Abiotic stresses management on Verdejo wine composition: cluster thinning in different water regimes

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Abstract

BACKGROUND: The effects of cluster thinning (TH) applied in different water regimes (WR) on Verdejo wine composition were studied. TH was applied on three WR (R0, rainfed control; R25, drip-irrigated at 25 % ETo, and R50, drip-irrigated at 50 % ETo) over 2012-2014 seasons.

RESULTS: TH advanced grape maturity although this behavior was only significant in 2012. A significant effect of TH on R0 was observed in 2013, the wettest season, increasing the concentration of alcohols, esters, acetates, and lactones. The same trend was shown in 2012 for all groups of volatile compounds in R0. In contrast, a trend to increase all volatile families was observed in 2014, the driest season, when TH was applied to R50. WR*TH interactions were found in most wine chemical parameters.

CONCLUSION: The variation of wine volatile composition among vintages observed shows the capacity of influence of TH depends not only on the water regime but also on annual conditions.

Keywords: crop load; irrigation; volatiles; wine composition.

1. INTRODUCTION

Plants produced a wide spectrum of metabolites as response to abiotic stresses.[1] In grapevine, abiotic stress modifies growth and development of all plant organs. The abiotic stress at the berry level induces the accumulation in berry pulp, seeds and skin of secondary metabolites as a line of defense. Grape quality, particularly that of winegrape varieties, is largely dependent on secondary metabolites, mainly polyphenols and volatiles contributing to wine colour, taste and aroma.[2]

Viticultural practices are known to influence secondary metabolites by microclimate modification and they can be managed to control stress plant response in order to increase secondary metabolite concentrations, reflecting on an enhancement of must quality.[2]

One of the most important factors in the quality of white wines is the aroma and their concentration can be modified by factors that affect its biosynthesis and accumulation. The concentrations of volatile compounds and precursors in grape berries and wines are highly influenced by viticultural practices. Among these cultural practices, several authors showed that grape volatile compounds were responsive to cluster thinning[3,4] and water regime.[5,6,7]

Regulated deficit irrigation to improve the water use efficiency and reduce canopy vigor, is an important practice for sustainable agriculture, especially in arid and semi-arid areas. Previous studies showed that water deficit influenced physiological parameters of the vine, changed berry composition and improved sensory attributes of wines by increasing fruity aromas and decreasing vegetal aromas.[8] Several studies reported that deficit irrigation has an effect on the aroma of

Cabernet Sauvignon[9] and Merlot[10,11] grapes. A recent study performed in a semi-arid area from Spain demonstrated that the deficit irrigation treatment was linked to Cabernet Sauvignon wine aroma.[12]

Cluster thinning is a standard viticultural practice with the goal of reduce the crop load to advance grape ripening and improve the quality of grapes and wines.[13] The effect of cluster thinning on grape and wine aroma was studied by several authors.[4,14,15,16] Cluster thinning produced Pinot Noir wines less herbaceous and higher black pepper, cherry and currant descriptors.[14] Also, the thinning treatments produced Pinot Noir wines with higher marks in the descriptors fruity, spice, sweet, and body.[17] Higher levels of monoterpenes in Riesling grapes were observed when cluster thinning was applied[4]. However,[15] concluded that reduction of crop level in Vidal and Riesling vines not substantially affect to ice-wine aroma composition.

The combined effect of water status and crop level on grape and wine quality was studied by several authors, mainly focused on agronomic and physicochemical aspects.[18-22] However, few works were addressed to study the interactive effect of water status and crop level on grape and wine volatiles. In this sense, a study developed on Tempranillo wine volatiles[23] showed that rainfed and cluster thinning treatments increased the majority of individual aromatic compounds quantified as well as total odor activity value (OAV).

Considering the lack of studies developed with the aim to determine how these factors, cluster thinning and deficit irrigation, affect to grape and wine quality, the objective of this present study was to know the influence of crop level on three water regimes on white Verdejo wine volatiles and its implications for the wine composition.

2. METHODOLOGY

2.1. Treatments and experimental design

The experimental trial was conducted over the period 2012-2014 in a commercial vineyard (*Vitis vinifera* L) owned by Grupo Yllera S.L., located in Medina del Campo (Valladolid, Spain), within the Rueda D.O. The vineyard was planted in 2006 and trained as a vertical trellis, with North-NW row orientation, vine distance of 2.60 × 1.25 m (3077 plants ha^{-1}), pruned to bilateral Royat cordon with eight 2-bud spurs per vine. Verdejo variety scions were grafted onto 110R rootstock.

This work belongs to a project with two objectives: the first one was to determine the impact of water availability on the yield components and the must and wine composition from *Vitis vinifera* L. cv. Verdejo, published in a recent article.[7] The second one was to know the influence of crop level in three water regimes on white Verdejo wine volatiles. This is the current manuscript. Therefore, the same vineyard, same vintages and same controls of the previous work were used to develop this study.

Six treatments were tested through the trial, obtained by three types of water regime (WR), rainfed (R0), and drip irrigated at 25 % ETo (R25) and 50 % ETo (R50), and two types of crop level, cluster thinning (TH) and control. Then, the treatments applied were R0, R0-TH, R25, R25-TH, R50 and R50-TH. Drip irrigation was applied weekly from the end of vegetative main shoot growth until harvest. Crop reference evapotranspiration (ETo) was calculated by the Penman-Monteith model (FAO 1990). The irrigation treatments and phenological dates have been described in recent earlier paper.[7]

The study of the application of cluster thinning (TH) was based on the regulation of the number of clusters, compared to a reference treatment. The cluster thinning was applied at the beginning of veraison, trying to approach it to a reduction of a third part of all clusters, in such a way that the final adjustment resulted in an average reduction of 27 % of clusters with respect to the reference treatment, so that the reference vines have retained 36 % more clusters than the thinned vines. The cluster thinning was applied alternately to the highest rank and to the lowest rank in the different shoots of the vine.

The experimental design consisted of four randomized blocks for the first factor, the water regime (WR), and subdivided plots for the second factor, the crop level (four replications for each treatment: R0, R0-TH, R25, R25-TH, R50, R50-TH), with single experimental plot of 20 control vines disposed in two adjacent rows, surrounded by a row of vines on each side for buffer effect. Over the years 2012, 2013 and 2014, the timing and applied irrigation amounts were, respectively, as it was described:[7] first irrigation, 9, 15 and 14 of July; last irrigation, 8 of October, 23 and 22 of September; amount of water applied (R25/R50), 125/250, 104/208 and 108/216 mm; annual rainfall, 140, 407 and 329 mm; rainfall between April 1 and September 30, 84, 118 and 103 mm. The irrigation was conducted after harvest because some controls post-harvest were made.

2.2. Yield components and berry sampling

The following yield components were determined in each vine at harvest: yield (kg vine^{-1}), number of clusters per vine and cluster weight (g). A sample of 200 berries from all the vines of each experimental replication was collected on the harvest date to measure average berry weight (g). Berry sampling was made in every replication from most clusters of each vine and all parts of the cluster. The number of berries per cluster was calculated taking into account both the cluster weight (g) and the berry weight (g).

2.3. Winemaking process

This section has been described in earlier paper.[7] The grapes harvested from each experimental treatment were vinified in the experimental winery of the ETSIIAA (Technical High School of Agronomic Engineering) in Palencia (Spain), following a classical pattern of development of white wine. Two replications were carried out by treatment with exception for R-50 and R50-TH in 2013 vintage because healthy grape there was not enough due to *Botrytis* infection in these treatments.

The must of each treatment replication was processed separately in 50-liter stainless steel tanks and sulfur dioxide was added up to 50 mg/L to help the static settled at 8 ºC for 24 hours.

Commercial neutral yeasts were added to carry the alcoholic fermentation. LALVIN EC 1118 (Lallemand; Quebec, Canadá) *Saccharomyces cerevisiae* was the selected strain. Monitoring of alcoholic fermentation was performed by daily control of density and temperature (it was always kept below 18.5 ºC).

Reducing sugars were determined when density level reached 990-995 g cm^3 and fermentation was finished when sugar levels were below 3 g L^{-1}. Sulfur dioxide was then added to the wines in order to reach 30 mg L^{-1} of free SO_2. Cold stabilization was performed by settling the tanks in a cooler at 4 ºC. A week later, the wines were bottled.

2.4. Grape and Wine composition

Sugar concentration, pH and titratable acidity in must and pH, titratable acidity, ethanol, total polyphenol index (TPI), hydroxycinnamic acids and flavonoids in wines were analyzed in duplicate after bottling. The must and wine composition were determined by the official methods of the International Organization of Vine and Wine.[24]

2.5. Wine volatile composition

In a 10 mL culture tube, 8 mL of wine, 3 μg of internal standard (4-nonanol) and a magnetic stir bar (22.2 mm x 4.8 mm) were added. Extraction of volatiles was done by stirring the sample with 400 mL of dichloromethane, according to the method of Oliveira *et al.*[25] with modifications[7]. After cooling at 0 °C for 10 min, the magnetic stir bar was removed and the organic phase was detached by centrifugation (5118 *g*, 5 min, 4 °C) and the extract was recovered into a vial, using a Pasteur pipette. The aromatic extract (200 μg L^{-1}) was dried with anhydrous sodium sulphate and placed in a new vial. Extractions of volatile compounds from each of the respective wines were performed in triplicate.

Gas chromatographic analysis of volatile compounds was performed using an Agilent GC 6890N Chromatograph coupled to mass spectrometer Agilent 5975C. A 1 μL injection was made into a capillary column, coated with CP-Wax 52 CB (50 m × 0.25 mm i.d., 0.2 μm film thickness, Chrompack). The temperature of the injector was programmed from 20 °C to 250 °C, at 180 °C min^{-1}. The oven temperature was held at 40 °C, for 5 min, then programmed to rise from 40 °C to 250 °C, at 3 °C min^{-1}, then held 20 min at 250 °C and finally programmed to go from 250 °C to 255 °C at 1 °C min^{-1}. The carrier gas was helium N60 (Air Liquide) at 103 kPa, which corresponds to a linear speed of 180 cm s^{-1} at 150 °C. The detector was set to electronic impact mode (70 eV), with an acquisition range from 29 to 360 *m/z*, and an acquisition rate of 610 ms.

Identification was performed using WSearch Free Software, by comparing mass spectra and retention indices with those of pure standard compounds. Pure standard compounds were purchased from Sigma-Aldrich (Darmstadt, Germany) with purity higher than 98 %. Semi-quantitative data were obtained by calculating the relative peak area in relation with internal standard (4-nonanol).

2.6. Statistical analysis

All data were analyzed using statistical package XLSTAT-Pro 2017 (Addinsoft, Paris, France). The data were subjected to two-way variance analysis (ANOVA) to evaluate the differences among treatments, water regimes (WR) and cluster thinning (TH) and interaction WR*TH. The mean differences between treatments by year were calculated according to the least significant difference from Fisher's test (LSD).

3. RESULTS AND DISCUSSION

3.1. Meteorological conditions

Meteorological data from the weather stations of the locations of Medina del Campo and Rueda (Valladolid), which were both 6 km apart from the experimental trial, across the three consecutive seasons (2012-2014) were used to calculate climatological parameters during the study (Figure S1). During the experimental season (1 April to 31 October), the average temperature (ºC) was 16.9 in 2012, 16.5 in 2013 and 17.7 in 2014 (Figure 1). During berry ripening (from *veraison* to harvest) the average temperature (°C) was 20.2 in 2012, 17.8 in 2013 and 19.9 in

2014. However, the maximum temperature (°C) for the same period was 28.3, 25.6 and 27.5 respectively. From 1 April to 31 October, ETo was 977, 940 and 1039 mm and the rainfall 154.9, 177.5 and 117.3 mm in 2012, 2013 and 2014, respectively. Therefore, 2013 was the wettest season with the lowest mean temperature and the lowest temperature in the ripening period. In contrast, 2014 was the driest season with the highest mean temperature. The highest ripening temperature was observed in 2012 season, which was also a quite dry viticultural year (data not shown).

3.2. Yield components and fruit composition

Table 1 shows the yield components and must chemical composition by season when cluster thinning (TH) was applied to three different water regimes (WR). Two-way ANOVA with interaction (WR*TH) by season is also shown.

As expected, clusters by vine and yield decreased significantly when the TH was applied in all water regimes and in all seasons with exception for R0-TH in 2014, where the same tendency to yield decrease was observed. For the whole of treatments grape yield became reduced around 19 % on interannual average when the cluster thinning was applied. In general, the cluster weight has shown a tendency to increase in the thinned treatment, to a greater or lesser extent depending on the water regime and year. Statistically significant differences between treatments were only found in R25 in 2013 where TH increased the cluster weight in 14 %. Therefore, the cluster weight would explain some compensation in grape production, since the number of clusters per vine has been reduced directly by 27 % on interannual average, by applying the thinning treatment. In this sense, some authors have shown that berry weight compensation occurs when the berries are in phase I of development, during the first 3-4 weeks following fruit set. It is during this stage that cell division occurs in the berry, and removal of competing clusters may to allow for greater berry weight. Additionally, not all cultivars experience berry size compensation. Thus, if there are no nutritive limitations, such water availability, crop thinning after fruit set may not result in increased berry weight compared to non-thinned vines.[26,27] In our work, it has been observed certain tendency to increase berry weight when the vine water status increased and this increase was higher in all three seasons when cluster thinning was applied, although the differences were not found statistically significant in any case. These results indicate that water regime and cluster thinning could have an additive effect on berry weight.

In the same way, the number of berries per cluster has shown a trend to be slightly higher when TH was applied in R0 (2012-2013) and R25 (2013-2014), having found no statistically significant differences between treatments. As to berry weight, no significant water regime x cluster thinning interaction was found in any yield components, with exception of the clusters per vine in 2014 season, showing in general that the effect of TH on the yield components was equivalent in all WR.

On the other hand, fruit composition at harvest was consistent from year to year (Table 1). Climatic conditions of the growing season did have an impact on sugar concentration and total acidity (TA), resulting in lower ºBrix and higher total acidity in the coolest and wettest season (2013). In contrast, the high temperatures in the ripening period of 2012 produced higher ºBrix values and lower TA. Temperature differences were the major cause of the variation in sugar accumulation among seasons in Cabernet Sauvignon under cluster thinning treatment.[22] In the present work, TH induced an increase of sugar content (ºBrix) and a decrease of total acidity in all three seasons but this effect was only significant for R25-TH and RO-TH respectively in 2012 season, when higher ripening temperatures were observed.

Similar results were achieved by Reynolds *et al.*[28] showing that *veraison* cluster thinning applied in Chardonnay advanced fruit maturity increasing sugar content (ºBrix) and decreasing total acidity. According to total acidity of must, pH value showed a trend to increase when TH was applied, being only significant in the wettest season (2013) for R25-TH and R50-TH. Our results, obtained in warm and dry site, have shown that cluster thinning may be effective for hastening fruit ripening in contrast to other authors.[29] In general, grapes exposure caused by thinning treatment will increase fruit temperature and enzymatic activities can be produced. Temperature is the main environmental factor affecting malic acid concentration in berries.[29] Thus, the post-veraison decrease of malate concentration in grapes by respiration increases with high temperature.[30] Changes in pH and acidity by application of cluster thinning were observed in several cultivars and years.[13] Thus, the pH decreased and acidity increased with increasing crop level in several cultivars and years. Some studies have clearly demonstrated that cluster thinning advances grape maturity, diminishes acidity and increases soluble solids and pH in juice.[31,] Also, cluster thinning improved phenolic content and volatile composition of Shiraz grapes.[32]

No significant WR*TH interaction was found for any grape chemical parameter in Verdejo must, with exception to total acidity in 2012 season. This interaction observed in 2012 season indicated that the crop load modified the total acidity with different intensity depending on water regime. In the same way, very few interactions were found between irrigation and crop load treatments on Cabernet Sauvignon in an arid climate, which means that cluster thinning did not influence the vine response to deficit irrigation.[22]

3.3. Wine basic chemical variables

Table 2 shows the basic chemical composition of Verdejo wines affected by cluster thinning (TH) on three different water regimes (WR) by year of study. Two-way ANOVA with interaction (WR*TH) results by season are also shown.

The behavior observed in Verdejo must was translated to the wine parameters, where the highest ethanol and lower TA levels were observed by 2012. There was a tendency to increase the pH value when cluster thinning was applied in all WR, but this increase was only significant in 2014 season. In contrast, a general trend to decrease the total acidity was observed in all WR when TH was applied, but only significant in R0-TH (2012) and R50-TH (2014). Cluster thinning induced an increase of wine ethanol levels in the different water regimes, but it was only significant for all WR in 2012, and R0 and R50 in 2013 and 2014 respectively. Similar results were observed by Gamero *et al.*[33] when applying cluster thinning in Tempranillo vines.

In general, no noticeable effect of thinning treatment was found on TPI. Cluster thinning induced a significant increase of TPI only when it was applied in R0 for 2012 (season with the highest ripening temperature). In contrast, cluster thinning induced the increase of TPI in Tempranillo wine when irrigation was applied at 25 % and 100 % ETo.[33]

Regarding hydroxycinnamic acids, the results showed a significant increase of their concentrations when cluster thinning was applied in R50 for 2012 and 2014 seasons and in R0 for 2013. Also, the flavonoids concentration increased in R0 when TH was applied in 2013. The WR*TH interaction only was significant for total acidity in all seasons studied. However, this interaction was also significant for hydroxycinnamic acids and flavonoids in 2013 and 2014, for ethanol in 2012 and

2013 and for TPI in 2014. Some studies stablished that cluster thinning improve the fruit composition,[34] however others authors have observed that the effects of seasonal conditions, especially temperature, were greater than the impact of cluster thinning and this effect not always is translated into the final wine. [35]

3.4. Verdejo wine volatiles by groups of compounds

Figure S2 shows the combined effect of cluster thinning and water regime on Verdejo wine volatile concentration by groups of compounds (alcohols, C_6 compounds, volatile acids, ethyl esters, volatile phenols and lactones) by year (a) and average of the three seasons 2012-2014 (b).

The volatile compounds in wine arise from several sources: grape berry, yeast and bacterial metabolism, chemical reactions upon storage and enzymatic or chemical reactions of nonvolatile precursors during winemaking process.[36] Grapes contain different groups of non-volatile aroma precursors as unsaturated lipids, phenolic acids, carotenoids, S-cysteine conjugates, glycoconjugates and S-methylmethionine. These non-volatile, odorless constituents are susceptible to transformation into volatile varietal aroma compounds during the biotechnological sequence of wine, from the cellular disorganization. Glycoconjugates precursors are the origin of monoterpenes, C_{13}-norisoprenoids, volatile phenols, alcohols, C6 compounds and others.[37]

In addition to glycosides and S-conjugates, several nonvolatile precursors are present in grapes, which may lead to potent wine aroma compounds during winemaking process, acting as precursors of volatiles. These include amino acids, which serve as precursors aldehydes, fusel alcohols, fatty acids, which can be enzymatically converted to C_6 compounds and others.[38] Secondary metabolites under genetic control, some of them associated with specific cultivars, but they are also dependent on the several factors as situation, soil, climate or viticultural practices.[37]

Alcohols were the mayor group of volatiles in Verdejo wines for all treatments studied, followed by volatile fatty acids and ethyl esters. When the effect of cluster thinning in different water regimes on volatile groups were studied season by season (Figure S2a), a significant cluster thinning effect was only observed in 2013, the wettest season, where TH applied on R0 water regime increased the concentration of alcohols, ethyl esters and lactones. In addition, a trend to increase volatile fatty acids was observed for R0-TH with respect to R0 in the same season, 2013. The same trend was observed in 2012 season for all volatile groups. In contrast, TH decreased all volatile groups in R0 water regime in the driest and warmest season, 2014, with exception to lactones. A trend to increase all volatile families was observed in 2014 season when TH was applied in R50. No significant differences between R25 and R50 when TH was applied were shown.

Most of the aroma compounds are influenced by the grape exposure to sunlight, thus thinning increases the cluster exposure, increasing the aroma compounds concentration.[39,40] The major ethyl esters identified in Shiraz wine showed higher concentration when cluster thinning was applied, however the fatty acids content was lower in thinned samples.[32] Increase of ethyl esters and fatty acids was observed in Pinot noir wines when cluster thinning was applied in the vineyard.[40] With respect to water availability, results obtained by Talaverano *et al.*[12] showed that a lower water supply had a negative effect on the aromatic potential in Cabernet Sauvignon wines.

Regarding to the three-season average (Figure S2b), no significant influence of crop level was shown on volatile groups in different water regimes. However, there was trend to increase the concentration of all volatile groups in Verdejo wines when TH was applied in R0. A trend to increase the all volatile groups level in TH vs control treatment also was observed (Figure S2b). Other studies[23] showed the combined effect of both factors, water regime and cluster thinning, on volatile composition of Tempranillo wine. They concluded that the combined effect of rainfed and cluster-thinning treatments (T0–TH) increased the majority of individual aromatic compounds quantified in red Tempranillo wines.

3.5. Verdejo wine volatiles by individual compounds

Tables 3-6 show the results of the influence of cluster thinning on individual volatiles when this treatment was applied in different water regimes. As regards the aroma compounds, limited and contradictory information is present in the literature about the effect of cluster thinning and water regime. The present findings suggest that crop level in different irrigation regimes slightly modified the volatile composition of Verdejo wine. These observed changes were higher when cluster thinning was applied in strict deficit irrigation (R0-TH) affecting to some alcohols, volatile fatty acids, ethyl esters and acetates, C_6 compounds, phenol volatiles and lactones. Thus, from 33 volatile compounds identified and quantified in Verdejo wine, only 12 compounds were affected significantly by the treatments applied, mainly ethyl esters and acetates. Strong correlation between the extend of cluster thinning and wine volatile composition of Pinot noir was observed by other authors.[39] However, no consistent effects of cluster thinning on Cabernet Sauvignon wine quality and aroma were found by other authors.[41,42]

The highest treatment effect was shown in the wettest season (2013), where cluster thinning treatment applied in R0 water regime (R0-TH) increased significantly the concentration of nine volatile compounds (2+3-methylbutanoic, 2+3-methyl-1-butanol, ethyl butyrate, isoamyl acetate, phenyl ethyl acetate, Z-3-hexen-ol and γ-butirolatone). Only 4-vinylphenol showed the same behavior in 2012, increasing their concentration in R0-TH. In contrast, in 2014, the driest season, only hexadecanoic acid and Z-3-hexen-1-ol were affected significantly when TH was applied in R0, decreasing their concentration. Moreover, in the driest season, 2014, R0-TH showed a tendency to decrease the concentration of all volatile fatty acids, higher alcohols, some ethyl esters, C_6 compounds and phenol volatiles. In plants several alcohols, aldehydes, acids, esters and lactones are derived from fatty acids via α− o β-oxidation or by lipoxygenase pathway. C_6 compounds (aldehydes and alcohols) are the mayor aroma compounds derived from fatty acids in grapes via lipoxygenase pathway and they are involved in plant defense against pests and diseases.[43] The alcohols with six carbon-atoms, hexenols and hexanols, supply vegetal and herbaceous nuances to the wine and fruit maturity has been previously associated with lower levels of these compounds.[25]

Six volatile compounds showed a tendency to increase their concentration when TH was applied in R0 independently of the season, and they were 2,3-butanediol, ethyl hexanoate, ethyl decanoate, diethyl succinate, phenylethyl acetate and γ-butirolactone. The other major grape-derived compounds with a fatty acid origin are the γ-and δ-lactones that are derived from the corresponding 4- or 5-hydroxy carboxylic acids.[37]

On the other hand, when cluster thinning was applied in R50 in 2012, it was observed the significant decrease of two acetates (isoamyl acetate and hexyl acetate). Moreover, a trend to decrease the concentration of all ethyl esters and acetates was observed in R50-TH in 2012 with exception of ethyl succinate. The concentrations of the acetate esters had moderate to strong negative correlations with the extent of crop thinning in Pinot noir wines.[39] In the same study, when considering alcohols and C_6 alcohols, there were no consistent treatment effects, with exception to 1-hexanol, where a strong negative correlation with crop thinning was observed. Regarding the driest season, 2014, it was observed a trend to increase in most of the compounds quantified when TH was applied in R50, but this increase was only significant for 4-vinylguaiacol. In grape berry, most of volatile phenols occur as glycoconjugates, which are more abundant than the free fraction. In wine, they are release of their aglycons through hydrolysis.[44,45] Moreover, the vinyl derivatives, 4-vinylphenol and 4-vinylguaiacol, are also formed during the alcoholic fermentation by decarboxylation of the cinnamic acids with a cinnamate decarboxylase of *Saccharomyces cerevisiae* yeasts.[46]

Chapman *et al.*[47] concluded that Cabernet Sauvignon aromas and flavors respond to yield manipulation but only when yield is altered early in fruit development. Thus, it suggested that cluster thinning at *veraison* did not increase the wine value. However, King *et al.*[48] observed that grape ripeness, must and wine composition tended to respond more from crop removal at *veraison* than at other times. In our study the cluster thinning was applied at the beginning of *veraison*, trying to approach it to a reduction of a third part of clusters, resulting in an average reduction of 27 % of clusters with respect to control vines in all water regimes.

A significant WR*TH interaction was found for eight compounds (2+3-methyl-1-butanol, 2+3-methylbutanoic, isoamyl acetate, Z-3-hexen-1-ol, 4-vinylguaiacol and γ–butirolactone) in 2013 but it was found for only one compound (4-vinylguaiacol) in 2014.

4. CONCLUSIONS

This study, conducted across three consecutive seasons (2012-2014), allowed to determine the grape agronomical and wine chemical effects of cluster thinning applied in three water regimes in Verdejo cultivar grown in Rueda D.O. The cluster thinning, applied at the beginning of *veraison*, reduced a third part of clusters, so the grape yield to a lower extent, in spite of the slight cluster weight compensation. In the three seasons TH induced the increase of must sugar content (^{o}Brix), so the wine ethanol, and the decrease of total acidity in both must and wine for all water regimes. Analysis of volatiles suggested that cluster thinning applied in R0 water regime induced an effect on Verdejo wine volatiles increasing the concentration of alcohols, ethyl esters, acetates and lactones, and this effect was higher in the wettest and coolest season (2013). A general trend to increase the concentration of volatile compounds caused by the treatment of cluster thinning was observed in R0 water regime, mainly for most of ethyl esters and acetates. Also, TH showed a tendency to increase the concentration of most compounds when it was applied in R50 but only in the driest season, 2014. Interactions between water regime and cluster thinning were found in most wine chemical parameters indicated that cluster thinning influenced the vine response to deficit irrigation, which was mainly observed in the wettest and coolest season. The application effects of irrigation and cluster thinning together depend on the climatic conditions and the fertility level of the current year. Since there is a productive loss due to the effect of cluster thinning and non-application of irrigation, the criterion for executing the combination of both practices should be search of the balance that improve the grape composition in each season. Taking into account our results and those found in the bibliography, more studies about cluster thinning would be advisable in different condition of grape growing to obtain more consistent conclusions.

ACKNOWLEDGEMENTS

This research was supported by FEDER and INIA funds through Project RTA2011-00100-C05 and help of Project RTA2014-00049-C05. We thank Dr. Manuel Marcos for the GC/MS service from Scientific and Technological Research Assistance Centre (CACTI, Vigo University, Spain).

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