

The Latest Research on Grapevine Viruses and Phytoplasmas

Highlights of the 18th Meeting of the International Council for the Study of Virus and Virus Like Diseases of the Grapevine

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THE 18TH MEETING OF The International Council for the Study of Virus and Virus Like Diseases of the Grapevine (ICVG) was held in Ankara, Turkey, September 7-11, 2015. The ICVG meeting is held once every three years to promote collaboration and interaction among pathologists who specialize in viruses, viroids and phytoplasmas that infect grapevines. The next meeting will be held in 2018 in Chile.

The meeting in Ankara was well attended with more than 109 scientific presentations from different grape-growing areas across the world. The two field visits were of interest because Turkey is one of the main genetic and domestication centers of the wild Eurasian grapevine *Vitis sylvestris*. Furthermore, biodiversity of the cultivated form *Vitis vinifera* is found in this country. The delegates visited the **Ankara University Agriculture** vineyard to learn about genetic diversity, planting selections and local disease issues. The second vineyard visit was held near Cappadocia, a region thought to be the site where the oldest vineyards in the world are found. Drs. **Feliz Ertunc** and **Birham Marasali** provided informative presentations on the Turkish viticulture and wine industry.

There were many interesting sessions in which a broad range of research was presented. The complete articles can be found by searching the author's name in the meeting abstract book, found at http://icvg2015.org/data/icvg_2015_abstract_book.pdf.

Here, we refer to a number of novel achievements discussed at the meeting.

New Findings on Known Viruses and Viroids

Grapevine Rupestris vein feathering virus (GRVfV) was reported from New Zealand. GRVfV is quarantined in Australia, but it appears to be an inert virus. Grapevine yellow speckle viroid (GYSVd-1) and Australian grapevine viroid (AGVd) were reported from Turkey. Researchers from Iran (**Zakiaghl** and **Izadpanah**) reported AGVd-induced stunting in cucumber; stunting, leaflet deformation and mottling in tomato; twisting and leaf edge sharpening in *Gynura aurantiaca*; and mottling and faint vein banding in *Nicotiana glutinosa*.



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FIGURE 1: Corky bark-like symptoms (known to be caused by GVB) on Shiraz (Syrah) infected with *Grapevine virus A*.

The full-length sequence of three grapevine viroids, AGVd, GYSV-1 and Hop stunt viroid, was detected in a 10-year-old bottled wine (Habili et al.). This may bring biosecurity risk to a new era as full-length viroids can be infectious following mechanical transmission. **Habili** and **Wu** presented research on the association of Grapevine virus A (GVA), rather than Grapevine virus B (GVB), with corky bark symptoms. Corky bark-associated GVB is a quarantined virus in Australia (**FIGURE 1**).

The beneficial effect of *Grapevine Rupestris stem pitting associated virus* (GRSPaV) to improve tolerance to drought conditions was reported by **Pantaleo** and colleagues. Under extended water stress conditions, infected plants developed more leaf area with taller and thicker canes.

Habili reported that GRSPaV might have originated from North America. This virus is present in most winegrape-growing areas of the world while

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FIGURE 2: Symptoms of Grapevine Pinot Gris virus on cv. Pinot Gris.

in Iran, where only local table grapes are grown, no GRSPaV was detected in over 60 samples tested. Meng *et al.* (2006) classified GRSPaV into four major groups of which group 1, represented by GRSPaV-1 which is adapted to *Vitis riparia*, and group 2 (GRSPaV-SG1) which is adapted to *V. rupestris*, are the most common groups. *V. riparia* and *V. rupestris* are two species native to North America and carry the virus as latent. When the pest Phylloxera *Daktulosphaira vitifoliae* accidentally entered Europe in the 1860s and devastated vast vineyards, the only solution to stop the pest was to plant *V. vinifera* scions on resistant American rootstocks and their hybrids.

Novel Grapevine Viruses and Diseases

Italian scientists P. Saldarelli and E. Angelini presented an update on Grapevine Pinot Gris virus (GPGV), the new Trichovirus associated with chlorotic mottling and leaf deformation in many wine and table grape varieties throughout Europe (FIGURE 2). The virus is genetically related to Grapevine berry inner necrosis virus (GINV). Interestingly, GPGV has been detected in both symptomatic and asymptomatic vines, suggesting that both virulent and avirulent variants may exist even in the same vine. A survey in northern Italy reported that most of the infected vines showed no symptoms. Additionally, Malagnini demonstrated significant clustering of diseased vines resulted from the spread of GPGV by the eriophyid mite, *Colomerus vitis*. Preliminary data indicate that *C. vitis* is able to acquire the virus from GPGV-infected vines and transmit it to healthy vines (albeit inefficiently). Under controlled conditions, seven out of 34 cv. Traminer plants became infected. The occurrence of GPGV in California was recently reported.

Al Rwahnih and co-workers at the University of California, Davis reported the identification of a novel Reovirus named Grapevine Cabernet Sauvignon reovirus (GCSV) using next-generation sequencing (NGS). The virus was found in a leafroll diseased vine infected with a combination of different Grapevine leafroll associated virus (GLRaV-2, and GLRaV-3), Grapevine virus A (GVA), and Grapevine fleck virus (GFkV). The novel reovirus was graft transmitted to a healthy vine. Further research is needed to determine the economic impact and the symptoms associated with GCSV in the vineyard.

Two presentations by M. Fuchs from Cornell University summarized the progress on Grapevine Red Blotch-associated Virus (GRBaV), a virus threatening the Australian wine industry. The first presentation elegantly showed, by recombinant DNA technology, replication of the virus in the recipient plants as confirmed by sequencing analysis. The study allowed the completion of “Koch’s Postulates” and has shown that GRBaV can cause Red Blotch symptoms in infected red grape plants derived from micro-shoot tip culture. Here, we propose with confidence that the acronym GRBaV must be changed to GRBV, “Grapevine Red Blotch Virus,” by deleting “a” for “-associated” from its name. The virus is not necessarily associated with red vein symptoms, as green veins have also been observed as a typical symptom of this virus in the absence of any leafroll virus infection. Additionally, the virus causes yellow blotch in white grape varieties and red patches in red varieties (symptoms in many commercial grape varieties are available at this website: www.eurofinsus.com/sta-laboratories/grapevineplant-disease/grapevine-red-blotch-photo-gallery/). Further field work is needed to demonstrate the effect of the virus on sugar production. The other presentation focused on the distribution of GRBaV in North American vineyards, including in wild *Vitis* plants in the vicinity of the vineyards. They detected GRBaV in



Reddened veins on underside of CS4/420A leaf with RBaV

six of the 28 non-cultivated grapevine samples tested. The viruses in both groups of vines clustered within the same phylogenetic (DNA) clade, indicating a common origin. The data suggests that these vines could function as a reservoir of the virus, although no insect vector for GRBaV has been identified to-date.

Virus Effects and Epidemiology

The effect of three different isolates of GLRaV-3 on the performance of Cabernet Franc plants grafted over nine different rootstocks was studied at UC Davis, California by Rowhani and coworkers. Cane length was significantly less for all three virus isolates compared to healthy vines while berry weight, total clusters and total yield were not significantly different from control. Among the rootstocks examined, significant virus effects were highest on 5BB, followed by 3309C.

Reynard and **Gugerli** reported the effect of GRBaV on vine physiology and fruit composition of field-grown grapevine cv. Gamay under cool-climate conditions. The rate of photosynthesis and transpiration was reduced by about 30 percent in GRBaV-infected vines even before the onset of virus symptoms. Fruits had lower sugar and a lower tartaric acid content but higher malic acid with an overall higher pH as compared with the healthy control.

The effect of *Grapevine fanleaf virus* (GFLV) on the yield of Gewürztraminer and Chardonnay cultivars was studied by **Vigne** and colleagues at **INRA**, Colmar. Yield loss was higher in Chardonnay (-63 percent) as compared to Gewürztraminer (-45 percent). These results were comparable to those obtained 20 years earlier by other researchers in the same viticultural area.

Research presented by **Hemmer** and colleagues in France demonstrated the successful antiviral activity of the GFLV-specific nanobodies that affect both mechanical and nematode transmission. Nanobodies are small peptides derived from the heavy chain antibodies found in the camel family.

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FIGURE 3: Pictured above is a close up view of characteristic patchy red symptoms associated with phytoplasma infection in a red table grape variety in Turkey. These symptoms can be confused with GRBaV infection.

Phytoplasmas

Phytoplasmas are wall-less bacterial pathogens that cause great damage to the grapevine. **Asunta Bertaccini** presented an overview on this topic. Fifteen of the phytoplasma subgroups are associated with the infection in grapevines worldwide. The grouping is based on the 16S ribosomal gene classification. It appears that each geographical area has its own phytoplasma subgroup. For example, in the Eastern United States, a phytoplasma disease infects grapevines in Virginia, which is called “Virginian grapevine yellows.” Its associated phytoplasma, *Ca. P. asteris*, belongs to 6SrI-A subgroup, which is specific for that part of the world. Two of the well-known diseases associated with phytoplasmas are Flavesence Dore (FD) and Bois Noir (BN) of which BN occurs in almost all grape-growing regions of the world, excluding Australia and the U.S. Since the vines affected by FD and BN can either recover or die, it has been advised that when only the plants are dead it is profitable to replace them but not when they undergo recovery. Replanting is more focused toward FD-affected vines as the active leafhopper vector



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Scaphoideus titanus is present and can transmit the pathogen from infected to healthy plants. Removing alternate hosts and infected vines can reduce the spread of phytoplasmas by eliminating the source of infection. **Contaldo** and co-workers reported the isolation and culture of grapevine yellows phytoplasma in specific agar media. This breakthrough will facilitate biological studies of these bacteria which will ultimately improve our knowledge on the epidemiology and management of diseases caused by the phytoplasmas.

Novel Techniques, Diagnostic Tools and Grapevine Clean Stock

Ackerer and coworkers reported that nanobodies were successfully used for the detection of GFLV in infected vines. **Blouin** reported the use of dsRNA-specific antibodies to enrich virus-associated dsRNA from infected cherry, grapevine and potato tissue for the detection of viruses using NGS.

Gianpetrucci and colleagues applied NGS to confirm the virus-free status of 20 grapevine scion and rootstock varieties that had undergone sanitary procedures and showed no evidence of infection of the following viruses: GLRaV-1, -2, -3, GFLV, ArMV, GVA, GVB and GFkV. The authors concluded that NGS has the potential to replace woody bio-indexing and could provide a standard technology for certification. Their point is that each country or certification program uses a different standard operating procedure with different methodologies and reagents. Furthermore, the results of the woody indexing are highly dependent on climatic conditions that could vary in each location. A similar study by Al Rwahnih compared the results of NGS and woody indexing in the **California Registration and Certification** program. The biological indexing technique has always been considered the “gold standard” as it is able to detect the presence of disease rather than a specific pathogen. In the California study, the biological indexing failed to detect viruses that were readily detected by NGS. An advantage of NGS is its ability to identify viruses to the species level, and it takes less time to complete. While the study suggests that NGS is superior to biological indexing, there are issues that will need to be solved before the technology is widely applied. The possibility exists that the findings of unknown or uncharacterized viruses could delay the registration or release of planting material from quarantine until their biological effects in the vineyard are better understood. Although only a few of the discovered viruses by NGS have been assigned to specific symptoms, others appear to be background viruses that do not cause disease in the vineyard. Two examples of these viruses are GRVfV and *Grapevine Syrah virus 1*.

Judit Monis presented information on the distribution and sampling guidelines for the detection of GRBaV in grapevines. The results revealed the presence of GRBaV in the following tissues regardless of the type of technique (conventional or qPCR) used: apical shoots, apical and basal leaves; petioles from basal and apical leaves; leaf blades or veins; lignified and green canes; flowers and fruits, and inflorescence rachis, etc.

Another study presented by **Sineaux** and co-workers indicates that the qPCR was more sensitive for the detection of GRBaV than conventional PCR. However, studies in the Monis lab indicate that the virus was detectable even after diluting the grapevine extracts 1 million times either by conventional or qPCR methods. **WBM**

References:

Meng B, Rebelo A.R, Fisher H. 2006. Genetic diversity analysis of Grapevine rupestris stem pitting-associated virus: Revelation of distinct population structures in scion versus rootstock varieties. *Journal of General Virology* 87, 1725-1733

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Rhonda Smith and Dr. Monica Cooper in collaboration with Dr. M. Sudarshana characterized leaf symptoms of red blotch disease in red & white cultivars infected with GRBaV. Their research associated the virus infection with consistent delays in fruit maturity. In this study, crop reduction at the onset of veraison did not improve juice chemistry at harvest. For additional information visit AVF.org or contact Drs. Smith and Cooper at rhsmith@ucanr.edu or mlycooper@ucanr.edu.

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