven. Due to the large expense of planting, the maximum possible samples should be tested prior to grafting. Planting a vineyard is very costly and the best approach is to plan ahead. Work closely with nursery and testing lab personnel as soon possible, to develop a testing plan that fits the vineyard's budget and quality goals.

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