Short Notes

Partial defoliation improves must quality of cv. Albariño infected by *Grapevine leafroll associated virus 3*

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Summary. The effect of leaf removal treatments around vine cluster zones on must quality were tested during 2003 and 2004 in Rías Baixas (Spain) in two Albariño vineyards infected by GLRaV-3. As expected, the main virus damage was decreased sugar content (2.1 Brix in 2003 and 0.9 Brix in 2004) in the musts compared with leafroll-free plants. Leaf removal improved must quality by decreasing titratable acidity by between 0.5 and 1.9 g L^{-1} of tartaric acid, depending on the experiment; it also increased the grape sugar content an average of 1 Brix. In vineyards with high incidence of GLRaV-3, partial defoliation at veraison, or 2 to 3 weeks later, had an improved must quality, counteracting the negative impact of the virus. This process is recommended to avoid penalties in wineries during years with poor ripening conditions.

Key words: leaf-removal, sugar content, titratable acidity, ampelovirus.

Introduction

Grapevine leafroll disease occurs in most regions of the world. At least nine different *Grapevine leafroll associated viruses* (GLRaVs), belonging to different genera within the *Closteroviridae* family, have been reported (Martelli *et al*., 2002). GLRaV-3 is the most widespread leafroll virus (Cabaleiro, 2009) and field data concerning its effects on grapevine yield and wine quality are available from many grapevine cropping countries and for many cultivars (Charles *et al*., 2006). The main physiological effect of GLRaV-3 on infected plants is the reduction of photosynthetic activity, which leads to delayed fruit ripening (Cabaleiro and Segura, 1996; Cabaleiro *et al*., 1999). Consequently, GLRaV-3 has been reported to cause up to 40% yield loss and, more often, poor quality of musts by decreasing the soluble solids content (Brix) and increasing the acidity (Charles *et al*., 2006). Moreover, the cool climate of Rías Baixas (between Region I and II on the Winkler Scale) may impair grape ripening over some growing seasons, characterized by particularly bad weather. Under such circumstances, grapes from highly leafroll infected vineyards may not either reach the minimum sugar content set by the wineries or receive severe penalties on the price paid for the grapes, as this depends on their sugar content.

The most commonly used cultural practices on grapevine canopies timely leaf removal, shoot thinning and cluster thinning (Jackson and Lombard, 1993). Canopy management practices modify the relationship between leaf area and production, leading to optimum levels of both (Smart *et al*., 1985). Partial defoliation of a number of leaves in the fruit zone of each vine, between fruit set and ripening, modi-

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fies surrounding microclimate and promotes the ripening process (Howell *et al*., 1994). Shaded clusters produce grapes with lower sugar content and higher acidity than those well exposed to sunlight (Crippen and Morrison, 1986; Chorti *et al*., 2010). Removal of basal leaves in a vigorous vineyard in temperate regions such as Galicia is not a major concern when plants have a well developed canopy exhibiting adequate photosynthetic activity. Furthermore, the photosynthetic capacity of leaves declines as they begin to age about 40 d after unfolding so their removal will not have negative impacts on plant performance (Bavaresco *et al*., 2008). An additional benefit of partial defoliation is the reduction in the risk of fungal attack on grape clusters due to improved air circulation, decreased humidity and better penetration of fungicide sprays (Pieri and Fermaud, 2005).

Since the main effect of GLRaV-3 is related to delayed ripening, and leaf removal improves ripening, defoliation could be used as an indirect control measure to counteract the negative effects of this and other leafroll-associated viruses in full production vineyards with high rates of virus incidence. As far as we know, the practice of partial defoliation has not been reported for this purpose. The objective of the current study was to evaluate the effect of leaf removal around vine cluster zones on quality parameters of must obtained from grapes from leafroll infected grapevine plants.

Materials and methods

Vineyards

Experiments were conducted in 2003 and 2004 in three plots from two vineyards in the Rías Baixas grape growing region (Pontevedra, Galicia, Spain). The Albariño variety is the main *Vitis vinifera* L. cultivated for quality white wines in Rías Baixas. The Beluso vineyard (hereafter designated "B", 46º 85'N, 110 m above sea level) has several plots of vines which are 15 to 20 y old. GLRaV-3 had been spread by *Planococcus citri* (Risso) throughout the vineyard, so and thus, all plots were almost 100% infected (Cabaleiro and Segura, 2006); plants in the vineyard were each trained on a vertical trellis with 4 m between rows and 3 m between vines along each row. The Meaño vineyard (hereafter designated "M", 46º 98' N; 110 m above sea level) was an experimental plot planted in 1988, which was found to be partially infected with GLRaV-3 in 1990 (Cabaleiro *et al*., 1999). In this case, each plant was trained on a horizontal trellis about 2 m high with 5 m between rows, 3 m between vines along each row, and two plants per hill. GLRaV-1 and 2 were not detected in both vineyards.

Climatic conditions

Climate data for the 2003 and 2004 growing seasons were obtained from two weather stations in the Environmental Information System of Galicia: Lourizán station (46º 95'N; 57 m a.s.l.) for vineyard B, and O Corón station (47º 14'N; 14 m a.s.l.) for vineyard M. Data collected were downloaded from the website www.meteogalicia.es and daily ambient temperatures were used to calculate Heat Summation or Growing Degree Days, that is the summation of mean daily temperatures minus 10ºC from April through October (Amerine and Winkler, 1944). This index and rainfall during the same period are reported in Table 1.

Experiments

Three canopy management schemes were tested; hand leaf removal was always performed only around the cluster zone of each plant on different dates depending on the test. The number of leaves removed was variable according to the canopy. Leaves were removed only from the west side of the vertical trellis in vineyard B. In vineyard M, a "hole" about 20 cm in diameter was opened around each cluster in the horizontal trellis (average of 182 leaves per plant). Available data from these and other vineyards show that GLRaV-3 does not affect cv. Albariño growth and development (Cabaleiro *et al*., 1999). Plants showing similar vigour and harvest level were only selected in order to avoid differences in must composition due to factors other than virus and defoliation.

Experiment 1: vineyard M

Twenty GLRaV-3 (+) and 20 leafroll free (-) plants were selected and assigned randomly as defoliation treatment (D) or as controls with no leaf removal (C). Leaf removal was carried out at veraison, on 23 August 2003 and on 21 August 2004. The respective harvest dates in the two growing seasons were 24 September 2003 and 23 September 2004.

Experiment 2: vineyard B-I

Sixty GLRaV-3 (+) plants, 15 years old, of similar growth and number of bunches were selected and

Table 1. Metereological data in August and September and growing degree days for 2003 and 2004 in the two climatic stations close to the experimental vineyard plots.

Meteorological data	2003		2004	
	B[a]	M[a]	B	M
August				
Average T (ºC)	22.2	21.7	20.1	19.8
Minimum T (ºC)	17.5	18.1	15.8	16.8
Maximum T (ºC)	28.6	27.0	25.1	23.1
Rainfall (mm)	67.6	48.0	146.0	126.0
September				
Average T (ºC)	19.4	19.4	19.6	19.1
Minimum T (ºC)	12.8	15.9	14.4	15.8
Maximum T (ºC)	26.1	24.3	26.3	23.9
Rainfall (mm)	39.6	33.4	76.6	16.4
GDD (ºC)[b]	1690	1676	1740	1597

[a]B, Beluso vineyard; M: Meaño vineyard.
[b] GDD, Growing Degree days.

randomly distributed among four treatments, including control plants without defoliation (C), plants with level 1 of defoliation before veraison with bunches not completely exposed (D1), plants with level 2 of defoliation before veraison with bunches exposed and about 35% more leaves removed than in D1 (D2), and plants with leaf removal carried out before veraison and 2–3 weeks later (2D).

In 2003, leaf removal was carried out on 8 August (1st intervention of treatment 2D) and 1 September. In 2004 the interval between the two defoliations in treatment 2D was less, on 24 August and 7 September, and the intensity of leaf removal was less. Harvest dates in the two seasons were 7 October in 2003 and 4 October in 2004.

Experiment 3: vineyard B-II

This test was performed only in 2004 to determine the effect of partial defoliation on older (25 years old) and more vigorous plants under conditions similar to Experiment B-I. Thirty GLRaV-3 (+) plants of similar vigour and number of bunches were selected and distributed at random as either control without leaf removal (C) or medium level of leaf removal (average of 57 leaves per plant) carried out after veraison (7 September). Harvest date was at 4 October 2004.

Test for GLRaV-3

Samples consisting of mature leaves with petioles were collected in July 2003 for Experiments 1 and 2 and in August 2004 for Experiments 1 and 3. In Experiment 2, the same plants were used in 2003 and 2004; the virus was detected in all plants in 2003 and they were not retested in 2004. Virus detection was carried out using DAS-ELISA with antibodies obtained from Bioreba AG (Basel, Switzerland), following instructions by the manufacturer.

Grape and must analysis

Fifty grapes were randomly picked from each selected plant. These grapes were weighed and stored at -20°C until analysis. Berries were hand-squeezed in plastic bags and measurements were made on Brix degree by refractometer, titratable acidity by titrating 10 mL juice with NaOH to pH 7, pH and potassium, only in 2004, (Ecoscan-ion 6 Ion/pH/mV Meter, Eutech Instruments Europe BV, Nijkark, Netherlands).

Statistical analyses

For Experiment 1, and for parameters analyzed both years, a three-way-ANOVA was carried out, with year, leaf removal, and virus as sources of variation. A two-way-ANOVA was carried out, with leaf removal and virus as sources of variation for parameters analyzed for one year. For Experiment 2, a two-way-ANOVA was carried out, with leaf removal and year as sources of variation for data, then means of the four treatments were compared by Tukey's HDS test. For Experiment 3, the analysis consisted of means comparison through a one-way ANOVA. All analyses were performed using SPSS 12.0 Statistical software (SPSS Inc., Chicago, IL, USA)

Results and discussion

The year, virus and defoliation all had effects on most quality parameters of the musts but not on grape weight in Experiment 1 (Table 2); this observation is consistent with reports on other *V. vinifera* cultivars as well as other grape species (Main and Morris, 2004). GLRaV-3, as expected, negatively affected grape quality in 2003 and 2004; this data, along with previous information collected from on leafroll-infected vineyards (Cabaleiro and Segura, 1996; Cabaleiro *et al*., 1999), confirm that GLRaV-3 effects on the cv. Albariño grape are a serious concern for the production of high quality white wines. The decrease in soluble solid content in infected plants was more severe in 2003 than in 2004, due to the heavy rains (Table 1), but this was the only significant interaction found (year × virus). When the comparisons were done factor by factor, only pH (2003) or titratable acidity (2004) were significantly different (Table 2). The differences were clearer in infected plants. When leaves were removed around the bunches, the quality of virus infected-plants was similar to those of virus-free plants (Table 2).

In Experiment 2 (Table 3), partial defoliation had differential effects depending on year and the manner in which defoliation was performed each year. These effects were more intense in 2003 than in 2004. Since there were no interactions between defoliation and year, 2003 and 2004 data were analyzed separately. There was a trend towards increasing pH and decreasing titratable acidity with greater leaf removal intensity, but the differences were not significant in 2003.

Table 2. Experiment 1. Mean plant and must quality parameters for grapevine plants with or without GRLaV-3 infections (+/-) and which received different leaf removal treatments (C/D) in 2003 and 2004. Data are means from 10 plants per treatment.

Year and variable	Treatment[a]				P^b
	C+	D+	C-	D-	
2003					
Weight 50 grapes (g)	66.5	68.9	64.6	66.1	0.4052 ns
pH	3.35	3.34	3.30	3.31	0.7671 ns
Titratable acidity (g L^{-1})	10.5 a	9.3 ab	9.9 ab	8.9 b	0.0054**
Soluble solids (Brix)	18.3 b	19.2 b	20.4 a	20.5 a	0.0001***
2004					
Weight 50 grapes (g)	68.5	68.7	67.9	67.0	0.8528 ns
pH	3.45 b	3.58 a	3.50 ab	3.54 ab	0.0074*
Titratable acidity (g L^{-1})	12.6	12.1	11.8	11.7	0.4179 ns
Soluble solids (Brix)	20.0 b	20.8 ab	20.9 ab	21.1 a	0.0170*
Potassium (mg L^{-1})	1752	1921	1582	1672	0.0634 ns

[a] C, control, no leaf removal; D, defoliation; +, GLRaV-3 (+); -, GLRaV-3 (-). Different letters indicate significant differences at $P<0.05$.
[b] *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<0.001$.

Table 3. Experiment 2. Mean fruit weights and must quality parameters from different grapevine defoliation treatements in 2003 and 2004. Data are mean values from 15 plants per treatment.

Year and variable	Treatment[a]				*P*
	C	D1	D2	2D	
2003					
Weight 50 grapes (g)	70.4	69.3	68.8	68.6	ns
pH	3.51	3.58	3.56	3.60	ns
Titratable acidity (g L^{-1})	11.3	10.9	10.8	10.3	ns
Soluble solids (Brix)	19 abc	19.5 ab	18.5 abc	18.1 c	*
2004					
Weight 50 grapes (g)	69.2	70.8	70.1	68.9	ns
pH	3.69 a	3.74 ab	3.81 b	3.83 b	*
Titratable acidity (g L^{-1})	9.2 a	7.6 b	7.8 b	7.3 b	*
Soluble solids (Brix)	18.1	19.1	19.7	20.0	ns
Potassium (mg L^{-1})	1830 a	1938 ab	1951 ab	2212 b	*

[a] See Table 2.
[b] *, significant at $P<0.05$; ns, not significant. Different letters in a column indicate significant differences at $P<0.05$ according to Tukey`s HSD.

Partial defoliation affected the soluble solid content, the greatest effect of which was observed in treatment D1 (19.5 Brix), whereas the least corresponded to treatment 2D (18.1 Brix), which was probably intense enough to have a negative effect on ripening. In 2004, leaf removal affected pH and titratable acidity with the greatest pH value and the least titratable acidity value being for treatment 2D (Table 3). Although leaf removal did not significantly affect ($P>0.05$) sugar content of grapes, there was a trend of increasing soluble solids with increasing leaf removal intensity. Potassium content was greater in defoliated plants and leaf removal had no effect on grape weight (Table 3). In Experiment 3 the quality of the musts improved for all analyzed parameters when compared to untreated controls. The pH and sugar content were greater, whereas titratable acidity and potassium content were less, and, similarly, grape size was not affected (Table 4).

In general, our experiments showed titratable acidity to be the factor in must that was most affected (decreased) by leaf removal. This phenomenon is probably related to an increase in temperature of clusters due to higher exposure to light, as has been concluded by other researchers (Ollat and Gaudillère 1988; Petrie *et al*., 2003). The increase in cluster temperature during the last phases of ripening causes an increase in respiration of malic acid (Blouin and Guimberteau, 2004), and an increase in the activity of malic degradation enzyme (Blouin and Guimberteau, 2004). 'Albariño' wines are acidic because its grapes may have between 8 to 10 g L^{-1} acidity (expressed as g L^{-1} of tartaric acid) at harvest, but most of that acid is malic acid. The level of reduction of titratable acidity obtained by leaf removal in cv. Albariño (approximately 1 g L^{-1}) may be enough to balance the characteristics in the must and offset the negative effect of the virus.

Walter (1988) showed that the increase in potassium content in the must of leafroll-infected plants was related to abnormal ion distribution through blocking the passage of this ion from petioles to leaves. In the current experiments, both leaf removal and GLRaV-3 affected potassium content, although results were conflicting depending on the experiment. Since data was available for one growing season, the results may be taken only as indicative.

In Experiments 1 and 3, the soluble solids content was significantly greater when leaves were removed

Table 4. Experiment 3. Mean fruit weights and must quality parameters from different grapevine defoliation treatements in 2004. Data are mean values from 15 plants per treatment.

Variable	Treatment[a]		P^b
	C	D	
Weight 50 grapes (g)	69.6	68.8	ns
pH	3.74	3.85	*
Tritatable acidity (g L^{-1})	8.7	7.2	**
Soluble solids (Brix)	18.8	19.9	**
Potassium (mg L^{-1})	2335	2135	**

[a] C, control, no leaf removal; D, defoliation.
[b] *, significant at $P<0.05$; **, significant at $P<0.01$; ns, not significant.

around the clusters. Depending on the cultivar, weather conditions and vegetative state, number of leaves removed around grape bunches and date of defoliation may have to be adjusted in order to avoid negative effects as in 2003. Veraison is usually the most appropriate time to conduct leaf removal in most cultivars (Hidalgo, 2002), and this likely applies to 'Albariño' as well, but one or two weeks later may be just as appropriate.

Partial defoliation is one of the techniques already used in winemaking to improve the quality of the musts (Jackson and Lombard, 1993). The current data prove that controlled defoliation can to some extent offset the negative effects of virus. In other grape growing regions, delayed harvest in infected (symptomatic) plants has been suggested (Golino *et al.*, 2006), but for white grape cultivars such as 'Albariño', the leafroll symptoms are not so easy to distinguish by non-experts (Cabaleiro *et al.*, 2008). Besides, as rainfall at the end of the summer is a frequent occurrence in the region under study, the risk of a delayed harvest is not easily undertaken. In Albariño vineyards of the Rías Baixas region, leaf removal may, in addition to counteracting leafroll virus damage, reduce *Botrytis* and other fungal threats, and improve efficiency of fungicide sprays (Jackson and Lombard, 1993).

In conclusion, the quality of must from grapes of cv. Albariño is consistently affected by the presence of GLRaV-3, which decreases Brix degree and increases titratable acidity. Leaf removal at veraison or later was improved the quality of musts. Partial defoliation seems a good alternative to delayed harvest in white cultivars such as Albariño grown in cool grapegrowing regions as Galicia.

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