The Latest Research on Grapevine Virology

Highlights from the 17th Meeting of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine

Judit Monis and Nuredin Habili

Judit Monis is the plant health services division manager at Eurofins STA Labs. The lab provides state-of-the art disease testing and consulting, specializing in grapevine clean planting stock and vineyard disease management. Please direct queries to *juditmonis@eurofinsus.com* or 408-846-9964.

Nuredin Habili is a senior scientist at Waite Diagnostics, School of Agriculture, Food & Wine, The University of Adelaide, Australia. The lab provides advanced DNA testing for virus and phytoplasma in samples of grapevines and coconuts sent from growers, as well as famous institutes around the world. Please direct queries to *nuredin.habili@adelaide.edu.au* or + (61) 88313-7426.

THE LATEST RESEARCH ON grapevine virology was presented at the 17th annual meeting of the **International Council for the Study of Virus and Virus-Like Diseases of the Grapevine** (ICVG). The meeting, held every three years to promote collaboration and interaction among grapevine pathologists who specialize in viruses, viroids and phytoplasmas, took place in October 2012 in Davis, California and was well attended, with more than 120 scientific presentations representing different grape-growing areas around the world.

Two field visits were organized to learn about local disease problems, planting selections and disease prevention. The delegates visited the **UC Davis Foundation Plant Services** laboratory and foundation blocks, as well as the **USDA ARS Germplasm Repository** vineyards in Davis and Vacaville, respectively, where they heard presentations on breeding, clonal selection and California clean stock programs. As it has been a tradition for many years, **Giovanni Martelli**, chairman of the ICVG, presented the introductory keynote talk with an overview of the progress in grapevine virus research in the past three years.

There were nine main sessions presented at the ICVG in which a broad range of research was presented. Highlights are summarized below.

Fanleaf and Other Spherical Nematode-transmitted Viruses

This session opened with an overview of fanleaf and other spherical viruses by Dr. **Marc Fuchs** of **Cornell University**, who discussed the transient expression of antiviral constructs (segments of genes) that interfere with grapevine fanleaf virus (GFLV) multiplication. The research promises to speed up the use of genetically engineered resistance to this virus in grapevines. Based on the transient work, grapevine rootstock varieties, transformed with the most efficient antiviral constructs, are being evaluated in a naturally infected vineyard.

Leafroll Disease and Related Viruses

A study in France on the natural transmission of grapevine leafroll disease highlighted the risk of establishing new vineyards adjacent to diseased ones. Two Pinot Noir vineyards in Burgundy were surveyed for a period of eight years. One of the vineyards—planted with certified plant material—displayed typical leafroll symptoms that appeared to spread rapidly from the edges of the vineyard to the entire block. Further analyses demonstrated that the virus and its vectors originated from the adjacent GLRaV-1 infected blocks, which were also heavily infested by mealy bugs (*Phenacoccus aceris*). The second vineyard displayed none or very little incidence of leafroll disease. This vineyard was not surrounded by mealy bug-infested or leafroll-infected vineyards. The researchers noted that Grapevine Virus A (GVA) and B (GVB), often found in combination with GLRaVs, are transmitted by the same mealy bugs and scale insects.

Another study in France reported the dispersal of *Parthenolecanium corni*, the vector of GLRaV-1 and GVA, in a vineyard surrounded by infected grapes. Their healthy trial block was set among infected vines, and the results showed that the healthy vines were rapidly infected by the virus. The results support those reported for GLRaV-3 from New Zealand in 2009, in which healthy vines were planted several years later in a fallow block after removal of a leafroll-infected vineyard. All the new vines were infected due to the survival of mealy bug vectors on root remnants.

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Close up view of a grapevine accession at the USDA ARS Repository Germplasm collection—note the disease symptoms related to leafroll disease.

In another study, the transmission of GVA was found to be negligible in the absence of a leafroll (helper) virus. When *Planococcus ficus* mealy bugs fed on Chardonnay vines doubly infected with GLRaV-3 and GVA, the mealy bugs transmitted both viruses to healthy Pinot Noir plants in a higher proportion (31 percent) than either GLRaV-3 (24 percent) or GVA (2 percent) alone.

Preliminary results of a long-term study on the effects of GLRaV on yield, vine performance and grape quality in California were presented. Cabernet Franc vines budded onto nine different rootstocks were inoculated with different GLRaVs and planted in the field to monitor the symptoms, plant growth, yield and berry qualities. The data collected in 2011 showed that the mixed infection of GLRaV-1 and GVA had detrimental effects on Cabernet Franc plants propagated on 420A, Freedom, 3309C and 101-14 rootstocks—many of these vines died within a few months after inoculation. In addition, GLRaV-2 positive vines grafted on Freedom and 5BB were extremely weak, showing red leaves and short internodes. This study will provide further information on the severe damage of a combination of different leafroll viruses on vine and wine quality.

South Africa has pioneered the control of GLRaV-3 infections in red varieties with vine removal, insect control and planting disease-free vines. This strategy is more difficult to apply in vineyards with infected white varieties. Because symptoms are less obvious, the confirmation of virus infection relies on laboratory diagnosis before vine removal.

The molecular diversity of Pacific Northwest GLRaV-2 field isolate, from own-rooted, asymptomatic Sangiovese grapevine (designated as GLRaV-2-Sg), was determined to be closely related to the isolate from Oregon and South Africa but distantly related to the variants known to cause graft incompatibility, such as the "Red Globe" or "RG" isolate from USA and "BD" isolate from Italy. It was noted that "asymptomatic strains" can induce disease in other situations (i.e., after being grafted), signaling the importance of rigorous testing in clean plant programs to ensure that elite virus-tested propagating materials are provided to nurseries and grape growers.

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Healthy rootstocks planted at the UC Davis Russell Ranch foundation block promise clean planting stock for the future.

Rugose Wood and Related Viruses

A study by **Gambino**, et al. suggests that the long co-existence between grape and Grapevine Rupestris Stem Pitting-associated Virus (GRSPaV) resulted in a favorable mutual adaptation between this virus and its host, which led to the lack of disease development in grapevines. The absence of symptoms in many own-rooted Australian varieties singly infected with GRSPaV has been observed for many years (N. Habili, unpublished). The analysis of several American and European grapevine accessions never subjected to grafting suggests that an ancestor of GRSPaV infected the *Vitis* species a long time ago since it is found both in cultivated (*Vitis vinifera ssp. vinifera*) and wild (*V. vinifera ssp. sylvestris*) grapevines. It is interesting to note that GRSPaV was not detected in a number of vineyards in the Old World, providing evidence that the virus has an American origin and entered Europe in the 19th Century via infected American rootstock hybrids.

Next-Generation Sequencing technology (NGS), also known as deep sequencing, was used to study the etiology of Shiraz Disease (SD). Both South African and Australian researchers reported the effect of SD on the grapevine, especially Shiraz. The disease is of great concern for the grapevine industry in South Africa and Australia because it is highly destructive to premium cultivars, such as Shiraz and Merlot, and is spreading naturally in vineyards. Type 2 is the destructive form of GVA, which is associated with Shiraz Disease and has established itself in these two countries. Traditionally, GVA has been associated with the "rugose wood complex" disease.

Vines affected by SD are easy to identify in vineyards as the canes do not mature evenly and have a rubbery texture while the leaves turn bright red and/or purple and remain on the canopy through the winter. SD-infected plants never recover, gradually reducing the yield by 98 percent toward the final year of life (average lifespan is five years) when growers finally pull them out of the field. GVA is latent in most cultivars and rootstocks but can be transmitted easily from these to SD-susceptible grapevines through grafting on infected rootstocks or by top-working on existing GVA positive *Vitis* cultivars.

The detection of the Vitiviruses, Grapevine Virus E (GVE) and Grapevine virus F (GVF) by NGS in South Africa and in California, respectively, has added complexity to the virus status of grapevines.

A panic situation among growers should be avoided unless a relationship between the detected viruses and disease incidence in the vineyard is found.

New Viruses Discovery and Diseases of Unknown Etiology

As discussed above, the use of NGS has become a common tool for characterizing new viruses in an individual vine. The discovery of the presence of a circular DNA virus in symptomatic Cabernet Franc vines was independently announced by researchers at Cornell University and UC Davis. Although the genetic content of each virus is identical, the researchers characterized the virus with different means. Cornell University applied molecular cloning and sequencing techniques and named the virus Cabernet Franc associated Virus (CFaV) while UC Davis used NGS and named the virus Grapevine Red Blotch associated Virus (GRBaV). Eventually, both groups agreed on the name Grapevine Red Blotch associated Virus. This virus has a 50 percent genetic relationship to Geminiviruses, which destroy millions of dollars worth of tomato plants annually worldwide. A different type of circular DNA virus, Grapevine Vein Clearing virus (a Badnavirus) was reported in the Midwestern states (Missouri, Illinois and Indiana) and is infecting Cabernet Sauvignon, Chardonnay, Chardonel, Cabernet Franc, Riesling, Vidal Blanc and Corot Noir. It is associated with "vein clearing disease." Since this virus was found in mixed infections with GFLV, it was difficult to assess its independent effect on the vines.

A novel Vitivirus isolated from a black grape cultivar that displayed graft incompatibility in California vineyards was reported. This virus, designated as GVF, is highly related to GVA, a Vitivirus, associated with rugose wood complex disease. Field surveys and biological studies are in progress to show the possible involvement of this novel virus with graft incompatibility. Related to this work, the lab at **Eurofins STA** recently found this virus in a Chardonnay vineyard that displayed decline symptoms. It is interesting to note that the vines were also co-infected with GLRaV-3.

Diagnostics

Biological indexing, although a long-term approach, was firmly recommended in the meeting. Researchers compared different diagnostic methods and urged diagnosticians not to dismiss any technique, because it is not sophisticated enough, as there are methods that fit different applications. For instance, ELISA (a rapid serological test) is suitable to screen a large population of plants while NGS should be applied to a few specimens for research purposes. The bottom line is that any rapid diagnostics test must be confirmed by the long-term biological field indexing studies as the latter identifies disease development and not a particular pathogen.

In New Zealand, samples collected from commercial vineyards that had low ELISA reading allowed the discovery of two novel strains of GLRaV-3 and the development of specific RT-PCR for their detection. The study confirms the importance of using ELISA for the detection and analysis of virus variants. In California, at the Foundation Plant Services (FPS) Importation Program, the evaluation of GLRaV-2, GLRaV-3, Grapevine Fleck Virus (GFkV) and GVA were detected using ELISA, RT-PCR and RT-qPCR, and indicated that, in general, RT-qPCR had the highest sensitivity, followed by RT-PCR and ELISA. The low ELISA performance of GLRaV-2 was due to the use of "in-house" produced antibodies rather than high-quality, commercially available reagents. In spite of the low sensitivity of ELISA, in some cases GLRaV-3 was detected by ELISA but not by RT-qPCR. This is not a surprising fact as genetically diverse GLRaV-3 isolates are not detected by nucleic acid-based assays in other countries. The group reported potential cross contaminations in the lab by personnel who do not change gloves when handling different samples and/or detaching petioles during sample collection in the field.

The sensitivity and specificity of ELISA and RT-PCR were compared with RTqPCR (TaqMan) to determine if the latter technique could be applied in a commercial lab setting for the detection of important grapevine viruses. The work showed that both TaqMan and RT-PCR had the same sensitivity as measured by the limit of detection with serial dilutions of RNA of known infected grapevine samples. However, within the group of 251 field samples tested, the TaqMan probes missed the detection of samples known to be infected with GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, GFKV and GVB.

Real-time, high-resolution melting curve analysis on four variant groups of GLRaV-3 in South Africa showed that 88 percent of the samples were infected with multiple variants. In New Zealand the same virus existed as six variants, and each variant was detectible by Multiplex RT-PCR. In one root sample five different variants of the virus were detected. This confirms that grapevine GLRaV-3 exists as a quasi species in infected vines. It appears that the divergent strain of GLRaV-3 (GLRaV-3m, for being a mild strain) from Australia has a totally different genomic organization. This variant was not detected by RT-PCR, but it was detectible by ELISA.

Virus Effects on Viticulture Performance and Wine Quality

In a trial in Italy, GFkV (a widespread virus in grapevines) was removed while in another combination GLRaV-1 and GVA were removed from grafted Nebbiolo. The "virus-free" vines produced wine with a significantly higher concentration of anthocyanin and more intense color. However, berry weight of the "virus-free" vines was reduced by 38 percent. Researchers in Switzerland reported the influence of the GLRaV-1 and GFkV on Gamay grape and wine production. Previous reports indicated that grapevine leafroll disease caused by single or mixed infections of Ampelo and GLRaV-2 has detrimental effects on grape quality and yield. However, co-infection with GFkV did not have any significant effect on those parameters compared with infection by GLRaV-1 alone, but a synergistic effect between GLRaV-1 and GFkV was observed on fruit composition (reduction of sugar and nitrogen content) and wine quality. In France, the successful elimination of GLRaV-2 from premium Cabernet Sauvignon clones produced vines with strong vigor and a higher crop yield. However, the quality of the wine was not positively affected in this study.

The effect of irrigation on leafroll symptom development was studied in Israel. The intensity of leafroll disease symptoms ranged from non-symptomatic to severe infections. Interestingly, irrigated vines displayed more severe leafroll symptoms and higher Brix values than non-irrigated plants. The photosynthesis rate was lower in lightly irrigated vines. Vines that received a high irrigation from fruit set to veraison had a higher crop yield. These are preliminary results and suggest that it is possible to mitigate the effect of leafroll disease by altering the irrigation regime.

The effect of GLRaV-3 on photosynthesis was studied in own-rooted Merlot vines in a cool-climate setting (Washington state) before and after veraison. Before veraison, the vines were non-symptomatic while leafroll symptoms appeared after veraison. Before veraison, leafroll did not have a significant effect on the photosynthesis and sugar level in leaves while, after veraison, photosynthesis was reduced, and the sugar content in the leaves was increased by several folds. It appears that leafroll virus, being a tenant of pholem tissue, inhibits the free translocation of sugar into the berries with a negative effect on yield and a lower wine quality of Merlot vines.

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Meeting delegates from around the word enjoy a lunch break during the visit to the USDA ARS Repository Germplasm facilities in Vacaville, California.

Ancient grapevine varieties growing in the Greek Cycladic Islands were tested for viruses. Although all these varieties tested positive for leafroll viruses, none showed symptoms indicating the potential selection of mild viral variants over the years, enabling virus and vine to co-exist. However, we can speculate that exotic wine varieties grafted onto these native varieties may cause a synergistic effect with severe leafroll virus strains. This phenomenon was observed in Australia with a clone of a symptom-less Chardonnay called Mendoza naturally infected with GLRaV-1—high quality wine can be made from this clone. However, if a red variety is top-grafted onto this Chardonnay, severe leafroll symptoms will develop.

Conclusion

Growers that manage existing vineyards should always monitor the health status of their vines. It is important to analyze both in space and in time any unusual pattern of symptom spread. Any unusual symptom that persists for at least two seasons must be reported to a qualified consultant, and samples must be sent to diagnostic labs for analysis. Growers who are establishing new vineyards must plant the cleanest material available. The reader must be reminded that once a vineyard is planted with infected material, there are no curative methods. Prevention is the key to healthy vineyards.

The ICVG meetings are important because work done in all parts of the world is shared to encourage the understanding of grapevine viral disease epidemics. The October ICVG meeting was the 50th anniversary of the ICVG's establishment as a working group to study viruses and virus-like diseases.

For more details on the presentations, visit *www.icvg.ch/archive.htm*.
The next ICVG meeting will be held in 2015 in Ankara, Turkey. **WBM**