Figure I: Retarded growth of a young Chardonnay vine (Mildura, Victoria, Australia) tested positive for Grapevine leafroll virus type 2 (GLRaV-2). Healthy vines in background tested negative. Note symptoms of late bud burst in spring. If this vineyard is grafted to a new variety, results may be disastrous.

HIGHLIGHTS OF ICVG 2006 MEETING

Advances in Research: Grapevine Viruses

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The 15th Meeting of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (ICVG) was held in Stellenbosch, South Africa, April 3–7, 2006. The ICVG meeting is held every three years, alternating between the Northern and Southern Hemispheres. The next meeting will be in 2009, either in France or Canada. At the Stellenbosch meeting, 109 papers and posters were presented.

Readers can obtain a free copy of the abstract book from the following URL: http://www.racchangins.ch/doc/fr/chercheurs/collab_ext/ICVG/archives.html. Below is a summary of specific highlights for each virus and/or virus group that is of economic importance for the grapevine industry.

Although the conference focuses mainly on grapevine-infecting viruses, historically phytoplasmas have also been included. This is because, prior to their characterization (they are also known as mycoplasma-like organisms), they were thought to be virus-associated. A total of 25 papers (23%) covered grapevine phytoplasmas.

Giovanni Martelli, Chairman of the ICVG, began the meeting with an overview of the progress in grapevine virus research in the past three years. Of interest and relevance to grapevine certification and clean stock programs is the new European Community (EU) regulatory directive that recommends "the lowest possible level of harmful organisms in propagation material."

However the technical annex published in 2005 on the grapevine caused an uproar among the European Union scientific community, since it only lists the following as harmful organisms: *Grapevine fanleaf virus* (GFLV); *Arabis mosaic virus* (ArMV); *Grapevine leafroll-associated virus* (GLRaV) -1 and -3; and *Grapevine fleck virus* (GFkV) (only for rootstock material for the last virus).

The Italian Ministry of Agriculture has drafted a new directive that is more stringent and states that, in addition to the above viruses, the vines must be free of GLRaV-2 and viruses associated with the rugose wood complex, with the exception of *Grapevine Rupestris stem pitting-associated virus* (GRSPaV).

In the next few months, the ICVG will work on developing a compilation document that explains the detrimental effects of different viruses, to persuade the European Union and other regulatory agencies to develop more stringent regulations.[30]

Figure II. Symptoms of Australian Shiraz disease on a Shiraz vine infected with *Grapevine virus A*.

Leafroll-associated viruses

The various reports of sequence variants — "isolates or strains" — of GLRaV-1, -2, -3, -4; GFLV; GRSPaV; and GFkV confirm that genetic variability is common among viruses infecting grapevines.[2,3,19,21,32,39,43] One study suggested potential recombination events on GLRaV-1 infections.[31] It is known that GLRaV-2 is associated with graft incompatibility. Further evidence linking GLRaV-2 with graft incompatibility was provided by C. Pirolo.[36]

Single and mixed infections of GLRaV-2 variants were described in one study.[4] The study showed the effects of different GLRaV-2 strains alone or in combination, and showed the typical leafroll symptoms including graft incompatibility and increased mortality.[6] Damage caused by GLRaV-2 variants was most severe on Kobber 5BB, Teleki 5C, and 1103 Paulsen rootstocks.[3] A detrimental effect and high disease incidence of GLRaV-2 in Cabernet Sauvignon was reported in Argentina.[42]

The characterization of two sequence variants of GLRaV-4[1] provides more data supporting the work done in the USA, showing that a close relationship between GLRaV-4, GLRaV-5, GLRaV-6, and GLRaV-9 was found.[1] This raises the question: should these viruses be considered strains of the same virus or separate viruses? Attendees proposed that serological, molecular, and electron microscopy data must be available before a new "leafroll" virus can be named.

Grapevine virus A (GVA) was detected in Shiraz and Merlot vines showing Shiraz Disease (Figure II), a disorder reported to occur in South Africa and Australia.[20]

Vitiviruses

A study carried out in South Africa showed that only specific GVA variants are associated with this disease and that some of the GVA variants are latent in Shiraz.[14] These findings are different from studies in Australia that show a strong correlation between the presence of GVA and typical Shiraz disease symptoms.[18] Specifically the study showed that all GVA-positive Shiraz vines tested so far manifest Shiraz-disease symptoms.

Furthermore, the study indicates that the disease spreads naturally.[18] It appears that Australian Shiraz disease is different from the South African disease in that it does not kill the vines within the five years of surveillance (Habili, unpublished).

The complete sequence of the *Grapevine virus D* (GVD) genome, a virus associated with corky rugose wood symptoms, was reported.[37] GVD appears to be more similar to GVA than to GVB, although the expressed protein of ORF2 of GVD appears to be unique.

Two independent reports, both from Italy, described the presence of a high association between Grapevine Rupestris stem pitting associated virus (GRSPaV) and grapevine vein necrosis disease (GVND).[4,5] This information is important, especially for Australia and New Zealand, because GVND is a quarantinable disease, while the "associated virus" is widely spread in grapevines worldwide. M. F. Lima reported that GRSPaV is seed transmitted in the *Vitis vinifera* (up to 14%).[23]

Phytoplasmas

The European grapevine yellows agent, Flavescence doree, was reported for the first time in a southern Switzerland vineyard, in a region where the leafhopper vector, *S. titanus*, is well established.[16]

A report on the recovery of plants following infection by phytoplasmas was reported by Rita Musetti, who speculated that the systemic buildup of the antioxidant H_2O_2 may play a role in recovery of plants from phytoplasma infection.[33] Giovanni Martelli argued that classification and naming of different groups and sub-groups of phytoplasmas is getting too complicated, and something should be done about it.

Testing

A couple of reports related to grapevine disease diagnosis focused on "universal" or broad-spectrum detection of different grapevine pathogens. It was concluded that multiplex RT-PCR for the simultaneous detection of grapevine viruses is rapid and can drastically reduce the number of indicators in the biological assay.[7,9,11,12]

However, our experience at Waite Diagnostics (South Australia) suggests that the sensitivity of detection for each individual virus is reduced in the multiplex system (Habili, unpublished). Of high quarantine importance is the application of sub-group-specific detection of nepoviruses, including GFLV and ArMV.[7]

Post-entry screening of grapevine material in countries where nepoviruses are quarantined will be expedited if the methodology becomes adapted to different labs around the world. However, a preliminary trial proved unsuccessful (J. Monis, unpublished), suggesting the need for local method optimization.

In the case of many grapevine viruses, published diagnostic primers needed to be modified in our laboratories with local grapevine samples to be useful (Monis and Habili, unpublished). This can be explained by the genetic variation in specific sequences of the virus isolates or strains in different regions as discussed by different researchers at this meeting.

Identification of three mealybug species by multiplex PCR will facilitate the identification of species capable of transmitting viral diseases.[38]

Developing molecular protocols for reliable detection of phytoplasmas is still a challenge.[31] Single and multiplex real-time PCR for phytoplasma detection were presented.[12] Different strains of phytoplasmas could be detected using the primers designed from tuf genes, which appear to be more specific than the conserved 16S rRNA gene.[32]

Using specific primers, P. A. Magarey detected Australian grapevine yellows in non-grapevine hosts, including three weed species inhabiting wetlands around vineyards.[24] A report on the development of a microarray system for the detection of important grapevine viruses promises an alternative powerful and fast diagnostic method.[10]

Effects on wine quality

Several papers reported on the effects and advantages of virus elimination on vine performance and wine quality. The studies support that healthy vineyards are able to produce higher quantity (yield) and quality of grapes that can translate into better wine quality.

The use of new reagents for chemotherapy for the elimination of grapevine viruses seems to be promising. However, no data on the potential effects of the treatment on mature vines (such as juvenility, mutations, etc.) was presented.[34]

A report from Italy states that GFLV elimination significantly increases bud fertility and grape yield measured as higher bunch weight.[25] Studies performed in the same laboratory concluded that photosynthetic and nutrition activity was increased with the elimination of GLRaV-1 and GVA, translating to higher yields.[26]

Research in France showed that Chardonnay vines previously infected with GLRaV-1, -2, -3, GFkV, and GVB subjected to virus elimination using heat therapy and meristem culture showed increased vigor, fruit production, and sugar content (9% increase) while the juice acidity was reduced (3% decrease).[22] Fruit quality was improved most significantly when GLRaV-2 was eliminated from this variety (see also Figure I).

A study focusing on grape quality determined that White Muscat vines subjected to elimination of GLRaV-3 had higher fruit production and showed beneficial effects on the aroma of produced wine (higher soluble solids and higher amounts of free and bound terpenes), and higher fruit production.[27]

Another study with an Albarola clone reported an improved canopy, better ripening, higher soluble solids, and lower titratable acidity.[28] *Grapevine angular mosaic virus* symptoms were eliminated from regenerated plants using a combination of meristem tip culture and thermotherapy.[15]

Biotechnology

The advances and prospects of transgenic resistance were reviewed by M. Fuchs.[13] To summarize, many grapevine cultivars have been experimentally transformed with genes that confer pathogen-derived resistance (PDR), specifically coat protein (CP) or movement protein (MP), either as translatable or untranslatable, antisense or sense, single or multiple virus formats.

The following viruses have been targeted: ArMV, GFLV, Raspberry ringspot virus (RpRSV), GLRaV-2 and -3, GVA, GVB, and GRSPaV. *Vitis vinifera* cultivars include: Blaufrankish, Chardonnay, Lumassina, Nebbiolo, Red Globe, and Russalka.

Rootstock varieties transformed include: 3309C, Kobber 125-AA, Teleki 5C, 101-14MGT, Kobber 5BB, 41B, *Vitis rupestris* var de Lot, and *Vitis riparia* Gloire de Montpellier. Transgenic lines of grapevines transformed with the CP genes of GVA and GVB showed partial resistance to virus infection using mealybug transmission. The resistance was stronger for GVB than for GVA.[41]

Virus spread

Related to the epidemiology of grapevine viruses, studies were carried out to determine the spread of GLRaV-3 and the dispersal of its viruliferous mealybug vectors in South Africa.[35] A high correlation between the number of leafroll-infected vines present in a vineyard and the number of new infections was found. This shows the importance of disease pressure. The spread is most important along the same row, suggesting that spread occurs from one infected vine to the adjacent one, confirming what was already reported earlier.[17]

The dispersal of mealybugs by their own means, field equipment, birds, workers, or wind requires further study and would contribute to long-distance spread of the virus.

Sanitary practices (fallow periods, sanitation of equipment, clothing, etc.) are presently being evaluated to determine their effect on the reduction of leafroll disease spread from adjacent vineyards to healthy ones.

N. Douglas and K. Kruger reported that even a single individual of each of the two species of mealybugs, *Planococcus ficus* or *Pseudococcus longispinus*, were capable of transmitting GLRaV-3 to healthy grapevines in South Africa.[8] All the developmental stages of *Planococcus citri* were efficient vectors of GLRaV-3 in Spain.[6]

The conference included two field trips to observe the performance of clonal selections with the aim of keeping the vineyards virus-free. Delegates visited VitiTech, a company that produces nuclear and mother stock (free of pathogens) for distribution to South African nurseries. Government, university, and industry personnel have united to work together to reduce the spread of GLRaV-3 in South African nurseries. ■

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