IMPACT OF TOMATO BLACK RING VIRUS (TBRV) ON QUANTITATIVE AND QUALITATIVE FEATURE OF *Vitis Vilifera* L. CV. MERLOT AND CABERNET FRANC

Authors:Coralie DEWASME LAVEAU[1*], Séverine MARY[2], Guillaume DARRIEUTORT[2], Laurent AUDEGUIN[3], Maarten VAN HELDEN[4], Cornelis VAN LEEUWEN[1]

[1]*EGFV, Bordeaux Sciences Agro, INRA, Univ. Bordeaux, ISVV, 33882 Villenave d'Ornon, France*
[2]*Univ. Bordeaux, Vitinnov, ISVV, 1 cours du Général de Gaulle, 33170 Gradignan, France*
[3]*Institut Français de la Vigne et du Vin, Domaine de l'Espiguette, 30240 Le Grau du Roi, France*
[4]*SARDI Entomology, Urrbrae SA 5064, University of Adelaide, Australia*

*Corresponding author: coralie.dewasme@agro-bordeaux.fr

Abstract:

Context and purpose of the study – Fifteen nepoviruses are able to induce fanleaf degeneration in grapes which is economically the most imprtant viral disease. *Grapevine fanleaf virus* (GFLV) is the main causal agent of this disease worldwide and *Arabis mosaic virus* (ArMV) is the second most important nepovirus involved in this disease in Europe. A third nepovirus has been described in France. Indeed, Tomato Black Ring Virus (TBRV) was detected in vines for the first time in France on a multi-varietal plot in 2009. The objective of the study was to quantify the impact of TBRV on two varieties of this plot.

Material and methods – Quantitative and qualitative impact of TBRV assessment was carried out in 2010 and 2011. Over 200 vines were analyzed by ELISA tests in order to determine their virus status. Vines were distributed in four groups: 40 vines of Merlot TBRV positive versus 40 merlot vines virus free and 40 vines of Cabernet franc TBRV positive versus 40 free of the virus. For each vine, the presence of eleven other viruses was investigated. In 2010 and 2011 shoot length was measured. In 2010, grape composition was analyzed to determine technological maturity and phenolic maturity of each vine in relation with its virus status.

Results – Shoot length and total pruning weight is reduced in TBRV infected vines, while lateral number is increased. All yield parameters are affected by the presence of the virus. Vines affected by TBRV produce less bunches and berries and smaller berries compared to healthy vines. Yield loss is greater on Merlot compared to Cabernet franc. Grape quality parameters seem to be less affected by the presence of TBRV. These results provide essential elements for the management of the viral disease in the vineyard.

Keywords: Grapevine, virus, grape quality, yield

1. Introduction

Grapevines can be affected by approximately 75 virus or virus-like diseases but their impact is not equivalent. Some of them are more important due to their wide geographical distribution and their economic losses. Grapevine FanLeaf Virus (GFLV) is the most important virus in vineyards worldwide (Andret-Link *et al*., 2004). GFLV is the main agent of grapevine fanleaf disease and it is specifically transmitted by the ectoparasite nematode *Xiphinema index* (Hewitt *et al*., 1958) but it is also propagated by plant material. In Europe, Arabis mosaic virus (ArMV) is the second most important nepovirus involved in grapevine fanleaf disease. Grapevine Leafroll Virus (GLRV) and rugose wood diseases also affect plant vigor and yield and lead to economic losses. Grapevines are also affected by "minor" virus diseases (i.e., fleck, vein mosaic, rupestris stem pitting, etc.), whose impact is still unclear (Mannini & Digiaro, 2017).

Since the 1990s, the development of serological and biomolecular virus detection tests allow to set up experiments based on the comparison between healthy and virus infected vines (Cretazzo *et al*.,

2010). Several authors show the effect of GLRV on grapevine (Guidoni, 1997; Bertamini, 2004), there is less data on GFLV. The negative influence of GFLV on vine growth and grape yield as well as the variable influence on parameters of must quality potential such as color, sugar or acidity have been demonstrated. Crop losses caused by GFLV vary from moderate (30% of the crop) to high (80%) depending on the virulence of the virus isolate combined with the susceptibility of the varieties and with the effects of environmental factors (Bovey *et al.,* 1990; Martelli & Savino 1990; Legin *et al*., 1993). In a trial conducted by Martinez *et al.,* 2016, yield decreased by nearly 40% due to both a significant reduction in cluster number and berry weight. With regard to grape composition variables, GFLV infection caused significant increase of sugar contents, pH and colour intensity or no difference.

Fanleaf degeneration can cause symptoms such as internode shortening, leaf asymmetry, fasciation of shoots, mosaic discoloration of leaves and lead to a decrease in grape quantity and quality through flower abortion and millerandage and finally to plant death (Laveau *et al.,* 2013). The influence of virus strain is also acknowledged (Martelli & Savino, 1990; Bovey &Martelli, 1992; Walter & Martelli, 1996). Impact of virus on grapevine also depends on the environment and interaction with factors such as rootstock (Golino *et al*., 2003), cultivar (Credi & Babini, 1997; Legin *et al*., 1993), plant age, annual climatic conditions (Cretazzo *et al*., 2010), as well as training and pruning systems (Mannini *et al*., 2001; Clingeleffer 2002).

Tomato Black Ring Virus (TBRV) is another possible agent of fanleaf disease. In France, TBRV was detected for the first time on grapevine in 2009. Infected plants showed typical symptoms of fanleaf disease on the bunches that were very small and reduced drastically the vine productivity (Laveau *et al.,* 2013).

The aim of this work is to evaluate the effect of the presence of TBRV in two grapevine varieties, evaluating the effects on grape yield and berry quality potential. Yield, berry weight and grape composition have been recorded over two vintages as well as the architecture and vigor of the vines. The strength of the study is that the incidence of the virus has been studied in a same plot, so with the same soil and climatic conditions, and that both varieties were grafted on the same rootstock.

2. Materials and Methods

Plant material

In this study, 80 vines of Merlot and 49 vines of Cabernet franc have been studied in the same plot. All the vines are grafted on 3309 C rootstock and planted in a gravelly soil in Pessac-Léognan appellation in the Bordeaux area and the climate is a typical oceanic climate. Vines are guyot-pruned. In 2011, vines were thinned to a maximum of eight bunches. TBRV infected vines never had a bunch number greater than eight, so that only TBRV free vines were thinned. The measures have been carried out between spring 2009 and winter 2011 and agronomic observations and measurements were carried out in 2010 and 2011 which were both warm and dry vintages.

Serological detection

In 2009, vines showing symptoms of fanleaf degeneration, but negative for the two main fanleaf viruses, Grapevine Fanleaf Virus (GFLV) and Arabis Mosaic Virus (ArMV), were screened by ELISA (Enzyme-Linked ImmunoSorbent Assay) (Bioreba, Switzerland, CH-4153) for other nepoviruses that could potentially explain the symptoms. Tests revealed the presence of Tomato Black Ring Virus (TBRV) on these vines. Following, a survey of plants around the positive vines was carried out. A total of 170 Merlots (MN), 88 Cabernet franc (CF) and 115 Cabernet-Sauvignon (CS) were tested for TBRV. ELISA tests revealed presence of TBRV on 2 CS, 21 CF and 41 MN. The tests were repeated on leaves the following spring. In 2010, when vines were pruned, a short piece of pruning wood was collected on each vine to carry out serological tests by ENTAV (French National Technical Institution for the Improvement of Viticulture, FR30240) in order to test each vine for TBRV and ten other viruses such as Nepovirus ; Grapevine Fanleaf Virus (GFLV), and Arabis Mosaic Virus (ArMV) but also Strawberry latent ringspot virus (SRLV) and Raspberry Ringspot Virus (RpRSV) ; Leafroll associated viruses (GLRaV-1, GLRaV-2 and GLRAV-3), Fleck disease virus (GFkV), Rugose wood associated virus (GVA and GVB). The tests confirmed the presence of TBRV. None of the ten others viruses were detected by ELISA tests except GFkV. The vines

contaminated by GFkV were excluded from the experiment. In 2011, wood and leaves were tested for a third time to control a putative contamination of vine to vine of TBRV during the experiment as the vector, nematode *Longidorus attenuatus*, was detected in the soil of this plot. Some vines were uprooted during the 3 years. So only the data of vines remaining after the 3 years of the experiment were analyzed and if the ELISA tests revealed at least once the presence of TBRV, the vine was considered as contaminated (positive).

Vine vigor, grape yield and composition

A total of 129 vines were monitored during two consecutive years (2010 and 2011). The number of vines analyzed for yield components, grape composition and vegetative expression varied according to the possibilities of sampling in the plot. Table 1 indicates the number of vines per type of measurement or observation and year.

Table 1 - Number of vines per type of measurement and virus status for MN and CF, respectively.

Year	Cultivar	Virus status	Vigor measurements on pruning wood	Yield measurements on grapes	Grape composition measurements
2010	MN	TBRV -	46	16	13
		TBRV +	34	29	18
		Total	80	45	31
	CF	TBRV -	22	10	10
		TBRV +	27	21	21
		Total	49	31	31
2011	MN	TBRV -	16	15	8
		TBRV +	22	25	9
		Total	38	40	17
	CF	TBRV -	14	10	8
		TBRV +	24	21	9
		Total	38	31	17

To determine yield components, all bunches produced were harvested from each vine and the number and weight of bunches, the number of berries and their weight were determined. The grape composition was analyzed from a sample of 200 berries /vine. Sugar content, total acidity, weight of 100 berries but also malic acidity, nitrogen content, total and extractable anthocyanins, as well as maturity index of seeds and skins were assessed by a commercial laboratory according to standard methods. Vegetative expression was measured by assessing total pruning weight per vine and consequence of virus infection on growth was evaluated by measurements of the number, the weight and the length of laterals shoots. The Ravaz index (Vasconcelos & Castagnoli, 2000) was calculated to evaluate the balance between the yield and the vegetative expression of the plant.

Harvest date was determined according to winery specifications and all the vines were harvested in the same week, irrespective of grape sugar content.

Data analysis

The effect of virus status was tested separately for each year and cultivar. All response variables were analyzed using linear models, except bunch number which was analyzed using a GLM (quasipoisson distribution for count data and a correction for over dispersed values). We checked that the residuals of both linear and generalized linear models complied with the assumptions of normality and homoscedasticity. All analyses were carried out using R Statistical Software version 3.5.1. (Foundation for Statistical Computing, Vienna, Austria).

3. Results

Impact on yield

Yield decreases for both varieties (Figure 2D). The losses were greater for Merlot than for Cabernet franc because of the greater number of Merlot vines without bunches (approximately 25% of the vines).

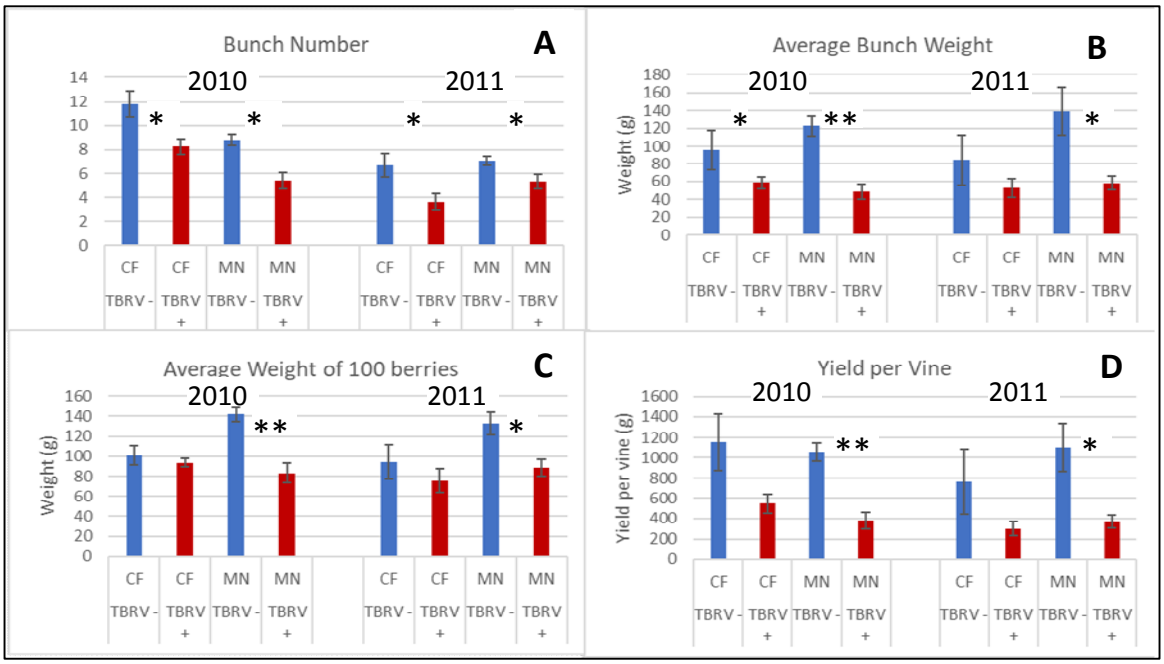

Figure 1 - Effect of TBRV (+ : positive virus status) on yield parameters. Bunch number (A), Average bunch weight (B), average weight of 100 berries (C) and Yield per vine. Error bars show Standard deviation. Statistical analyses were carried out per year and per variety (CF: Cabernet franc, MN: Merlot noir and ***p< 0.001 ; **p< 0.01 ; * p< 0.05).

All yield components were affected by the presence of virus in the vine. There were fewer bunches with a lower weight and fewer berries / bunch on TBRV infected vines than on TBRV free vines in both years. The impact is more important on Merlot than on Cabernet franc except for the bunch number in 2011 (Figure 1A, B, C).

For bunch weight, infected Cabernet franc showed 39% and 37% decreases while infected Merlot had 60% and 58% of losses in 2010 and 2011respectively. The mean weight of 100 berries was significantly different for infected Merlot with 41% reduction in 2010 and 33% in 2011 whereas non-significant berry weight losses of only 7% and 20% were recorded on infected Cabernet franc. The final result was a decrease in yield of 52% and 61% on Cabernet franc in 2010 and 2011respectively, and a decrease of 64% in 2010 and 66% in 2011 for Merlot (Figure 1).

The effect of TBRV on grape yield was greater on Merlot compared to Cabernet franc. Differences in grape shatter have been observed between varieties. Grape shatter was scored for each vine from 0 to 4 according to the importance with a score of 4 representing vine with no berries. On average Merlot score was 50% higher compared to Cabernet franc (Table 2).

Table 2 - Average score of grape shatter according to year, variety and sanitary status (Scoring ranges from 0 to 4)

Year	Status	Variety	Average score
2010	TBRV -	CF	0.70
2010	TBRV +	CF	1.81
2010	TBRV -	MN	1.13
2010	TBRV +	MN	2.66
2011	TBRV -	CF	1.10
2011	TBRV +	CF	1.81
2011	TBRV -	MN	1.40
2011	TBRV +	MN	2.56

Impact on must quality

The effect of TBRV on grape sugar content, juice titratable acidity and pH, malic acid content, richness in polyphenolic compounds (tannins and anthocyanins) and yeast assimilable nitrogen are shown in Figures 2 and 3. Sugar content was higher on TBRV positive vines for both varieties and years, but the difference was statistically significant only for MN in 2010 (Figure 2A).

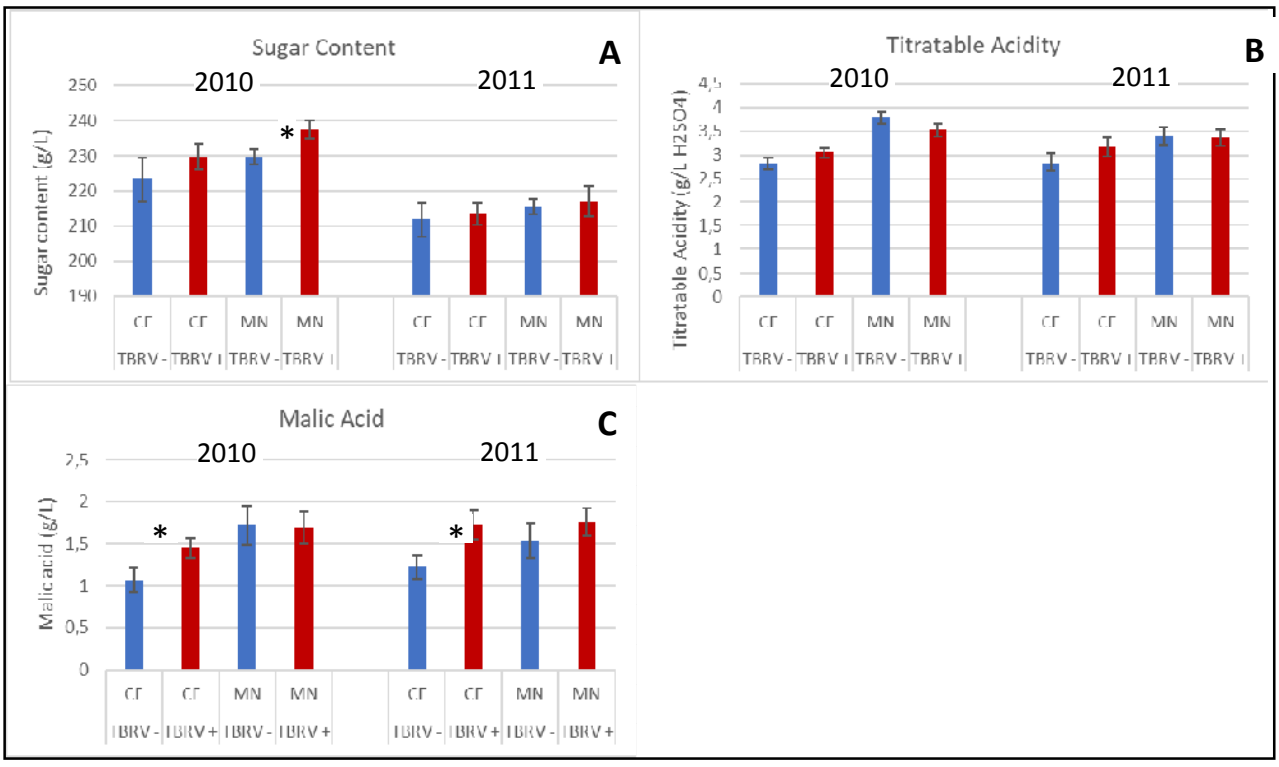

Figure 2 - Effect of TBRV on grape composition: sugar content (A), titratable acidity (B) and Malic Acid (C). Error bars show Standard deviation**.** Statistical analyses were carried out per year and per variety (CF: Cabernet franc, MN: Merlot noir and ***p< 0.001 ; **p< 0.01 ; * p< 0.05).

Titratable acidity (Figure 2B) and pH (data not shown) showed not uniform trends and was not significantly different between TBRV+ and TBRV- vines. There was more malic acid in the berries of infected vines in Merlot in 2011 and in Cabernet franc in both years; differences found in CF were significant (Figure 2C).

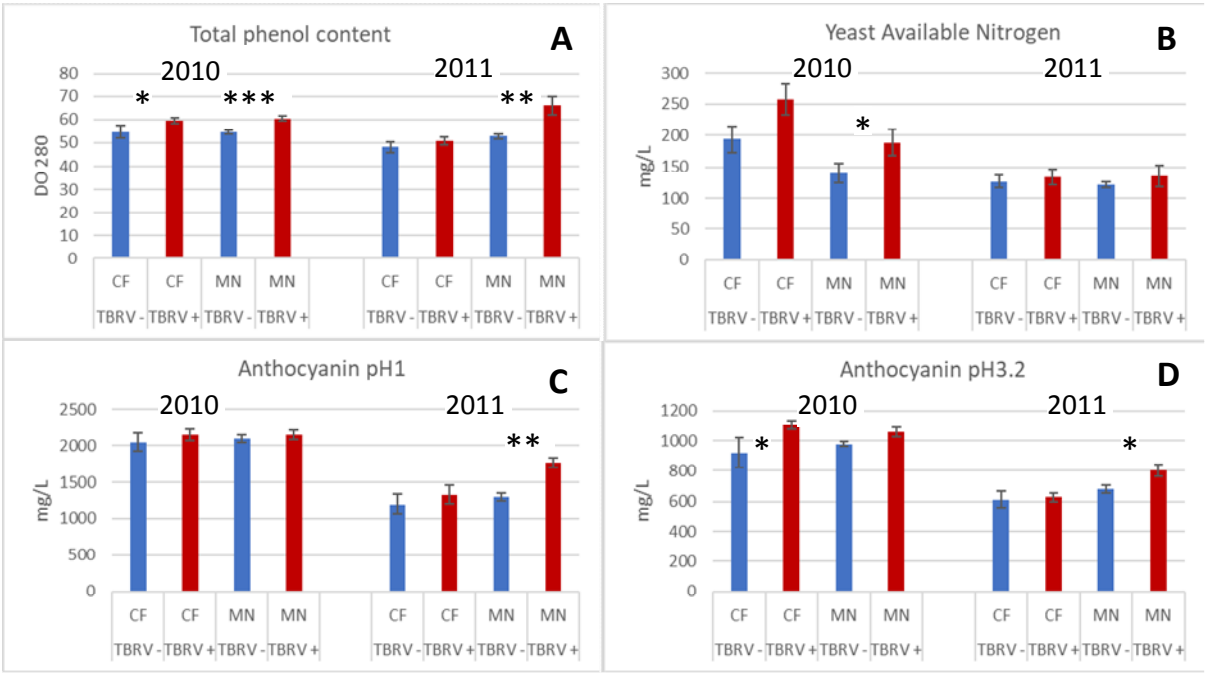

Figure 3 - Effect of TBRV on total polyphenols (A), yeast available nitrogen (B), anthocyanins (C) and extractable anthocyanins (D) in grapes. Error bars show Standard deviation.Statistical analyses were carried out per year and per variety (CF: Cabernet franc, MN: Merlot noir and ***p<0.001 ;**p< 0.01 ; * p< 0.05).

Significantly higher total polyphenol content was observed on TBRV+ vines of Merlot in both years and in Cabernet franc in 2010 (Figure 3A). There were also more total and extractable anthocyanins in TBRV+ vines in Merlot in 2011 (Figure 3D).
A significant increase for yeast assimilable nitrogen was found in the juice of TBRV positive vines of Merlot in 2010. There was more yeast available nitrogen in the juice of TBRV positive vines in 2010, but the difference was significant only for Merlot (Figure 3B).

Impact on vigor
A reduction in pruning weight was observed for TBRV positive vines of the two varieties in both years. This reduction was significant for Merlot in 2010 and for CF in both years. Concerning vegetative expression, CF was more impacted than MN with 31% and 26% decrease of pruning weight in 2010 and 2011 respectively, while this reduction was 19% and 13% for MN.
Pruning weight was lower on positive vines than on negative ones but a greater development of the lateral shoots was observed on TBRV positive vines.
The average length of lateral shoots of TBRV infected vines was greater than those of the negative vines for both MN and CF but differences were not significant.
This increased growth of lateral shoots leads to a two-fold increase in terms of pruning weight relative to primary shoots. No significant difference between Merlot and Cabernet franc on vine growth was observed in terms of average length of lateral shoots.

The Ravaz index was significantly different between TBRV- and TBRV+ Merlot vines both years. In this case, the value of the Ravaz index was almost halved.

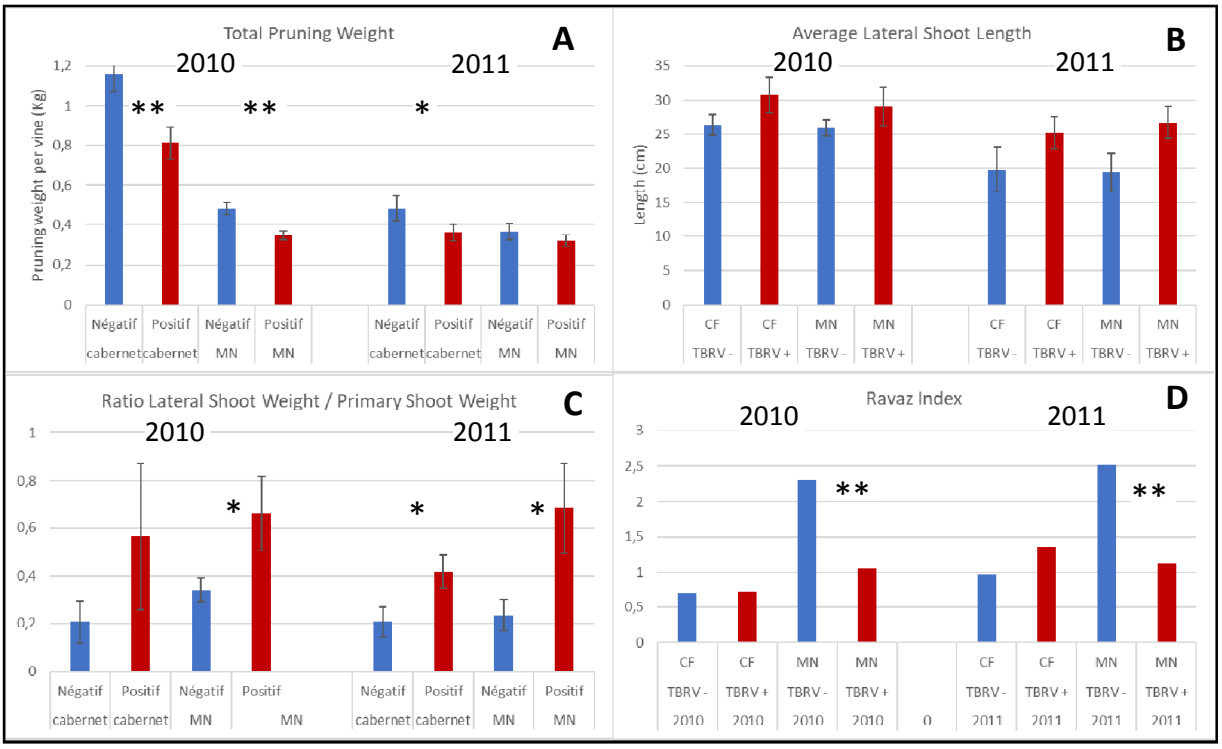

Figure 4 - Effect of TBRV on vine vigor: total pruning weight (A), average lateral shoot lenght (B), ratio between lateral and primary shoot weight (C) and Ravaz Index (D). Error bars correspond to Standard deviation.Statistical analyses were carried out per year and per variety (CF: Cabernet franc, MN: Merlot noir and ***p< 0.001 ; **p< 0.01 ; * p< 0.05).

4. Discussion and conclusion

Investigations on the sanitary status of *V. vinifera* cv. Merlot and cv. Cabernet franc of a same plot confirmed the presence of Tomato Black Ring Virus (TBRV) by ELISA tests. The results showed the impact of this virus on vine development, yield and grape composition. In this study, a very significant impact on the vegetative growth and fruit production of vines infected by TBRV was found.

The major impact of the presence of TBRV in vines was the very significant yield decrease of infected vines compared to virus free vines. The losses were over 50% for Cabernet franc and up to 66% for Merlot. The real effect of the virus was even greater, considering the fact that the virus free vines had been thinned to a maximum of 8 bunches per vine, which is the standard practice of the estate where this study was carried out. TBRV+ vines rarely reached 8 bunches or more per vine and were almost not thinned. The effect of grape shatter was stronger for the Merlot vines. All yield parameters were affected by the presence of the virus. Bunch number, average bunch weight and average berry weight were decreased on TBRV+ vines compared to TBRV free vines. This impact of viruses of infectious degeneration is well known. Andret-Link *et al.* (2004) evaluate crop losses caused by GFLV between 30% when the impact is moderate and up to 80% in highly impacted vines. This study shows that TBRV had a high impact on yield for both varieties considered, and that this impact was greater for Merlot than for Cabernet franc.

The effect on grape composition has also been recorded. The impact on grape quality potential was quite moderate. The results of this study showed no significant differences between healthy vines and infected vines in terms of titratable acidity and only slight differences on sugar content, malic acid content and yeast available nitrogen, depending on the variety and the year. However, on infected vines, grape sugar content, malic acid, available nitrogen, polyphenols and anthocyanins showed a tendency to increase in both varieties and years. The increase of total polyphenol was greater on Merlot than on Cabernet franc for both years and this was the major effect on the quality potential of the grape must. There was also an increase of anthocyanins and especially for those extracted at pH3.2,

representing the extractable anthocyanins during vinification. Hence, the wines made with grapes of infected vines could potentially have more color. In the literature, however, the viruses of infectious degenerations are often described as affecting the quality potential of grapes especially through decreases in sugar and total acidity. Cretazzo *et al*. (2010) demonstrated that GFLV infection may lead to a decrease of anthocyanins in berries despite the decrease of berry size and yield. There is probably an interaction between the infection by the virus and the variety. In this study, as the berries were much smaller on infected vines, the increase of quality potential can be a consequence of yield reduction rather than a direct effect of the infection. It is possible that berry size reduction results in a concentration of compounds. It would be interesting to compare the composition of grapes from infected vines versus that of non-infected vines with different yield levels, in order to measure the real impact caused by the infection on grape quality potential.

The third impact observed in this study was the reduction of vegetative expression. Several measurements have been implemented to characterize the vegetative development of the studied vines. The infected vines were visually bushy with anarchic development of shoots. The total pruning weights were measured with a focus on lateral shoots. Number, length, and weight of the laterals were measured. Total pruning weight of infected vines was lower than that of healthy vines. The reduction of vegetative expression has reached 30% in Cabernet franc, while reached only 19% in Merlot. Despite the reduction of vegetative expression, there were more and longer lateral shoots on infected vines. Hence, the ratio of lateral shoot weight to primary shoot weight was much greater for TBRV infected vines. This result is important in terms of vineyard management. In particular, in cool to moderately cool climates, laterals in the fruit zone need to be removed. Hence, production cost can be supposed higher in TBRV+ vines. In addition, the pruning will be more difficult to implement because of a lack of well-developed primary shoots. From a qualitative point of view, the development of lateral shoots is often considered to have a negative impact on the quality potential of the grapes. However, this study showed an increase in most quality-related parameters of the grapes in the infected vines while these vines showed an increased growth of the lateral shoots. The negative effect of laterals is supposed to be due to competition for nutrients between clusters and secondary shoots which may still be growing after veraison. In the context of this study that involved a soil with low water holding capacity and two dry vintages, the secondary shoots stopped their growth early in the season, which may have reduced negative impact of laterals on fruit quality potential. Finally, the infected vines were vigorous compared to the fruit weight they carry. Indeed, yield was highly impacted by grape shatter. Hence, source sink ratio was high in TBRV vines considering regular leaf functioning in infected vines.
The very low Ravaz index calculated for the TBRV positive vines reflect this particular development of the virus infected vines.

This study demonstrates the importance of TBRV which impact on yield is at least as important as that of GFLV. The slight positive effect observed on grapes quality potential in infected vines is not sufficient to counterbalance the strong negative impact on yield. To date, this virus has been detected in several plots of the French vineyard and is not eliminated during the sanitary selection of vines in nurseries. Given the results of this study, further research on the impact of this virus is needed, as well as potential transmission in vineyards and nurseries.

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