This vine is infected with grapevine red blotch-associated virus. Photo: Judit Monis

Grapevine Registration and Certification Program Under Review

Red blotch-associated virus in California certified planting material opens discussion

BY DR. JUDIT **MONIS**

Some years ago I wrote an article and presented information on grapevine clean planting stock and the California 2010 Registration and Certification (R&C) program administered by the California Department of Food and Agriculture (CDFA). Since the adoption of the new regulations, known as Protocol 2010, a new foundation block was planted and progeny material is slowly becoming available to growers.

This article will focus on the challenges of certification and registration programs, and provide guidelines to growers for planting healthy vines.

CALIFORNIA GRAPEVINE CERTIFICATION PROGRAM

The California R&C program was revised and a new state law written in 2010. The Protocol 2010 regulations include specific rules for traceability back to the original foundation blocks, distance of increase

AT A GLANCE

- Due to the discovery of Grapevine red blotch-associated virus, the California Registration and Certification (R&C) program is being reviewed
- New technology may replace the current biological indexing technique considered to be the gold standard in grapevine certification and quarantine programs.
- Planning ahead for new plantings allows growers to order diagnostic tests using the correct sample type during the appropriate season.
- Testing composite samples comprised of cuttings from many vines can dilute the virus below detectable levels.

blocks from commercial fruit-bearing (non-registered) vineyards, and disease diagnostic tests. With funding from the National Clean Plant Network (NCPN), a new block was planted at the UC Davis Foundation Plant Services (Russell Ranch Foundation Block) with vines propagated using the "shoot tip" tissue culture technique (a method that eliminates viruses and bacteria).

Protocol 2010 includes a testing program: All vines are to be field- and laboratory-tested to assure that a comprehensive list of diseases and pathogens are absent in the vines released. In other words, the traditional field index technique, a method that involves grafting the prospective foundation vine onto indicator hosts that may detect specific diseases such as leafroll or fanleaf, is complemented with laboratory testing to detect pathogens using specific methodologies.

The R&C program is voluntary and open to registered participants who follow the required rules. Nurseries enrolled in the program further propagate the planting stock to create increase blocks that will become certified grafted or rootstock rooting plants that are distributed to growers. With the exception of the bacteria *Xylella fastidiosa* and *Agrobacterium vitis*, the R&C program limits testing efforts to viruses. To assure the health status of the vines, foundation and nursery increase blocks, as well as certified grafted plants, need to be inspected and tested routinely.

REVISIONS NEEDED

Certification programs must be dynamic and adapt quickly to the discovery of new pathogens and improvement of diagnostic methods. As an example, the recent report of Grapevine red blotch-associated virus (GRBaV) in California certified planting material has opened discussion on needed revisions of the current R&C program. The CDFA hosted several public meetings in 2014 and 2015 to collect feedback from growers and industry representatives concerned about issues regarding the 2010 regulations. Specifically, the vineyard industry is concerned with the traceability of stock, location of increase and certified blocks, eligibility requirements, standards for suspending or canceling certification, and the detection of GRBaV in certified planting material, among other issues.

As a result, a committee will be assigned by the California Secretary of Agriculture to work on solving these issues and developing regulations that are acceptable to the California grape industry.

TESTING CERTIFIED MOTHER BLOCKS

Members of the NCPN are petitioning that regulators making decisions on the California R&C and quarantine programs allow the testing of plants using the next generation sequencing (NGS), also known as deep sequencing technology. NGS uses computer algorithms to analyze the many genomic signatures generated from the candidate vine to be tested. When the grapevine-specific sequences are subtracted, NGS is able to detect a diversity of viruses (and other pathogens) and specify the number of copies that are present in a vine. This technique has the potential to replace the current biological indexing technique considered to be the gold standard for the detection of viral diseases in certification and quarantine programs worldwide.

There are clear advantages in using this technology, as the NGS process can be completed in a relative short period of time (weeks compared to years) and is able to detect all types of pathogens in grapevine propagation material. The expectation is that this modern technology will expedite the release of imported and certified selections. However, the disadvantage is that the data generated from NGS testing will find other viruses that may appear alarming but actually have no effect on grapevine health.

One needs to approach this issue cautiously, as new viruses and mutants of known viruses could be detected before sufficient data is available to determine if they are able to cause disease. While NGS can rapidly discover new viruses, the biology and potential effect of these viruses in the vineyard will take much longer to understand. Therefore, unless regulators specify a list of harmful pathogens to be excluded, the detection of unknown viruses using NGS may slow down the registration or importation of new planting material rather than accelerate it.

ROUTINE TESTING PROGRAMS

Regardless of how the California R&C program produces source grapevine material, pre-planting testing is necessary. Increase blocks and certified stock still are and will continue to be produced from plants grown in the field, where they are exposed to disease-causing pathogens. Therefore, pathogen-free tested planting stock could become infected at any point after release from an R&C program. Of particular concern are diseased mother blocks that become a source of infecting pathogens that are inadvertently graft-transmitted to other vines.

Grapevine growers and nurseries must develop routine testing programs to complement the current and future R&C diagnostic programs in order to avoid the potential transmission and spread of pathogens. Determining the vineyard's planting needs (clone and rootstock selections) in advance allows the testing of mother vines, rather than the finished bench grafts that were derived from these plants.

The main advantage of testing mother vines is that the number of vines needed to be tested is reduced compared to the hundreds that would be required when testing grafted plants (i.e., one mother vine can provide buds to produce 100 or more progeny vines). Many nurseries are able to trace the tested mother vines and specifically collect their cuttings, providing assurance of the health status of

the material used in a specific grafting project.

It is most important to understand sampling requirements, disease incidence and frequency of detection of different pathogens (this topic was discussed in an article published in the August-September 2014 issue of this magazine) and how it may affect detection in the testing laboratory. It is alarming that the recent trend has been to request the laboratory to test composite samples consisting of cuttings from many vines.

While this appears to be a cost-savings strategy, it sometimes backfires, depending on the biology of the pathogen to be detected. For example, the detection of viruses such as GRBaV and Grapevine fanleaf allows combining material from multiple vines in one sample for diagnostic testing, while other viruses that cause leafroll disease do not allow this practice. When composite samples are tested for pathogens, there is the potential of combining one infected vine with many others that are not infected, thereby diluting the concentration of the virus to below detectable levels. The results would show no evidence of infection (a false negative), while one or more of the vines included in the composite could have been positively infected.

This concept explains why the vineyard in the above photo was planted with infected vines, in spite of the assurance from the supplier that "every vine in the block was tested" prior to grafting. The shared responsibility of nurseries and growers in adopting testing protocols that detect important grapevine pathogens in propagation material will assure the health of future vineyard plantings and avoid the movement and transmission of diseases.

This 1-year-old vineyard was planted with healthy-looking vines that were later determined to be infected with Grapevine leafroll-associated virus-3. Photo: Judit Monis

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