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Severe trimming and enhanced competition of laterals as a tool to delay ripening in Tempranillo vineyards under semiarid conditions

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Abstract

Aim: An advance in grapevine phenological stages (including ripening) is occurring worldwide due to global warming and, in the hottest seasons, already results in a lack of synchrony between sugar and phenolic ripeness, leading to unbalanced wines. In order to cope with this fact, a general effort is being made by researchers and growers aiming at delaying ripening through cultural practices, particularly under warm growing conditions, where these effects are more deleterious. The aim of this work is to evaluate to which extent severe trimming and enhanced competition of laterals can delay ripening in Tempranillo vineyards under semiarid conditions.

Methods and results: The experiment took place during two consecutive seasons in Traibuenas (Navarra, Spain) in a cv. 'Tempranillo' vineyard trained to a vertical shoot positioned (VSP) spur-pruned bilateral cordon. Severe mechanical pruning was performed ca. 3 weeks after fruit-set in order to reduce leaf-to-fruit ratio, and in the trimmed plants, three irrigation doses were applied until harvest aiming at enhancing lateral growth, hypothesized to compete with ripening. All measurements were performed in six 10-vine replicates per treatment. Trimming significantly reduced leaf area and yield, resulting in higher water availability in trimmed plants. The whole ripening process was delayed by trimming: mid-veraison was delayed by about 5 days, and the delay in sugar accumulation and acid degradation was longer, differences being more marked in malic than in tartaric acid concentration. The use of increased irrigation levels compensated the losses in yield caused by trimming, enhanced laterals' growth and implied an additional delay in ripening.

Conclusion: trimming and increased irrigation had an additive effect in terms of delaying ripening, and they can be used jointly when that delay is needed.

Significance and impact of the study: this study proves the potentiality of the joint use of trimming and increased irrigation to delay ripening, although it is necessary to analyze the implications the obtained delay has on other quality aspects. The lower anthocyanin and phenolic values observed in trimmed vines were not solely due to delayed ripening, as lower values were observed even when data were compared for a given total soluble solid content.

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Introduction

Adaptation to climate change is a major challenge for the wine grape growing sector, since climatic conditions affect not only the crop's sustainability, but also its *typicity*, i.e. the specific characteristics that make a wine produced in a given region or terroir singular. During the last decades, most of the world's highest quality wine-producing regions have shown a warming trend during the growing season (Duchêne and Schneider, 2005; Jones *et al.*, 2005). This change has led to an advance in phenology, which, jointly with some changes in cultural practices, resulted in earlier harvest dates, higher sugar concentration in grapes, and higher alcoholic concentration in wines (Duchêne and Schneider, 2005; Ramos *et al.*, 2008; Tomasi *et al.*, 2011; Neethling *et al.*, 2012; Webb *et al.*, 2012; Bock *et al.*, 2013; Koufos *et al.*, 2014; van Leeuwen and Darriet, 2016). Higher berry sugar content usually implies higher must pH, which results in less stable, less colored, leaner wines (Ribéreau-Gayon *et al.*, 2000).

Moreover, advanced phenology indirectly implies that physiological ripening processes are occurring at increased temperatures, which can have a direct impact on grape composition (Keller, 2010). With regards to aromatic compounds, several studies suggested that, at equivalent sugar concentrations, higher temperatures lead to lower levels in white aromatic grape varietals, thus potentially reducing aromatic intensity (Mira de Orduña, 2010). In red grape varieties, high temperatures during ripening have also been shown to decouple sugar and phenolic maturity (Sadras and Moran, 2012; Bonada *et al.*, 2013; Teixeira *et al.*, 2013), resulting in altered organoleptic profiles.

Although climate change can favor grape growing in some regions (Fraga *et al.*, 2012; Hannah *et al.*, 2013), this is not the case for most wine regions in Spain and Portugal, where climatic change can negatively impact grape growing (Malheiro *et al.*, 2012; Resco, 2015; Lorenzo *et al.*, 2016). The change in climatic conditions during the last decades is a matter of fact. For instance, in La Rioja and Navarra, two wine regions in Northern Spain, all bioclimatic indices relevant to viticulture have changed significantly between 1951-1980 and 1981-2010 (Figure 1). As a consequence, the abovementioned detrimental effects of advanced ripening on grape and wine composition are becoming an increasing problem that needs to be addressed (Alonso and O'Neill, 2011; Martinez de Toda *et al.*, 2014).

Looking at the past to understand the future

When facing a new climatic scenario, winegrowers can display a wide set of cultural techniques in order to minimize its effects (Neethling *et al.*, 2016). Among them, adapting the training systems and canopy management operations, planting vineyards at higher altitudes, and changing vinifera/rootstock varieties and soil management practices can be regarded as the most powerful tools (Battaglini *et al.*, 2009; Duchêne *et al.*, 2010; Neethling *et al.*, 2016). However, some of the changes made in Navarra and Rioja (and in many other areas in Spain) in the past decades (particularly in the 1980-2000 period) have led to a certain degree of *miss-adaptation* to climate change, despite being associated to the introduction of irrigation:

Table 1. Effect of trimming and irrigation treatments on trunk cross sectional area (TCSA), shoot characteristics, cluster number, yield and carbon isotope ratio ($\delta^{13}C$)

Treatment	TCSA (cm^2 $vine^{-1}$)	Main shoot length (cm)*	No. laterals main $shoot^{-1}$	Total lateral length (cm $shoot^{-1}$)	Cluster number $vine^{-1}$	Yield (kg vine-1	$\delta^{13}C$ (‰)
Control	18.3	83.4 a	1.20 b	12.6 c	12.1	2.73 ab	-25.18 a
Trim + R1	18.2	56.7 b	1.41 ab	19.2 c	10.8	2.36 b	-25.97 b
Trim + R2	17.9	58.4 b	1.43 ab	36.4 b	11.0	2.57 ab	-26.94 c
Trim + R3	18.2	61.9 b	1.57 a	49.7 a	11.5	2.98 a	-27.44 d
P	*0.844*	*<0.001*	*0.016*	*<0.001*	*0.474*	*0.032*	*<0.001*
Year							
2014	17.7	--	--	--	11.3	2.24	-26.01
2015	18.7	--	--	--	11.6	3.09	-26.75
P	*0.005*	--	--	--	*0.583*	*<0.001*	*0.013*
Interaction							
P_{int}	*0.924*	--	--	--	*0.975*	*0.648*	*0.205*

*main shoot length, no. laterals and total lateral length were measured only in 2015; data were analyzed through a one-way ANOVA

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- (i) New vineyards were frequently planted in irrigated areas, moving from poor but deep soils to more fertile but shallower soils, where irrigation water was available. When the maps of vineyard locations in Navarra in 1956 and 2012 are compared (Figure 2), it can be seen that the altitude of vineyards has changed significantly (Figure 3). At a regional level, the decrease accounts for 20 m, with a particularly important change (18 m) in the warmest sub-area (zone VII).

- (ii) In addition to location change, the traditional low-water-consuming gobelet training system was shifted to vertical shoot positioned (VSP) systems

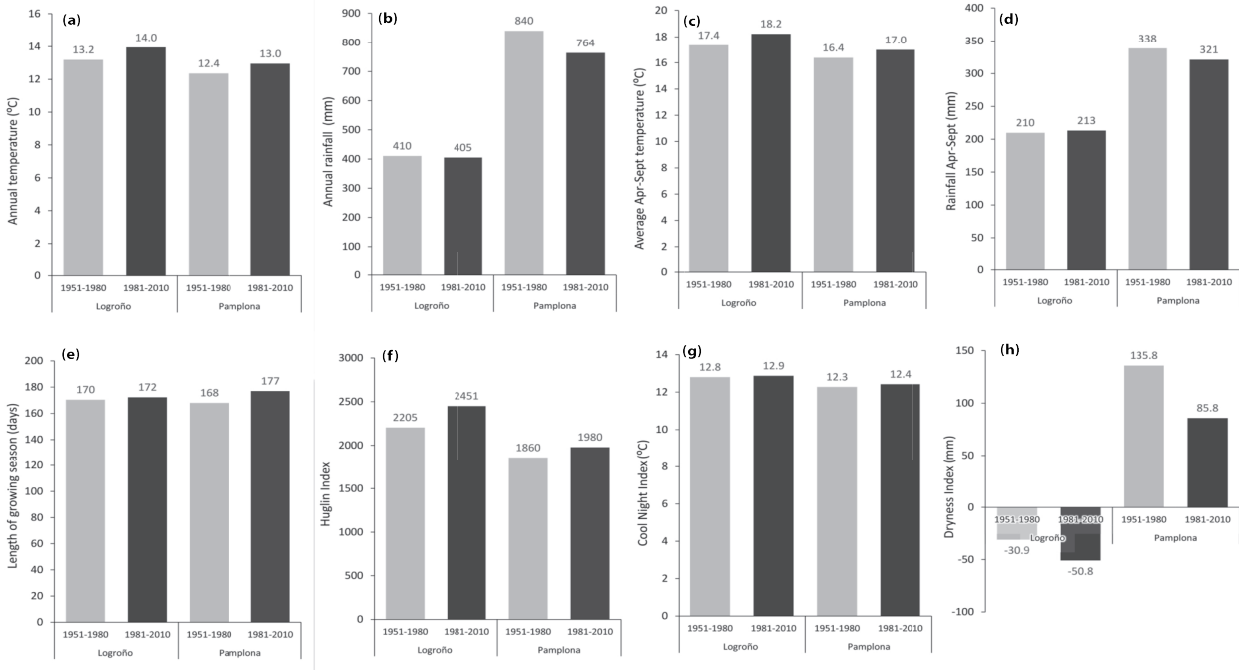


Figure 1. Comparison of bioclimatic indices for the 1951-1980 and 1981-2010 periods at Logroño-Agoncillo (La Rioja) and Pamplona (Navarra) observatories. Elaborated using the Daily Dataset for European Climate Assessment (Klein Tank *et al*., 2002) available at http://www.ecad.eu.

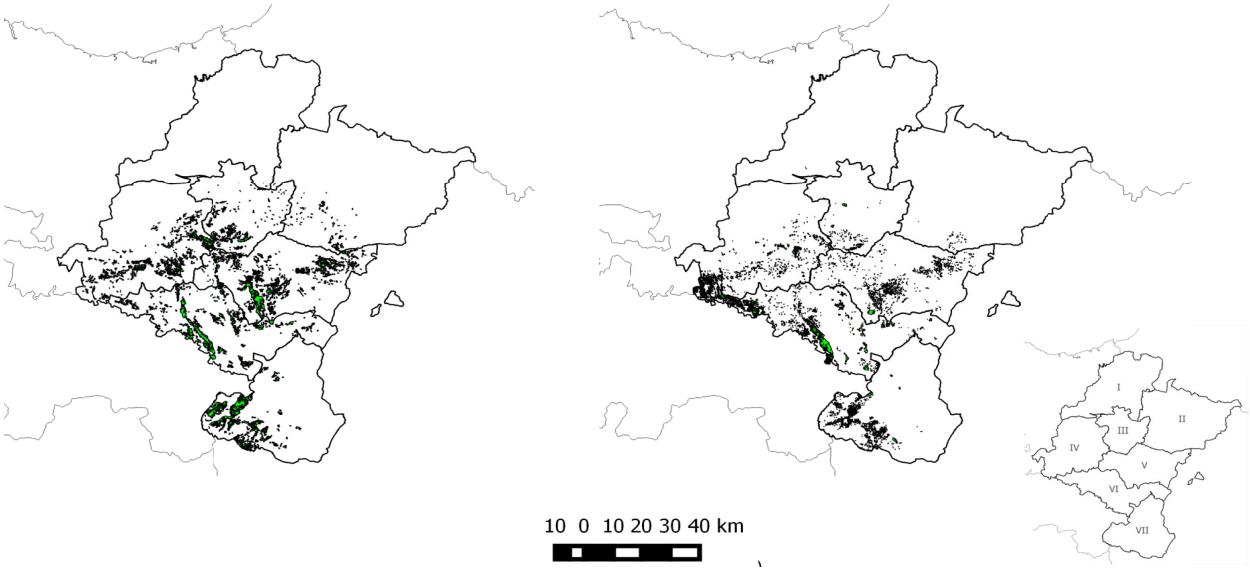


Figure 2. Comparison of the 1956 (left) and 2012 (right) land use maps of Navarre; agricultural areas are indicated as roman numbers. Elaborated using data provided by Navarre Territorial Information System (SITNA) distributed under Creative Commons Licence at http://idena.navarra.es/.

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where, apart from the increased water needs (Reynolds and Heuvel, 2009), clusters are much more exposed to solar radiation and therefore subjected to higher temperatures.

(iii) Last, there was a varietal shift from Grenache N to Tempranillo (Figure 4), tolerant and sensitive to water deficit, respectively (Santesteban *et al.*, 2009; Martorell *et al.*, 2015), with the former tending to give lower pH juices than the latter (Garcia *et al.*, 2011). Tempranillo, as an autochthonous variety, is well adapted to its original habitat (i.e. the cooler areas in Rioja), but during this varietal shift, it was also used for planting most vineyards in the warmest areas of the region.

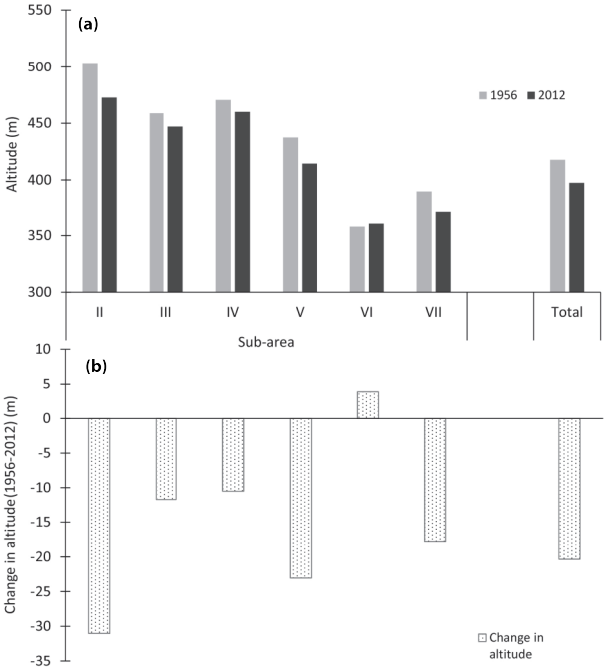


Figure 3. Mean altitude of vineyards in Navarra agricultural areas in 1956 and 2012 (a) and altitude change in that period (b). Agricultural areas correspond to those indicated in Figure 2. Elaborated from land use maps and terrain elevation models provided by Navarre Territorial Information System (SITNA, http://idena.navarra.es/) using QGIS 2.12. and GRASS open access software packages.

Although the introduction of irrigation made it possible for vineyards to be located in shallower soils (using a more drought-sensitive cultivar with a more water-demanding training system), other side-effects appeared, like unwanted high sugar and pH values, berry shriveling and uncoupled sugar and phenolic ripening. In this context, a reversion of the variety change is starting to be implemented, with the growing presence of the low pH, high color cv. Graciano for blending. A big effort is also being made to find cultural practices that can help the growers to adapt to climate change (Martínez de Toda and Balda, 2013; Martinez de Toda *et al.*, 2013; Martinez de Toda *et al.*, 2014).

The aim of this work is to evaluate to which extent severe trimming and enhanced competition of laterals can delay ripening in Tempranillo vineyards under semiarid conditions.

Materials and Methods

1. Experimental design

The experiment took place during the 2014 and 2015 growing seasons in Traibuenas (Navarra, Spain) in a 4.2 ha 'Tempranillo'/110 Richter vineyard (42º 23' 7'' N, 1º 37' 29'' W, 350 m a.s.l.). Vines were 17

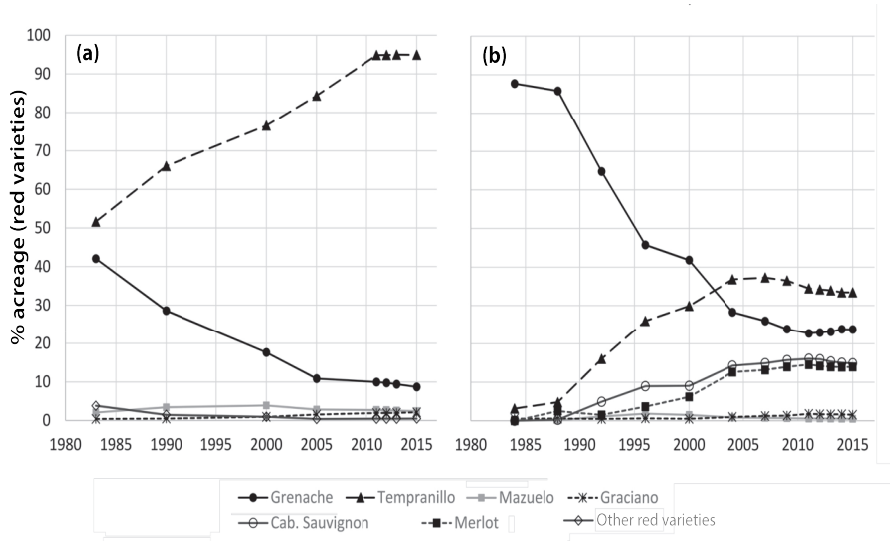


Figure 4. Evolution of the relative acreage of the red varieties grown in AO Rioja (a) and in AO Navarra (b) in the 1983-2015 period. Data were provided by the Regulatory Boards of both AO.

years old at the beginning of the experiment, planted at 3 x 1 m spacing, and trained to a VSP spur-pruned bilateral cordon, with three 2-bud spurs per arm.

Control vines (CTRL) were subjected to standard practices and compared to three treatments. In all treatments, severe mechanical trimming (TRIM) was performed ca. 3 weeks after fruit-set (pea-size) by cutting shoots at ca. 55-65 cm. The trimmed treatments differed in the irrigation level: Trim+R1 plants received the same amount of water than control plants (ca. 36 L vine^{-1} once a week from pea-size to harvest), whereas Trim+R2 and Trim+R3 plants were irrigated two and three times a week for 4-5 weeks after trimming (ca. 72 and 108 L vine^{-1} every week, respectively). None of the treatments was irrigated between harvest and pea-size. The hypothesis supporting this approach is that reduced leaf-to-fruit ratio and enhanced lateral shoot growth can delay ripening through, respectively, decreased leaf-to-fruit ratio and increased competition. The experimental layout was set-up at a nearly commercial scale (2.4 ha of vines as a whole), measurements being taken in six 10-vine replicates per treatment.

2. Measured variables

In winter, trunk cross sectional area (TCSA) was calculated after measuring trunk diameter 30 cm above-ground, and total shoot growth determined measuring main shoot length, the number of laterals, and lateral's length (only in 2015). Veraison was determined by careful visual inspection of 30 clusters per replicate twice a week from the onset until the end of veraison.

Yield was determined at harvest by counting and weighing all the clusters produced in the 10 vines in each replicate. Grape composition was determined weekly between veraison and harvest in one 300-berry sample per replicate. Samples were processed according to standard laboratory procedures in order to determine berry weight (BW), total soluble solids (TSS), pH, titratable acidity (TA), and malic (MalA) and tartaric (TarA) acid concentration. Phenolic ripeness was evaluated using the Cromoenos® method (Bioenos, Cariñena, Spain, http://www.bioenos.com/cromoenos/index.php), which allows determining total anthocyanins (TAnt), total phenolics (TP), and a phenolic maturity index (PMI) after a fast extraction in buffer solutions.

Plant water status was monitored weekly between fruit-set and harvest. Stem water potential at midday (Ψ_{midday}) was determined for three healthy leaves per replicate, bagged 1.5 hours prior to measurement using zip-bags covered with a metalized high-density polyethylene reflective film. Measurements were carried out with a Scholander pressure bomb (P3000, Soil Moisture Corp., Santa Barbara, CA, USA). Finally, berry carbon isotope ratio ($\delta^{13}C$) was determined in 50-berry samples collected at harvest, oven-dried, ground into a fine homogeneous powder, and analyzed using an Elemental analyzer (NC2500, Carlo Erba Reagents, Rodano, Italy) coupled to an Isotopic Mass Spectrometer (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany) as detailed in Santesteban *et al.* (2012).

3. Data analysis

Data were analyzed using linear regression and two-way ANOVA (trimming/irrigation treatment x season). All analyses were performed with computing environment R (R Development Core Team, 2015).

Results

1. Vegetative growth and yield

Both trimming and additional irrigation achieved their goal in terms of vegetative growth (Table 1). Trimming caused a significant decrease in main shoot length and a slight increase in the number of laterals, whereas increased irrigation resulted in longer laterals. Trimming significantly decreased yield, but this was compensated by the increased irrigation treatments.

2. Plant water status

The evolution of water status followed a similar pattern in both years (Figure 5), with slightly lower midday stem water potential values in 2015. Increased irrigation resulted, as expected, in higher stem water potential, with a clear gradation between Trim+R1, Trim+R2 and Trim+R3 treatments; the same trend was observed when carbon isotope ratio values ($\delta^{13}C$) were compared (Table 1). Trimming also had a relevant effect on vine water status since Trim+R1 plants showed higher water potential and lower $\delta^{13}C$ than control plants, indicating that trimming alleviated to a certain extent the water deficit.

3. Ripening dynamics

Trimming and increased irrigation caused a delay in veraison in both years (Figure 6). In 2014, trimming caused the greatest differences, inducing a 4-day delay in mid-veraison, whereas increased irrigation delayed it an additional day. In 2015, a greater delay was observed, with four days due to trimming, and an additional three days after increased irrigation.

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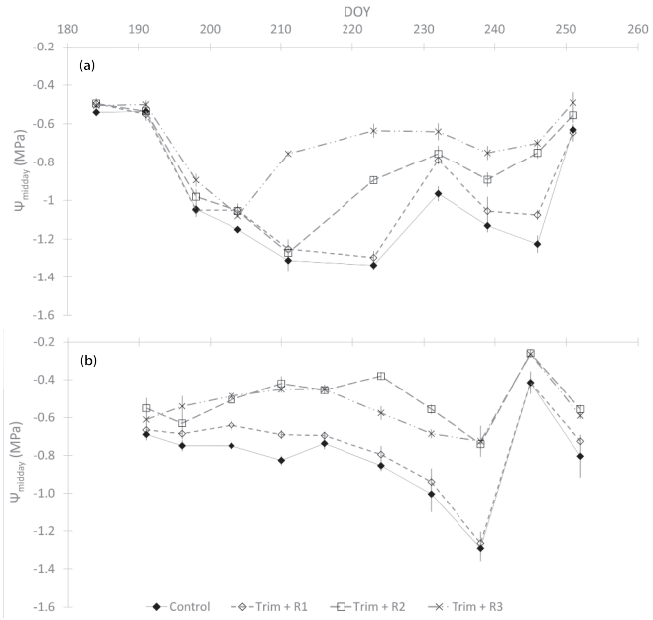


Figure 5. Evolution of stem water potential at midday (Ψ_{midday}) in 2014 (a) and in 2015 (b).

Treatments also affected berry size and ripening dynamics (Figures 7, 8 and 9). Control vines produced smaller berries in both years, and irrigation increased berry size (Figure 7a, c). Both trimming and increased irrigation caused a remarkable delay in sugar content (Figure 7b, d). If we take 23.5 ºBrix as a reference value, trimming resulted in a 9- and 10-day delay in 2014 and 2015, respectively, while increased irrigation additionaly induced 5 days of delay in 2014 and 7 in 2015.. These changes in ripening dynamics were also reflected in acidity parameters (Figure 8): untrimmed vines tended to have an advanced ripening, whereas increased irrigation resulted in an additional delay, showing higher titratable acidity and malic and tartaric concentration, and lower pH for a given date. Last, with regards to phenolic compounds, no clear trends were observed in 2014 (only three sampling dates are available), whereas in 2015 the effects of trimming and increased irrigation were much clearer (Figure 9). On the one side, trimmed vines showed lower anthocyanin and phenolic concentration, and lower PMI values, indicating an advance in phenolic maturity. On the other side, increased irrigation tended to cause lower anthocyanin and phenolic content, and increased PMI values, indicating a delay in ripening.

Discussion

Trimming and additional irrigation induced significant changes in ripening dynamics, resulting in a later onset of ripening and delayed maturity, proving the soundness of our hypothesis. The effect

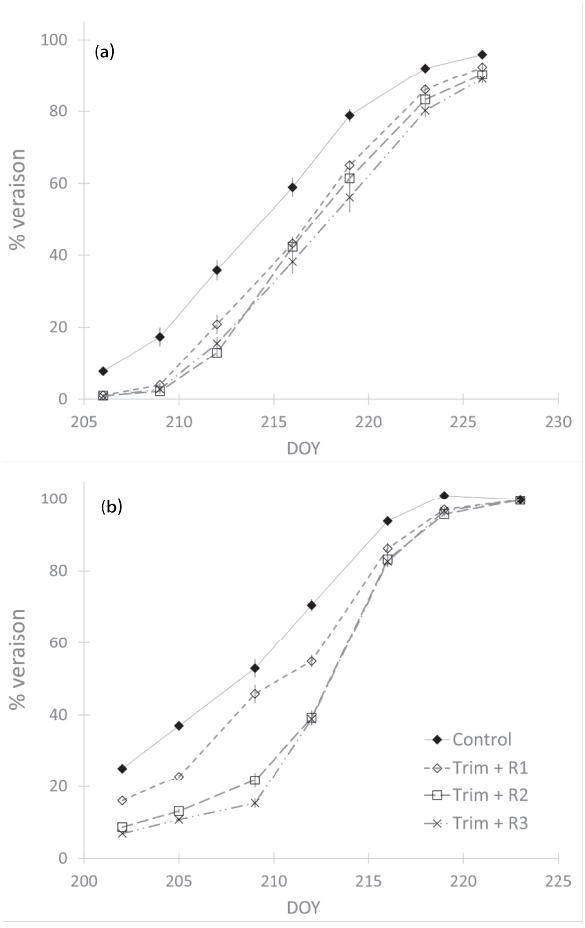


Figure 6. Evolution of veraison percentage in 2014 (a) and in 2015 (b).

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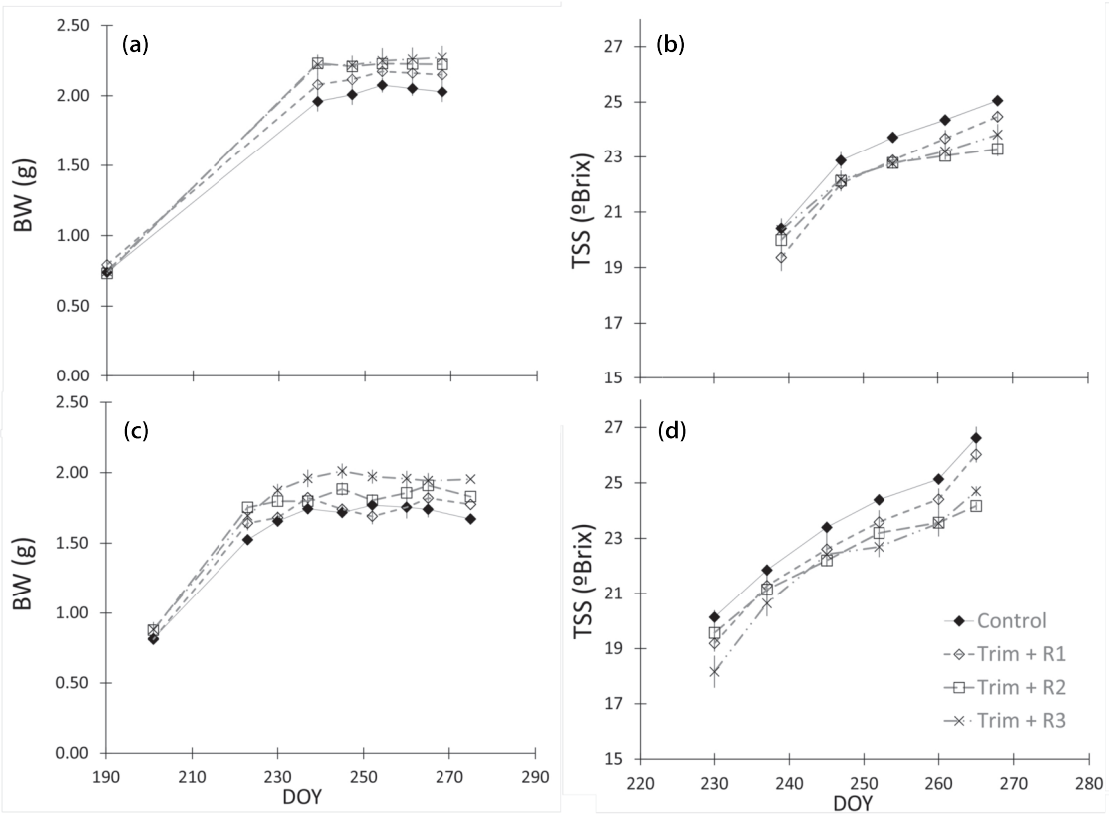


Figure 7. Berry weight (BW) and total soluble solid content (TSS) evolution in 2014 (a, b) and in 2015 (c, d).

of trimming (estimated as a 5-7-day delay) can be explained by a reduced leaf-to-fruit ratio, which, as proved by earlier research, limits plant photosynthesis (Keller *et al.*, 2005; Poni *et al.*, 2013). As a result, yield was also decreased, not due to a reduced berry size, but rather to a lower berry number. Trimming can cause some damage in clusters and, in the second season, decrease cluster and flower differentiation due to an unfavorable carbon balance (Santesteban *et al.*, 2011a; Dayer *et al.*, 2013; Intrigliolo *et al.*, 2016). In fact, the berries of trimmed plants were bigger, probably as a consequence of a lower fruit load and higher plant water availability. Reduced leaf area can certainly decrease water use, especially in cvs. such as 'Tempranillo', known to have poor stomatal control and relatively high night transpiration. However, it should be taken into account that under non-limiting solar radiation latitudes, leaf-to-fruit ratio plays a relatively limited role in carbon balance (Santesteban and Royo, 2006). Wounds resulting from trimming are known to promote a complex response in plants (Schilmiller and Howe, 2005; Delano-Frier *et al.*, 2013; Böttcher *et al.*, 2015). In grapevines, trimming has been shown to modify root hydraulic conductivity through aquaporins and interfere with the normal signaling process (Vandeleur *et al.*, 2014) and could therefore be a factor enhancing stomatal control.

The use of additional irrigation increased the delaying effect of trimming. This delay can be assumed to be due to the enhanced competition of laterals, since higher water availability does not induce by itself a delayed ripening in warm climates, but rather promotes sugar accumulation (Santesteban and Royo, 2006; Chaves *et al.*, 2007; Valdes *et al.*, 2009). The enhanced growth of laterals could be the cause of the higher malic acid content observed, as higher leaf area in the cluster zone reduces cluster temperature (data not shown), which in turn decreases malic acid degradation. However, this does not explain the delayed sugar accumulation in 'trimmed + supplementary irrigation' vines. The observed delay in ripening cannot be due to increased yield, which is known to delay sugar accumulation (Bravdo *et al.*, 1985; Santesteban *et al.*, 2011b), since the yield in Trim+R2 plants was similar to that in control vines, and the delay was remarkable. In all, our results show the potential of lateral growth-promoting techniques as a tool to delay ripening and to adapt to climate change. The originality of our approach is that

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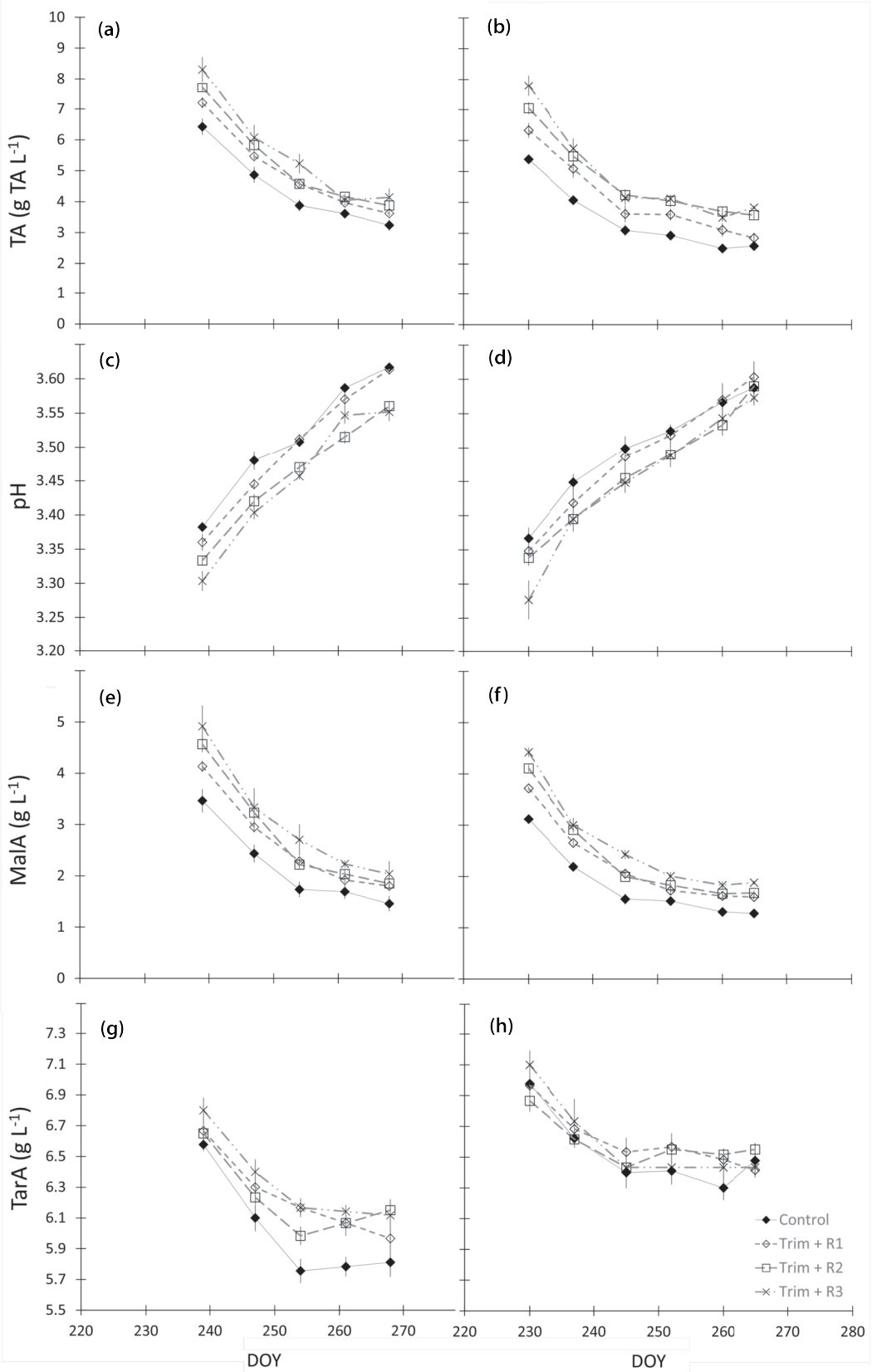


Figure 8. Titratable acidity (TA), pH, and malic (MalA) and tartaric (TarA) concentration evolution in 2014 (a, c, e, g) and in 2015 (b, d, f, h).

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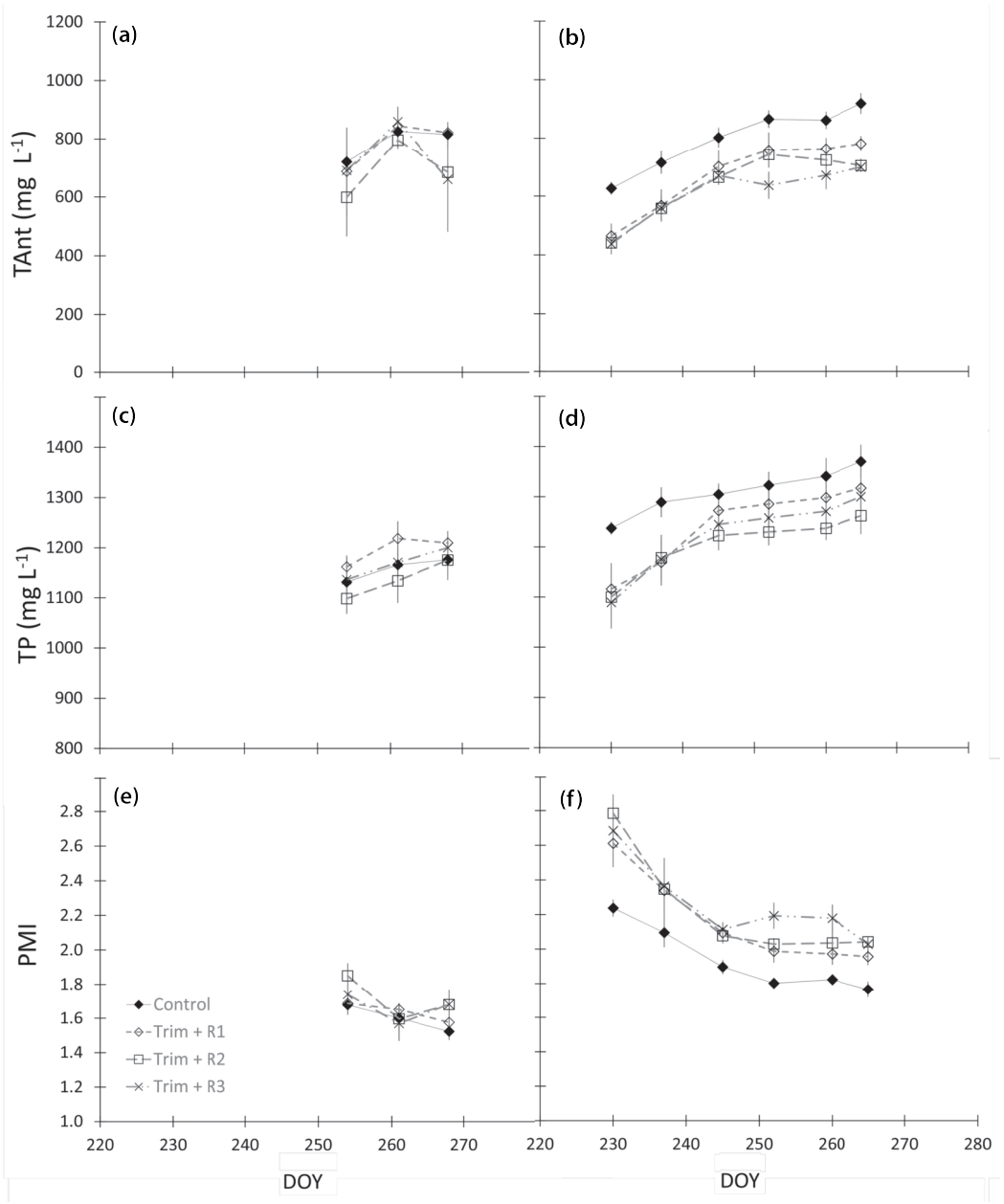


Figure 9. Total anthocyanins (TAnt), total phenolics (TP) and phenolic maturity index (PMI) evolution in 2014 (a, c, e) and in 2015 (b, d, f).

reduced leaf area was combined with enhanced lateral growth.

The results obtained are globally satisfactory since the techniques tested were able to delay ripening. However, it is necessary to test to which extent the effects on ripening balance are favorable from an enological point of view. According to berry ripening dynamics summarized in Figures 8 and 9, berries from trimmed and supplementary irrigated vines had, for a given date, lower sugar content, higher acidity and lower anthocyanin and phenolic content, which can be due to delayed ripening but also to changes in ripening balance. In fact, when we plot grape composition variables against soluble solid content (Figure 10), it can be seen that for a given TSS value, control vines had similar pH, lower titratable and malic acidity, higher anthocyanin content and better phenolic maturity (lower PMI values). Thus, apart from delaying ripening, trimming and trimming + supplemental irrigation intrinsically increased acidity (due to reduced malic degradation), reduced anthocyanin content and resulted in grapes with lower phenolic ripeness. Therefore, it is necessary to be cautious before implementing these techniques at

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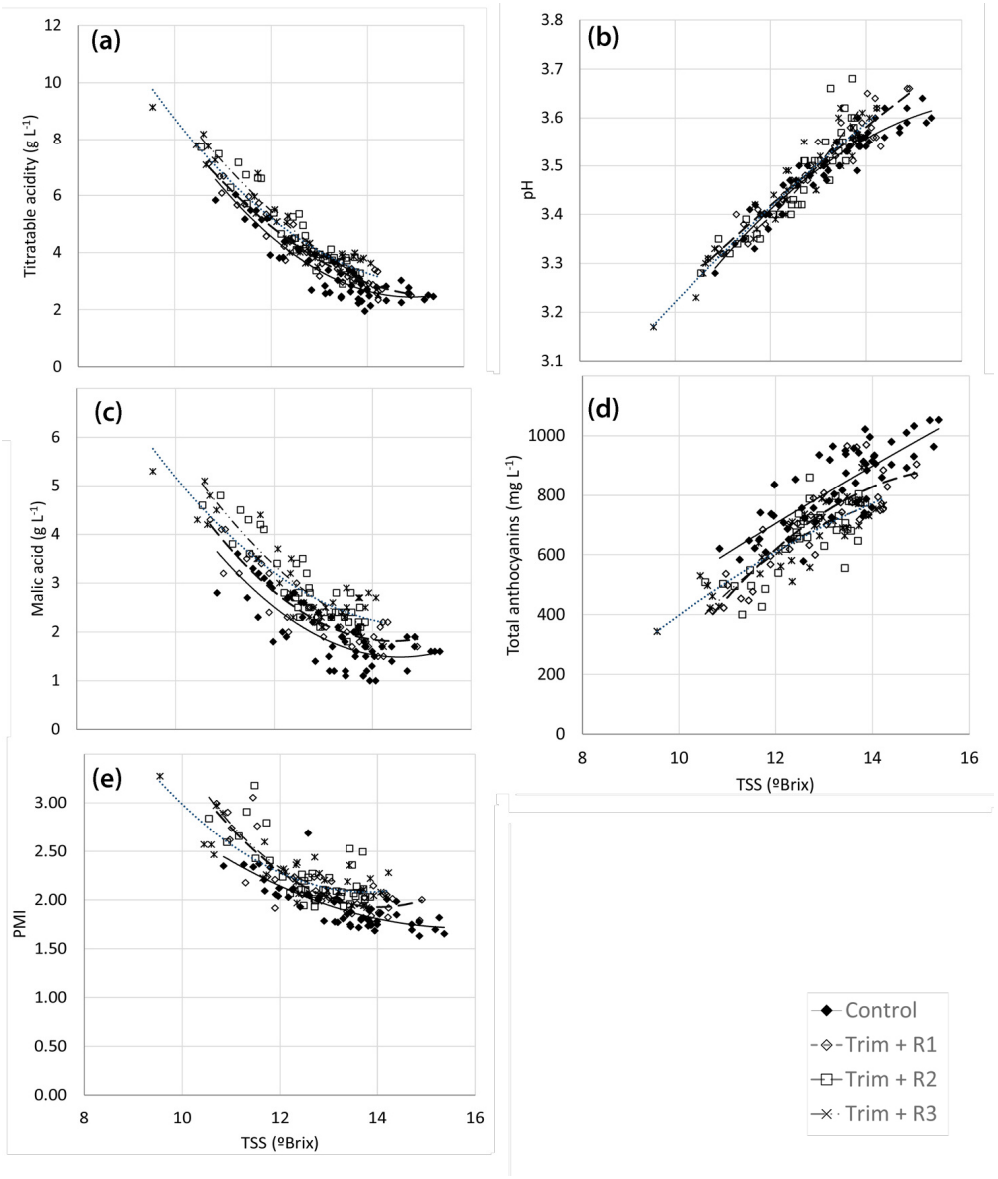


Figure 10. Comparison of total soluble solid content (TSS) and (a) titratable acidity, (b) pH, (c) malic acid, (d) total anthocyanins, and (e) phenolic maturity index (PMI).

a commercial scale. The most positive effect of trimming and increased irrigation is that delayed ripening would allow full ripening under cooler temperatures, more favorable for aroma and phenolic synthesis (Mira de Orduña, 2010). However, if its use implies a decreased anthocyanin and phenolic accumulation, its introduction would not be justified. Besides, in some areas it may not be possible to increase irrigation level (even for a few weeks) due to water scarcity. It is therefore necessary to test the implications of these techniques on the composition and organoleptic properties of the wines produced prior to making any specific recommendation to growers.

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