The latest research on grapevine virology

Highlights of the 20th International Council for the Study of Virus and Virus-like Diseases of the Grapevine meeting

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Figure 1. Delegates at the 20th ICVG meeting held in Thessaloniki, Greece, on 25-29 September, 2023. Photo courtesy Professor Varvara Maliogka

The 20th meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG) was held in Thessaloniki, Greece, on 25-29 September, 2023. The ICVG meeting is usually held once every three years. The last ICVG meeting was held in Santiago, Chile, in 2018, but due to the COVID-19 pandemic, the subsequent meeting was delayed. The next meeting will be held in New Zealand in 2026. The scope of the ICVG is to promote collaboration and interaction between pathologists who specialise in viruses, viroids and phytoplasmas infecting grapevines.

The meeting in Thessaloniki was attended by 110 delegates and featured 44 oral and 48 poster presentations presented across six sessions by research entities located across various grapegrowing regions throughout the world. The conference also included a visit to the archaeological site of Vergina, showcasing the royal tomb of Philip II, and Kyr Yanni Vineyard and Winery where delegates observed typical leafroll disease symptoms on the red Greek variety Xinomavro (Figure 2, see page 66).

In this article we will describe some of the novel achievements discussed at the meeting. Full proceedings of the meeting can be found at https://icvg.org/proceedings.cfm

NEW FINDINGS ON KNOWN VIRUSES AND VIROIDS

The meeting opened with a keynote presentation by the ICVG's president Professor Marc Fuchs, from Cornell University, USA. Fuchs presented updates and highlights of grapevine virology since the last meeting in 2018. He revealed that to date, 101 viruses from 21 families have been identified in various Vitis germplasm worldwide. This count is higher than the number of viruses found in any other crop. However, not all are responsible for disease development. He referenced novel approaches to virus diagnostics, including the use of surface acoustic wave sensors to detect fanleaf and leafroll viruses and the research aimed at training dogs to detect grapevine leafroll and grapevine red blotch diseases.

The second keynote speaker was Professor Stefanos Kounddouras, of Aristotle University of Thessaloniki. He emphasised that due to global warming, the shift to earlier harvest dates could potentially disrupt vector activity and alter the rate of virus infections.

DISEASE ETIOLOGY AND EPIDEMIOLOGY

In 2022, grapevine red blotch virus (GRBV) was found for the first time in different commercial vineyards in Australia. A survey was completed in an Australian germplasm collection to investigate the presence and potential spread of GRBV. The researchers detected GRBV in three different varieties using a modified nested PCR. Surprisingly, an inconsistency in the detection of the virus was encountered. We wonder if the challenge of detecting GRBV (which Judit Monis has not

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had issues detecting in California) might be due to Australian climatic conditions or how the samples are processed. Further, reports from authorities in Australia note that the grapevine infections did not show typical red blotch symptoms.

In contrast, work in British Columbia by Jose Ramon Urbez-Torres' team, reported typical symptoms in Canada. The virus was detected in cambial scrapings from dormant wood (fall/winter) and basal leaves collected in the summer.

In California, the spread dynamics of GRBV was studied by Madison Fiasco (Cornell University) who performed yearly surveys for the presence of *Spissistilus festinus* (the vector of GRBV) and the distribution of infected plants in different Cabernet vineyard blocks. Despite the high infection rate in a Cabernet Sauvignon block, it was hypothesised that the spread of the virus was low due to a 10 times lower population of the insect vector compared to an adjacent Cabernet Franc block.

Syrah decline is a disorder characterised by the reddening of leaves, with grooving and swelling of the graft union found in certain Syrah clones. At the extremes, clone 383 is highly susceptible while clone 473 has been reported to be resistant to Syrah decline. Anne-Sophie Spilmont (IVES, France) presented data showing that Syrah decline is caused by genetic characters and not by a pathogen. In one study, self-pollination was performed on clones 383 and 473 grafted onto 110R rootstock. By the fifth year after grafting, typical Syrah decline symptoms were observed only in the plants derived from the sensitive clone at a Mendelian segregation rate of 1:3 (suggesting the presence of a dominant gene). In contrast, no symptoms were observed in the population derived from the 'resistant' clone.

In a poster presented by Darko Voncina (University of Zagreb, Croatia), experiments to transmit grapevine leafroll associated virus (GLRaV-3) using the vine mealybug *Planococus ficus* as a vector did not yield any positive infection in the 411 herbaceous and woody plant species tested. Vector transmission was only achieved through *Vitis* to *Vitis* species.

Figure 2. Symptoms of leafroll disease on the Greek variety Xinomavro at Kyr Yanni vineyard, Naoussa, Greece. Photo courtesy Professor Baozhong Meng

DIAGNOSTICS

The diagnostic session started with a presentation by Hano Maree (Stellenbosch University, South Africa) who discussed the consequences and responsibilities of using high throughput sequencing (HTS) for plant virus discovery. He noted that in grapevines, HTS has led to the discovery of economically important viruses such as grapevine Pinot Gris virus (GPGV) and GRBV. However, a large number of viruses have been reported with no association to disease, notably, the discovery of many vitiviruses (e.g. grapevine virus A, B, D, E, F, G, H, I, J, K, L, M , N, O) but only grapevine virus A (GVA), GVB and GVD have been reported to be associated with rugose wood disease, as well as Shiraz Disease (GVA). Clearly, more viruses will continue to be discovered using this powerful technology and science will need to provide biological data to help regulators decide which viruses should be kept out of planting material and which ones are considered innocuous.

Related to virus sequencing and discovery, Mamadou Fall (Université de Sherbrooke, Canada) described a modified, double-stranded RNA isolation method that would allow for the characterisation of actively replicating viruses in fungi and plants. Bhadra Vemulapati (Brock University, Canada) presented work on the development of drop digital PCR (ddPCR) for the detection of GPGV, which promises to be a sensitive technique that allows determining the virus copy number without needing a reference sample. Sudarsana Poojari, from the same university, spoke about the application of an amplification-free assay for the detection of GLRaV-3 using CRISPER (Clustered Regularly Interspaced Palindromic Repeats) Cas13a. Michel Hilly (Université de Strasbourg France) presented work on the application of datamining to determine the historical evolution of GPGV. Hilly's work showed that GPGV originated in Asia with China being its likely country of origin.

The presentation by Robin MacDiarmid (New Zealand Institute for Plant and Food Limited) was related to field visual detection of GLRaV-3 using RBG (red, blue, green) photography and machine learning. MacDiarmid described a study where a detection model was developed by analysing 26,000 field photographs of healthy and diseased (symptomatic) vines collected during three growing seasons using a phone app. The researchers hope that the technology will allow for the diagnosis of red virus symptoms through the analysis of photos taken from a camera mounted on a tractor or autonomous robot.

CERTIFICATION AND DISEASE MANAGEMENT

This session opened with a presentation by Maher Al Rwahnih (University of California, Davis, USA). Rwahnih described the regulatory process that allowed the Foundation Plant Services to replace the woody indexing procedure with the application of HTS in

quarantine and certification programs.

A series of presentations and posters focused on clonal selection and sanitation.

A study on the application of HTS to determine the virome of six grapevine varieties and subsequent pathogen elimination was reported by Vanja Miljanić (University of Ljubljana, Slovenia). The HTS results (confirmed by RT-PCR and Sanger sequencing) revealed the presence of viruses belonging to the *Nepovirus*, *Ampelovirus*, *Tymovirus* and *Trichovirus* genera plus two viroid species. The sanitation protocol employed a combination of thermotherapy (36-38°C) along with the micrografting of meristem tips of 0.1-0.2mm onto *in vitro* propagated rootstock seedlings. This process successfully eradicated all viruses but was ineffective against the viroids.

Dunja Leljak-Levanić (University of Zagreb, Croatia) described the successful use of somatic embryogenesis on virus elimination, while Eva Varallyay (Hungarian University) reported on the use of chemotherapy. In the Hungarian study, different concentrations and combinations of ribavirin, zidovudine and 2-thiouracil were used in grapevines grown *in vitro*. Based on the preliminary results, the researchers concluded that none of the treatments were able to increase the efficiency of virus elimination.

Gábor Jakab presented the use of BABA (β-amino butyric acid) to induce different plant defence mechanisms in grapevines. Interestingly, the results indicated that the treatment eliminated arabis mosaic virus (ArMV) and GLRaV-1 but had no effect on grapevine fanleaf virus (GFLV) and GVA infections.

VIRUS CHARACTERISATION AND DIVERSITY

Baozhong Meng (University of Guelph, Canada) presented research that aimed to elucidate the molecular and cellular biology of GLRaV-3. The construction of a full-length infectious clone coupled with agro-inoculation of tissue culture-grown grapevine plants allowed the demonstration of Koch's postulates for this Ampelovirus. After regeneration and a dormancy period, the infected grapevines showed typical leafroll symptoms. Consequently, it is proposed to drop the word 'associated' so that the name of the virus is grapevine leafroll virus.

Qi Wu and colleagues (University of Adelaide) reported on the genetic diversity of grapevine rupestris stem pitting-associated virus (GRSPaV) in 15 varieties grown in South Australia. Phylogenetic analyses indicated that groups 1, 2a, 3 and 4 of GRSPaV were present.

Grapevine leafroll virus 3 is present all over the world, but it is symptomless in most grapevines. Complicating matters, grapevine leaf mottling and deformation (GLMD) symptoms associated with GPGV infection can be confused with those caused by Nepoviruses (*e.g.* ArMV, GFLV). Anne-Sophie Spilmont (IFV - French Wine and Vine Institute) reported 32% infection of GPGV in a survey of 117 vineyard blocks around France. Further, a low correlation of GPGV infection with GLDM symptoms was found, questioning the relationship between GPGV and GLMD. In Australia, Nepoviruses are not present in commercial vineyards, therefore, if a GPGV-positive vine shows GLMD symptoms, this would be associated with the virus. However, very few GPGV vines have shown GLMD symptoms in Australia or the USA. Furthermore, when these symptomatic vines were subjected to HTS, a few other viruses were detected (K. Kaur, Agriculture Victoria, Australia, personal communication).

Olufemi Alabi (Texas A & M University, USA) described the molecular characterisation of divergent isolates of GRBV in the recently released Pierce's Disease tolerant interspecific hybrid Blanc du Soleil. Genetic mutations and recombination events were reported to be responsible for the generation of the divergent isolates of GRBV in the Blanc du Soleil variety. The study points to the need for screening breeding material to avoid the introduction of infected material into winegrowing areas. Research by Mate Carija and colleagues (Croatia) showed that the response of different grapevines to GLRaV-3 infection is variety and virus-variant dependent. In the study, Merlot was the fastest variety to display viral symptoms regardless of virus variant, while the Croatian red grape Tribidrag appeared to be more resistant to leafroll infection.

Figure 3. A view of the Kyr Yanni vineyard, Naoussa, Greece. Photo courtesy Professor Marc Fuchs

PLANT-VIRUS AND VECTOR INTERACTIONS

Urbez-Torrez reported that two species of treehoppers, *Stictocephala basali* and *S. bisonia,* were able to transmit GRBV in artificial transmission experiments in the laboratory. Greenhouse and field experiments will follow to determine the ability of these tree hoppers to transmit the virus *in planta*.

In the USA, the alfalfa three-cornered treehopper *Spissistilus festinus* is known to transmit GRBV in a circulative, non-propagative manner.

Victoria Hoyle (Cornell University, USA) presented data on the feeding preferences of *Spissistilus festinus* in the vineyard ecosystem. The analysis of the DNA

extracted from the insect's gut indicated that the vector relies on many hosts for feeding and grapevine is not the preferred host. Therefore, even if *Spissistilus festinus* is present, the use of insecticides is not recommended as a disease management practice.

Munir Mawassi (The Volcani Center, Israel) and Emmanuelle Vigne (INRAE, France) presented work on the characterisation of isolates of GLRaV-3 and GFLV that cause mild or no symptoms in grapevines. The aim of the research is to use these mild strains on initial cross protection experiments. Cross protection is a method that has been used for many decades to control citrus tristeza virus in citrus crops. Scientists expect that cross protection would be accepted by consumers as it does not produce genetically modified vines. In theory, a vine already infected with a mild strain of the same virus could be protected against a severe strain that might be later introduced in the vineyard. The HTS methods can be used to identify mild virus strains established in vineyards.

In South Australia's Clare Valley, a symptomless Shiraz (Syrah) vine infected with GVA (Strain I) was found. We expect that this particular source of Shiraz may be tolerant to future infections by a severe strain of GVA (Strain II) that causes Shiraz Disease. Margarida Teixeira-Santos (INIAV, Portugal) reported the involvement of GRSPaV on the mitigation of the graft incompatibility of Syrah on 110R rootstock by silencing the virus with dsRNA prior to grafting. In South Australia, grapevine virus A (Strain II) severely affects Shiraz and Merlot varieties. Nuredin Habili and team have observed that if *Diplodia seriata*, a grapevine trunk disease fungus, is present in a vineyard, vines start to die back. However, in the absence of the fungus, symptoms similar to leafroll disease manifest.

Related to disease resistance, Olivier Zekri (Novatech, France) and Christophe Ritzenthaler (IBMP-CNRS-Strasbourg, France) presented work on the collaborative project between industry and a research institution to develop transgenic grapevine plants using nanobodies that confer resistance to ArMV and GFLV. Gérard Demangeat (Université de Strasbourg, France) presented data on the discovery of genetic resistance to GFLV found in two accessions of *Vitis silvestris.* The study showed that the gene(s) confers resistance to virus infection but had no effect on nematode populations. The research shows promise in producing GFLV-resistant plants using traditional breeding techniques.

CONCLUSIONS

The ICVG meetings provide an opportunity for researchers from all over the world to present, share and discuss their discoveries on grapevine viruses. From the 2023 presentations we learned that viruses continue to cause disease in vineyards. However, we need to learn more about the biological properties of newly discovered viruses before we decide to reject plant material for propagation, as many viruses that have recently been discovered have not been implicated in disease.

Judit Monis provides specialised services to help growers, vineyard managers and nursery personnel avoid the propagation and transmission of diseases caused by bacteria, fungi and viruses: www.juditmonis.com, juditmonis@yahoo.com

Nuredin Habili is an honorary Fellow at The University of Adelaide and The Australian Wine Research Institute (AWRI). *Nuredin thanks Affinity Labs at the AWRI for providing funding for the trip to Thessaloniki.*

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