Highlights of the 16th Meeting of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (Dijon, France, 2009)

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The 16th Meeting of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (ICVG) was held in Dijon, France on August 31-September 4, 2009. The ICVG meeting is held once every three years to promote collaboration and interaction amongst grapevine pathologists who specialize in viruses, viroids, and phytoplasmas. The next meeting marking the 50th anniversary will be held in 2012, in Davis, California. The last meeting was well attended with 161 scientific presentations representing different grape growing areas across the world. Two field visits were organized to learn about local disease problems, planting selections and disease prevention. The delegates visited vineyards in Côtes de Beaune, Hautes Côtes de Nuits, Davayé (Maconnais), and the Jura region (Chateau-Chalon) where they heard presentations on the national and regional clonal selection programs and hot water treatment to prevent spread of phytoplasma associated diseases through propagation.

There were ten main sessions in which a broad range of research was presented and these included:

1. Introductory keynotes
2. Detection, plant material and virus sources
3. Fanleaf, Fleck and other spherical viruses
4. Epidemiology – Survey of vineyards
5. Phytoplasmas
6. Molecular biology – New technologies
7. Virus effects – Control – Crop performances
8. Viruses of the Leafroll Disease Complex
9. Rugose Wood Complex viruses
10. Emerging diseases and diseases of unclear etiology

Following is a summary of the research presented during each session:

Introductory Keynotes

Giovanni Martelli, Chairman of the ICVG, began the meeting with a minute of silence to remember our highly-esteemed colleague, Dr. Rod Bonfiglioli, who passed away in May 2008 in New Zealand at the age of 55. As it has been tradition, Professor Martelli, presented an overview of the progress in grapevine virus research in the past three years. Other keynote speakers included Drs. Gugerli and Valerian Dolja. Dr. Gugerli presented the historical compilation of Grapevine leafroll associated virus (GLRaV-1-9) antibodies produced in the last 25 years; while Dr. Dolja's presentation focused on the evolution and gene function of grapevine Closteroviridae.

Detection –Plant material and virus source

Two research groups at the University of California (UC) at Davis and the Mississippi State University independently announced the discovery of a new virus member of the Marafivirus genus within the Tymoviridae family. Each virus originated from different vines with different symptoms; one from a Syrah vine expressing Syrah Decline while the other was found in a wild *Vitis* species with no symptoms. The accepted nomenclature for this virus is *Grapevine Syrah virus1* (GSyV-1). GSyV-1 was detected in only 19% of the Syrah symptomatic vines, therefore most likely is not the causal agent of Syrah decline.

The study at the UC Davis lab detected GSyV-1 in leafhoppers collected from symptomatic vines infected with GSyV-1, supporting the possibility of leafhopper transmission. The finding of this virus in asymptomatic wild grapevine species has relevant implications on the sanitary status of rootstocks used in viticulture because rootstocks do not express specific virus symptoms.

Three presentations focused on research studies comparing high throughput low density arrays, real time PCR, and traditional PCR. A South African study showed that real time PCR was found to be 8.9% more sensitive than ELISA. Dr. Gugerli presented information on the Grapevine Virus Collection at the Swiss Experiment Station at Nyon, Switzerland and urged representatives from each country or continent to contribute to produce a world-wide grapevine virus reference collection

Grapevines inoculated with *Grapevine leafroll associated virus (*GLRaV)-2, GLRaV-3, *Grapevine virus A (*GVA) and *Grapevine fleck virus (*GFkV) by grafting for validation of sampling strategy under Australian conditions showed that reliable testing can be conducted from late spring into autumn. It was interesting that GVA was not detected in any inoculated samples. In Australia green tissue can be used reliably for detection of the above viruses.

Fanleaf and other Nematode-transmitted spherical viruses

Recent molecular data analyses of *Xiphinema index*, which transmits *Grapevine fanleaf virus* (GFLV), from different geographical areas suggest a common Middle Eastern origin of this nematode. In contrast, the North American *Xiphinema* nematodes and their associated viruses: *Cherry rasp leaf virus*, *Peach rosette mosaic virus, Tomato ringspot virus,* and *Tobacco ringspot virus* appear to spread only across the North American continent. A study in France found that the capsid protein was responsible for specificity of transmission of GFLV and *Arabis mosaic virus* (ArMV) by *X. index* and *X. diversicaudatum*, respectively. The high virus retention and long survival of infectious nematodes in the absence of host plants suggest that control measures should focus on the development of virus and nematode resistant grapevines.

On the detection of GFLV the use of young shoots was recommended as this was more sensitive than testing dormant vine material.

Epidemiology – Survey of vineyards

Wild and native *Vitis* species were shown to be reservoirs of viruses that infect cultivated grapevines and may play a significant role in virus epidemiology. In particular *Grapevine Rupestris stem pitting associated virus* (GRSPaV), GLRaV-2, and *Grapevine virus B* (GVB) were detected in *V. californica* in the USA In Iran GVA was the most prevalent virus detected in wild and cultivated grapevines and wild vines were also hosts of GFkV, GLRaV-2 and GLRaV-6. In many countries mixed infections of various GLRaV species and GLRaV-3 were detected frequently. A high incidence of GRPSaV (96%) was reported in cultivated grapevines in Australia as well as in wild grapevines in California. However, this virus was not as prevalent in cultivated grapevines from the US Pacific Northwest (10%). GRSPaV is also the most common virus detected in California-based Eurofins-STA testing program (Judit Monis, unpublished) as well as by Waite Diagnostics in Australia. In the Lucca district of Italy an unusually high incidence of ArMV was observed and it was suggested this virus was introduced from the USA through infected planting material.

One study highlighted the importance of vectors and the possible influence of variety and rootstock in virus incidence and distribution in two areas in northern Spain. The incidence and distribution of GLRaV-2 and GFkV in both regions was likely to be associated with introduction through symptomless planting material. GFLV was more prevalent in Rioja and its distribution indicated the presence of a vector. Similarly, the incidence of GLRaV-3 in Galicia (north-west) was high as a result of virus transmission by mealybug vectors, while in Rioja (north-east), where the mealybug vectors are not present, no evidence of virus transmission has been observed for over 20 years. The presence of GLRaV-1 was variable within each region but it has decreased in the regions of Ribeira Sacra, even though vectors were present. It was suggested that the introduction of new varieties or rootstocks has influenced to this change in prevalence.

Phytoplasmas

The phytoplasma session included 11 oral presentations and 29 posters with most focusing on Flavesence dorée (FDp) and Stolbur (Bois Noir,

STOLp) phytoplasmas. The latter included Australian grapevine yellows phytoplasma. The detection of Aster yellows phytoplasma in the Southern Cape of South Africa was reported. Recent research showed that phytoplasmas may not be eradicated from Australian grapevines when daytime temperatures exceed 40°C, as had been previously hypothesized.

Phylogenetic analyses using multiple genes showed that FDp and Alder yellows (AldYp) phytoplasma strains were closely related and could be distinguished from other members of Group 5 (16SrV) phytoplasmas. The study also supported previous studies which suggested that FDp and AldYp may have a common origin, possibly in Alders. It was proposed that FDp and AldYp be classified into a new species known as *Candidatus* Phytoplasma cauldwelli. The species name honours Antoine Caudwell who first described FD disease in Europe in 1957 and contributed greatly to FD research during his career.

Analysis of the elongation factor EF-Tu (*tuf*) gene shows that the STOLp can be differentiated into three types, Tuf-types-I,- II and –III. Tuf-types-I and –II, which are common in grapevines, have distinct epidemiological cycles associated with their different alternative hosts, nettle and bindweed respectively, and different races of the plant hopper vector *Hyalesthes obsoletus*. As a consequence of these differences slightly different control strategies are required to prevent the spread of STOLp strains into vineyards. It was hypothesised that the type-I and -II STOLp strains have evolved independently on nettle and bindweed, respectively, and different races of *H. obsoletus* are specialising on these two hosts.

Wild rootstocks in Europe were shown to be a reservoir for FDp leaf hopper vector, *Scaphoideus titanus*, and may contribute to the spread of FDp. Recovered FD diseased grapevines were an unlikely source of FDp for acquisition by *S titanus* or for transmission through propagation. However, transmission in propagation material can occur and hot water treatment remains a recommended control strategy to prevent further spread of FDp and STOLp in Europe. Remission of Bois Noir disease was induced by the application of various commercially available chemicals such as Aliette, which stimulates natural host defenses. Remission was also induced through abiotic stress associated with partial uprooting, pollarding and by summer pruning.

Proteomic analyses and gene expression studies indicated changes in carbohydrate metabolism, photosynthesis and the phenyl propanoid pathway in FDp and STOLp infected grapevines. It was suggested these alterations may be associated with host defense responses to phytoplasma infection. Phytoplasma infection also altered the composition of the endophytic bacterial community of grapevines possibly as a result of plant host defense responses.

Molecular biology- New technologies

Two important technological developments were reported for grapevine virus identification. One, known as high throughput or deep sequencing, was used to identify the nucleotide sequences related to unknown viruses in diseased plants. This technology has become possible only after the complete sequence of grapevine genome has been released. Thousands of DNA clones are sequenced from both healthy and infected plants. These short sequences are analyzed in parallel and the DNA segments found only in the infected plant are screened for the presence of the possible pathogens. Using this technology Researchers in California attempted to determine the casual agent of Syrah decline, an economically important disease of unknown etiology reported from France and the USA. Although deep-sequencing is powerful, it is expensive. In this work, only one healthy and one diseased plant could be used for analyses. The other inherent problem is that it is a formidable task to prove the cause of disease (i.e., etiology) based solely on the derived sequence data.

A microarray detection strategy for multi parallel analysis involving 44 viruses using metagenomic approach was described. This technique is rapid and reliable. A low density PCR array (LDPA) based on TaqMan technology was examined in California for multi parallel grapevine virus detection in 29 varieties. The LPDA technique appears to be highly sensitive, being able to detect more viruses in the same samples as compared to the conventional RT-PCR assay.

Virus effects-control-crop performances

A three year field trial in the French Champagne region was carried out to determine the effect of rootstocks expressing the coat protein of GFLV on virus resistance. Although the genetically modified rootstock did not confer resistance to GFLV infection, a delay in symptom expression was observed in transgenic vines compared to vines grafted onto wild type rootstocks .

A study initiated in 2002, was designed to control the spread of GLRaV-3 in South African vineyards). A series of practices, including planting virus tested vines in *Vitis*-free sites (surrounded by infected vineyards), vine removal from adjacent infected vineyards and new vineyards (as soon as infection was detected), insecticidal treatment, sanitary control of tools and workers, led to drastic reduction of disease incidence and spread. The success of the project was due to heavy reliance on insecticides. Although the approach is not sustainable, the authors expect that South African growers will implement a more integrated pest management strategy as soon as the disease pressure is manageable.

A study in Northern Spain using micro-vinification of fruits collected from GLRaV-3 and GFLV infected and uninfected vines showed that the presence of virus had a drastic effect on both fruit set and quality. GFLV infection caused reduced alcohol, tartaric acid, and anthocyanins; while GLRaV- 3 caused a decreased alcohol content and color intensity. A study in Italy concluded that the presence of GFLV in the Nebbiolo variety significantly interfered with the physical and mechanical features of the berries. Another study indicates the beneficial effect of GLRaV-1 and GLRaV-3 elimination on vine performance (i.e., vigor, fruit quality and quantity) but questionable effects on wine quality. Previously, the same authors reported a significant increase in wine quality from virus free vines. In the present study they conclude that variation in wine quality depends on the clone, virus, environment, and viticultural practices. It is clear that more studies are needed to understand the interactions between viruses, vines and wine quality.

Researchers in California studied the effect of GLRaVs, GVB, GFkV, and GRSPaV in single and mixed infections on vine growth and fruit quality. The results showed a drastic reduction of sugar in GLRaV- infected Cabernet Sauvignon regardless of the rootstock used. There was no difference in fruit sugar content between healthy and GRSPaV infected vines. Foliar symptoms varied notably when one of the multiple infection treatments was grafted onto different rootstocks. The authors hypothesize variable tolerance to different viruses, suggesting that GVB infection has severe effects on certain rootstocks.

Viruses of the leafroll complex

The traditional taxonomic classification of the different virus species within the Closteroviridae family does not appear to fit the wide variation of genomic size and structure of described viruses A proposal to the International Committee on Taxonomy of Viruses (ICTV) will be made to change the status of GLRaV -4, -5, -6, -9 from virus species to strains. An alternative option would propose a new genus to include these viruses, but this seems improbable, since the retention of vector specificity as a "qualifying trait" to justify the existence of three Closteroviridae genera seems more logical. Yet another proposed option is to divide the Ampelovirus genus into two subgroups A and B. Subgroup A would include GLRaV-1 and GLRaV-3 with larger particles, a more complex genetic organization and are often associated with more severe symptoms, while members of subgroup B (GLRaV-4. -5. -6 and 9) are smaller in size with simpler genetic structure and are often associated with milder symptoms. The placement of GLRaV-7 in an appropriate genus will await transmission studies of the virus by whiteflies.

Strains of the same virus within a subgroup can vary considerably. For example, the South African GLRaV-3 isolates differ from the US isolates. Interestingly, an isolate reported from Chile is similar to the US GLRaV-3 isolates. Further, an isolate of GLRaV-3 in Crimson Seedless in Australia with desirable phenotypic characters exhibits mild symptoms, and can be detected only by ELISA, while a severe isolate of this variety induces severe symptoms and results in undesirable berry characters.

In recent years the spread of GLRaV-3 in South Africa, California and New Zealand has increased

dramatically with an exponential growth in some areas. This event resembles that of the phylloxera outbreak in Europe in 1860s. While in South Africa insecticidal treatments for mealybug vectors have been effective in an integrated control strategy this practice was not effective in New Zealand where beneficial insects were destroyed.

GLRaV-2 is the only known member of the *Closterovirus* genus which infects grapevines and it is the only leafroll virus that can be mechanically transmitted to *Nicotiana benthamiana* either as virus or as infectious cDNA clones. This virus, like many other grapevine viruses, consists of a number of distinct strains which are easily distinguishable by RT-PCR. Most of these strains are associated with graft incompatibility. In countries where grafting on rootstocks is required in order to control Phylloxera, infection by GLRaV-2 has become a challenge. Sanitary selection programs should aim to test and exclude this virus both in scions and in rootstock germplasm. The number of declined vines showing graft incompatibility increased by 83 % in certain rootstock/scion combinations as a result of the infection by GLRaV-2. In a grafting trial using Cabernet Sauvignon and a number of rootstocks, it appears that Paulsen 1103 and Rupestris St. George were most tolerant while Kober 5BB was the most sensitive rootstock when tested against a mixture of several viruses including GLRV-2 and -3

The existence of GLRaV-8 has been questioned and it is likely to be removed from the list of viruses infecting grapevines. This virus has only been reported once and its existence was based only on serology and a partial sequence (273bp) of the putative coat protein gene. It has since been demonstrated that the fragment was in fact amplified by PCR techniques from the genome of *Vitis vinifera.*

Multiple virus infections were frequent in ancient vineyards in Spain and Romania. These studies help identify the ancient relatives of grapevine viruses especially those of Ampeloviruses. A simplified evolutionary form of a leafroll virus has been identified in Greece which could shed light on the evolution of these viruses in the grapevine. A wild grapevine species in California, *Vitis californica*, an ornamental plant showing red leaves in autumn tested positive for GLRaV-2, GVB and GRSPaV, while in Australia the glory vine (*Vitis coignetiae*) tested positive only for GRSPaV (Habili, unpublished).

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Nymphs of *Heliococcus bohemicus* mealybugs in northern Italy could acquire three viruses, GLRaV-1, GLRaV-3 and GVA simultaneously, but only GLRaV-3 was efficiently transmitted. Two previously unreported soft scale vectors, *Coccus hesperidum* and *C. longulus,* were shown to transmit GLRaV-3. However, they may not play a significant role in the spread of the virus as they are poorly adapted to grapevine. The citrus mealybug, *Planococcus citri* was reported as an additional vector for GLRaV-1.

Rugose wood complex

Viruses of Rugose wood complex (RWC) belong to the three genera *Vitivirus, Foveavirus* and *Trichovirus*are which have now been proposed to be assigned to the new family, *Betaflexiviridae.*

Members of *Vitiviruses* include GVA, GVB, Grapevine virus D (GVD) and Grapevine virus E (GVE) play a major role in RWC. GVA is transmitted by mealybugs in association with *Ampeloviruses* GLRaV-1 and/or GLRaV-3. This virus is associated with Shiraz Disease both in South Africa and in Australia. In South Africa GVA and GLRaV-3 often appear together. Researchers suspect that GLRaV-3 may be needed for the GVA spread in South Africa. .However, in Australia GLRaV-3 has not been detected in any vine infected with Australian Shiraz Disease. Currently not much is known about the recently discovered GVE from Japan except that it is transmitted by *Pseudococcus comstocki* in the presence of GLRaV-3. Association of GVE with symptoms has not been observed (Nakaune, pers. communication).

Three strains of *Grapevine rupestris stem pitting associated virus* (GRSPaV-1, -2 and -3) have been described. These strains of GRSPaV were associated with two major grapevine diseases: An association of 93% was observed between GRSPaV-1 and grapevine vein necrosis; while 92% of vines displaying Rupestris stem pitting disease were infected with RSPaV-3. Both GRSPaV and GVA have been detected in seeds of three grapevine varieties tested.

Emerging diseases and diseases of unclear etiology

Studies in Iran confirmed the association of *Grapevine yellow speckle viroid 1* and/or *2* with *Grapevine fanleaf virus* induced yellow vein banding symptoms. This association was reported in California in mid 1990s.

The association of viruses with Shiraz disease in South Africa and Syrah decline in Europe remains unclear, although variants of GRSPaV and GVA have been implicated in previous studies. The studies presented during the conference provided no evidence that the causal agent of Syrah decline is a known virus or viroid. Phytoplasmas were not detected in Shiraz disease affected material in South Africa. Studies continue in South Africa and Australia to determine if t variants of GVA are associated with Shiraz disease. It is expected that more viruses and diseases associated with virus infections will emerge in the future.

As new viruses are discovered and characterized these will most likely be discussed in the next ICVG meetings to be held in the future

For further reference the reader can download the meeting's extended abstracts from the following URL http://www.icvg.ch/archive.htm

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Acknowledgements

Nuredin Habili's attendance at the ICVG was partly supported by the following bodies: Barossa Grapegrowers Vine Selection Society, Adelaide Hills Vine Improvement Society and Australian Vine Improvement Society. Fiona Constable would like to acknowledge the financial support given by Grape and Wine Research and Development Corporation and The Department of Primary Industries in Victoria for her attendance at the ICVG.

Fig. 1. Macon Vineyard 3
Panoramic view of typical Burgundy vineyards. Note the “Court noue” patchy yellowing caused by the infection of Arabis mosaic and Grapevine fanleaf viruses. (Photo by Judit Monis)

Fig.2. GLRaV-9 Shiraz Waite
Close up view of milder symptoms caused by Grapevine leafroll associated virus -9 in Shiraz (Photo by Nuredin Habili)

Fig. 3. Malbec CW14 LR3
Close up view of severe symptoms caused by Grapevine leafroll associated virus -3 in Malbec (Photo by Nuredin Habili)