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Considerations on grapevine selection and certification*)

by

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Summary: Different viruses and virus-like diseases can be detrimental to grapevines and their products. The protocols of the assays used to evaluate the impact of viruses have evolved with the progress of the knowledge in aetiology and diagnosis. A wrong interpretation of the data from experiments that compare the performance of virus-infected and non-infected vines may lead to erroneous conclusions. Even if some experiments show that virus infections induce an increase of sugar content, a better evaluation of the experimental data may show that the conclusion drawn is incorrect. The role viruses may play in clonal variability is also discussed.

Key words: sanitary selection, viruses, clonal variability, review.

Introduction

Preventive measures such as sanitary selection remain the most effective way to limit the detrimental impact of viruses in grapevines. Novel information on the aetiology and epidemiology of major virus diseases and the development of new detection techniques improve the efficient application of sanitary selection to the expanding worldwide exchange of propagating material.

To this effect, some critical questions need to be addressed:

- What is the impact of single viruses (*e.g.* any given grapevine leafroll associated virus *vs.* the leafroll complex as a whole)?
- Which viruses and virus diseases should be considered in a certification scheme?
- Which are the most sensitive techniques for a reliable detection of viruses and which are the most user-friendly techniques for a routine diagnosis of viruses?
- What is the most appropriate time of the year and what is the most suitable tissue for sampling?
- Can sanitary selection cause a genetic erosion of the cultivars?
- What is the impact of sanitary status on clonal variability?

Different opinions sometimes clearly appear between breeders and pathologists; *e.g.*, BECKER wrote in 1974: »We take a strong position against the notion according to which, together with the selection called "productivity selection", one has to proceed with "sanitary selection". The opinion according to which a clone cannot be regarded as such if it is not free from viruses can be as little correct as that attributing all differences in productivity of any cultivar to viral infections rather than to genetic differences«. In 1978, PONGRACZ wrote: »Do not trouble with virology«.

However, many reports indicate that viruses and virus-like diseases can be detrimental to grapevines and their products (for review: WALTER and MARTELLI 1997). Here, we will not detail the published literature which concludes to very detrimental effects of fanleaf, leafroll, rugose wood, fleck and other virus diseases; we will rather critically focus on the protocols of the assays used to evaluate the impact of viruses and the interpretation of the data.

Evaluation of the effects of viruses and virus diseases

In the sixties, in the first reports, the effects on yield were estimated by comparing the performance of symptomatic *vs* symptomless vines. This approach is not accurate because symptoms due to non-viral and viral origins can be mistaken (BOVEY *et al.* 1980). In addition, a given symptom may be due to different viruses, *e.g.* leafroll (GLRaVs).

Lateron, grapevine clones indexed for the presence or absence of a given disease were used to evaluate the impact of the diseases. However, in most cases, no information was available on the presence of virus diseases other than those which are of interest. The former might interfere with the effects of the disease which is studied. Again, this approach does not take into account the effects of single components (*e.g.* Rupestris stem pitting *vs.* Kober stem grooving or corky bark) or single viruses (*e.g.* GLRaVs).

Other experiments were done by comparing a given clone before and after heat treatment. Thermotherapy allows sequential elimination of viruses and diseases, that co-infect a vine, by increasing the duration of the treatment. In most papers reporting the elimination of a given virus, there is no information on the possible simultaneous elimination of other

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viruses or diseases than the virus or the disease of interest. Reportedly, treated clones have a significantly increased vigour and yield, higher sugar content in berries due to earlier ripening, lower acidity and higher pH if treated and non-treated clones are harvested on the same day. However, when treated and non-treated clones were harvested at the same level of ripening, treated clones showed higher total acidity and lower pH.

This approach may also be biased. It is generally thought that the yield increase following thermotherapy is due to the elimination of viruses, however, GOHEEN (cited by STELLMACH 1980) offered another interpretation. Heat treatment may revoke somatic mutations, thus restoring the original characters of the variety concerned. According to this hypothesis, infectious degeneration (fanleaf) would actually result from the combined effect of virus (GFLV) infection and somatic degeneration.

Finally, evaluations of virus impact were made by comparing vines before and after graft inoculation with a given virus. Here again one cannot be sure, in most cases, that the reported differences are due only to the virus which is studied because the grapevine used for graft inoculation can be co-infected by additional viruses or diseases. When possible, specific animal vectors (nematodes or mealybugs) or herbaceous selective hosts, from which virus can be transmitted to grapevines by heterografting, can provide a "pure" inoculum.

Experiments must be designed to answer the recurrent question raised by viticulturists and breeders: are all the components of a viral complex equally harmful?

A wrong interpretation of the data from experiments that compare the performance of virus-infected and non-infected vines may come to erroneous conclusions. Virus infections often result in lower yield and quality (i.e. sugar content and other traits) of the crop. Even when some experiments show that virus infections induce an increase of sugar content, a better evaluation of the experimental data may show that the conclusion drawn is incorrect. For example, BALTHAZARD (1993) demonstrated that the decrease in sugar content recorded in a heat-treated leafroll-free cv. Savagnin clone was only due to differences in yield between infected and sanitized plants. When regression coefficients were calculated using the same data, the alleged difference in sugar content was no longer significant, except for the first harvest (Table). It is dangerous and useless to envisage the utilization of viruses for regulating the vigour and yield of a vineyard as it has been suggested sometimes. There are safer types of intervention for managing growth and yield, such as proper choice of the rootstock, type of pruning, balanced fertilization, irrigation, thinning, etc.

The nature and severity of the effects of virus vary with respect to:
- Virus species
- Virulence of isolates of a given virus species
- *Vitis* species, variety, clone (?)
- Age of the infected vine
- Interaction with other viruses or pathogens
- Interaction with agro-climatic conditions
- Spread by vectors.

Hypovirulent virus isolates induce milder effects, but the existence of hypovirulent isolates cannot be taken into account during sanitary selection mainly because of three reasons:
(1) Hypovirulent isolates cannot always be easily identified by diagnostic tests.
(2) An isolate which is hypovirulent on a given cultivar may be more virulent on another cultivar.
(3) An hypovirulent isolate that causes mild effects on vigour and yield may have significant negative effects on quality of the must (WOLPERT and VILAS 1992).

Taking all these considerations into account, a group of virologists participating in an European network proposed the following viruses and diseases to be considered as the most detrimental and to be eliminatory for the sanitary selection and certification (WALTER and MARTELLI 1997):
- Infectious degeneration complex and relative agents (GFLV and other European nepoviruses)
- Leafroll complex and relative agents (closteroviruses)
- Rugose wood complex and relative agents (vitiviruses)
- Fleck
- Phytoplasma diseases.

Techniques for sanitary selection

Concerning the techniques for the detection and the identification of viruses and virus diseases, serological and molecular tools are increasingly used for nepoviruses, closteroviruses, vitiviruses and grapevine fleck virus (GFkV). For selection purposes, indexing remains the most appropriate detection technique. However, it has to be performed considering that: (1) indicator varieties are well chosen and identified; (2) grafting methods are selected with regard to the disease to be detected; (3) growing conditions are optimal for symptom expression; (4) reading and recording of symptoms are made frequently and for a sufficiently extended period of time. In open field indexing, indicators are inspected several times a year for at least 2-3 years. Indexing in a growth chamber or glasshouse under controlled temperature and light conditions favours symptom expression, and standardizes the procedure (MARTELLI 1993). The above mentioned European network made a first step towards the proposal of reference protocols for the detection of grapevine viruses and virus diseases by indexing (GARAU *et al.* 1997) and ELISA (BOSCIA *et al.* 1997).

An important issue of the clonal selection process is the optimal time when to proceed with sanitary evaluations. To our mind, vines that undergo field selection, prior to planting in performance plots for clonal evaluation, should be hot water-treated to eliminate phytoplasmas and should be checked for being free of disease with the following tests:
- Indexing on *V. rupestris* cv. St. George for fanleaf, Rupestris stem pitting and fleck
- Indexing on a sensitive red-berried *V. vinifera* variety for leafroll
- Indexing on Kober 5 BB for Kober stem grooving and graft incompatibility
- Indexing on LN33 for corky bark and LN33 stem grooving

Table

Effect of heat treatment on yield and sugar of Gewürztraminer (after BALTHAZARD 1993)

Year	Yield ($kg \cdot m^{-2}$)[a]			Sugar ($g \cdot l^{-1}$)			Potential sugar ($g \cdot l^{-1}$ for 1 $kg \cdot m^{-2}$)		
	infected	treated		infected	treated		infected	treated	
1990	0.53	0.70	**[b]	264	256	***	254	250	*
1991	0.97	1.27	***	227	215	***	226	223	ns
1992	1.44	1.78	***	224	217	***	230	228	ns

[a]) Data represent means from 36 plants in 1990, and 40 plants in 1991 and 1992 of each treatment.
[b]) F-test values: ns = not significant (P>0.05); * = significant at P<0.05; ** = significant at P<0.01; *** = significant at P<0.001.

- ELISA for nepoviruses present in the surveyed region
- ELISA for mealybug-transmitted closteroviruses
- ELISA for mealybug-transmitted vitiviruses.

Foundation blocks for the production of basic and certified material must be kept under continuous surveillance, especially for contamination by vector-transmitted viruses and phytoplasmas. For large-scale surveys, sensitive techniques such as ELISA and PCR must be made increasingly reliable and user-friendly.

Genetic variability and sanitary selection

Surveys for viruses and virus diseases made all over the world, have unanimously shown that they are widely spread. In some cases varieties were totally infected. In these instances valuable clones can be recovered by eliminating infectious agents by heat therapy or *in vitro* meristem tip culture, or a combination of the two. In general, there is enough genetic variability in grape populations, even if heavily infected, that a genotypic or phenotypic erosion caused by sanitary selection can hardly be envisaged. On the other hand, pomological selection is much more restrictive. SCHÖFFLING and DEROO (1991) reported that only 0.05 % of the candidate clones that undergo selection for agronomic and qualitative characters are registered and propagated in Germany. Thus, the conservative breeders engaged in clonal selection must make a point of preserving the widest possible genotypic/phenotypic diversity.

The role viruses may play in clonal variability is a much debated issue. Real differences between clones of the same cultivar are not always obvious. Sometimes, we have the impression that differences exist preferably at the label identifying the clone.

A plant clone is the vegetative descent of a cell and usually corresponds to a variety. In the case of grapevines a clone refers to the vegetative descent of a relatively large part of the plant, *i.e.* a cutting with several buds. The heterogeneity of old *V. vinifera* or rootstock varieties can be explained by their polyclonal origin stemming, *e.g.*, from different but genetically close seedlings. Another source of heterogeneity resides in the progressive accumulation of mutations, part of which is somatic, and/or from the rearrangement of chimeras. Finally, heterogeneity can also be explained by the presence of pathogens like viruses and, perhaps, viroids. For interspecific hybrids, it is generally accepted that the major, if not the only difference between the clones may be the relative sanitary status. The role of virus infections in the clonal variability of some *V. vinifera* varieties needs to be studied in more detail.

Conclusion

The presence of one or more virus diseases in a mother vine block or in a commercial vineyard can have quite variable consequences. Viruses affect wood production, graft take, rooting capacity, longevity of vines, quantity and quality of yield, and composition of must. The utilization of the ever increasing and deeper knowledge on grapevine virology in the selection process is made difficult by

- the unpredictability of the damage caused by certain diseases or association of diseases, and the insidious nature of some of them;
- the great variability of symptoms and damage as a function of the virus strain, grapevine variety, climatic conditions, seasons, etc.;
- the transmissibility of viruses through cuttings, grafting and vectors (nematodes, mealybugs).

Besides this complexity, selection and certification protocols must be as simple, homogeneous, and reliable as possible. Furthermore, their implementation must cause only moderate costs. There is a compelling demand from professional organizations and control services to (1) dispose of a scheme for sanitary selection and certification as simple as possible; (2) have ultimate information on whether all single incitants of complex disorders are equally detrimental (*e.g.* closteroviruses with leafroll or vitiviruses and the like with rugose wood); (3) dispose of sensitive, reliable, easy-to-use and harmonized protocols for laboratory diagnosis.

Grapevine virologists should participate in well designed experiments aimed at the most precise evaluation of individual viruses and complex disorders. They should also - following the work done by the European network - con-

tinue the development of harmonized protocols for the routine detection of viruses and diseases which are important for the sanitary selection and the certification. These issues are a challenge for the years to come.

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