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Effect of forcing vine regrowth on 'Tempranillo' (*Vitis vinifera* L.) berry development and quality in Extremadura

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Summary

In warmer regions, fruit ripening in the wine grape tends to take place during the hottest part of the growing season. This can have negative consequences on the qualitative characteristics of the grape berries at harvest. Forcing vines to regrow can be an aggressive but effective technique to delay the harvest date, but needs to be evaluated carefully in each growing condition. In 2017, in an experimental vineyard in Extremadura, forcing was conducted 3 (F1 treatment) and 22 (F2 treatment) days after anthesis (May 18 and June 6) by hedging growing shoots to seven nodes and removing summer laterals, leaves and primary clusters. Vines grown using conventional practices were used for the Control treatment. Forcing delayed the harvest date from August 22 (Control) to September 14 (F1) and October 19 (F2). Shifting the berry growth period modified the duration of the different fruit development stages. Compared to the Control treatment, the F1 and F2 berries were smaller at harvest, but had similar skin weight percentages; however, the seed weight percentage of the F2 berries was higher. The differences in grape composition observed at harvest between the various treatments were further accentuated in the wines. At harvest, the F2 berries had significantly higher total polyphenol and anthocyanin content than the Control and F1 berries, which had similar values. In the wines, both F1 and F2 characteristics differed considerably from the Control, most notably in the high F2 tannin concentration. These preliminary results from the first year of study indicate the potential of this technique to obtain wine grapes with very different characteristics, offering new viticultural perspectives in warm climate areas.

Introduction

The global temperature increases associated with climate change have significant effects on agricultural ecosystems at all scales (Howden *et al.* 2007, Stokes and Howden 2010, IPCC 2013, Hughes *et al.* 2015). Viticulture in southern Europe has been repeatedly identified as being especially vulnerable to climate change (Fraga *et al.* 2016, Jones *et al.* 2005, Moriondo *et al.* 2011, Ramos *et al.* 2008, Resco *et al.* 2016). High-quality wine regions in hot climates could be in danger (Moriondo *et al.* 2011, Resco *et al.* 2016) due to increased water needs, lower yield (Van Leeuwen *et al.* 2017), and changes in grape composition that could reduce the quality and typicity of the wine (Duchêne *et al.* 2010, Fraga *et al.* 2016, Resco *et al.* 2016, Van Leeuwen *et al.* 2017, Van Leeuwen and Darriet 2016).

Temperature is widely accepted to be the primary climatic factor that affects the phenology and quality of wine production (Winkler *et al.* 1974, Jackson and Lombard 1993). The effects will be dependent on the thermal regime and the phenological stage. Above-optimal temperatures in winter dormancy produce an early bud break and, consequently, an advance in the start of vegetative growth (Mullins *et al.* 1992). Prolonged periods of high temperatures during flowering and in early berry growth stages can cause premature veraison, grape abscission, enzyme inactivation and less grape flavour (Mullins *et al.* 1992 cited by Jones (2005)). Increases in daytime temperatures during the maturation stage may benefit the synthesis of some compounds such as tannins, sugars and grape flavours in cold climate conditions (Gladstones 1992), but could be harmful in warmer climate conditions. The advance of phenology, as a consequence of the increase in temperatures, is already causing shorter growth cycles with earlier harvest dates (Duchêne and Schneider 2005) and higher grape sugar concentration and pH (Neethling *et al.* 2012, Ramos *et al.* 2008, Tomasi *et al.* 2011, Van Leeuwen and Darriet 2016, Webb *et al.* 2012), increasing the alcohol content in a market that demands less alcoholic wines. It has also been found that elevated temperatures are decoupling the sugar ripening processes and the ripening of phenolic compounds, giving rise to unbalanced wines (Bonada *et al.* 2015, Sadras and Moran 2012).

This vulnerability to the effects of climate change requires high levels of adaptive responses (Howden *et al.* 2003). Growers need to adapt to this situation by delaying the period of grape ripening. This delay can be achieved by choosing varieties or clones of later maturation or the use of more vigorous rootstocks that can also delay ripening for a few days. Other possibilities include adjustments to the trellis systems which can be adapted by increasing the height of the trunk, modifying the distribution of the canopy (Baeza

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et al. 2005, Kliewer and Dokoozlian 2005) or installing direct shading structures (Caravia *et al.* 2016) in order to modify the vine microclimate. Cultivation practices can also be adapted to help modify the date of grape maturity. Such practices include minimum pruning (Martínez de Toda and González 1999), delayed winter spur-pruning (Friend and Trought 2007), intensive shoot trimming (Martínez de Toda and Balda 2013, Santesteban *et al.* 2017, Abad *et al.* 2019), and late cluster zone leaf removal (Poni *et al.* 2013, Buesa *et al.* 2018) although the maximum delay attained was only around two weeks.

The ability of the vine to bear fruit several times in the same year if the buds are forced out of dormancy soon after initiation of the inflorescence primordia as described by various authors (Dry 1987, Fang *et al.* 2000, Lin 1987, Lin *et al.* 1985, Pommer 2006), was used by Gu *et al.* (2012) to delay the berry ripening period from July-August to October-November and its significantly milder temperatures in an experimental 'Cabernet Sauvignon' vineyard located in Fresno, CA, USA. With this forcing technique, smaller-sized berries were obtained as well as musts with a lower pH, greater acidity and a higher content of anthocyanins, tannins and total phenolic compounds in comparison with the unforced vines. However, despite the significant benefits for must composition, there remain doubts about the most suitable phenological stage for the application of this technique, the impact on yield, the medium- and long-term effects on the sustainability of the vines, water needs and the effects of irrigation, as well as the possible variants of the technique itself. Currently, there is very little information to assess the suitability of this technique to minimize the impact of the effects of climate change on grape quantity and quality (Martinez de Toda *et al.* 2019).

The objective of this work is to evaluate the effect on yield components and berry and wine composition of applying, on two different dates, the technique of forcing vines to regrow in a vineyard of the variety 'Tempranillo' in southwestern Spain under semiarid climate conditions.

Material and Methods

Plant material, forcing treatments and experimental design: The study was carried out in an experimental vineyard located at Badajoz, Extremadura, Spain (38° 51' N; 6° 40' W; 198 m) in a 'Tempranillo' vineyard (*Vitis vinifera* L.) grafted on Richter 110 rootstock, trained as bilateral cordons in a vertical trellis system with a drip irrigation system of 4 $L \cdot h^{-1}$ per vine. All the vines were winter pruned to six spurs and two buds per spur. The rows are E-W oriented and row and vine spacing were 2.5 m and 1.2 m, respectively. The experiment considered three treatments, a Control (C) with vines grown under conventional practices (just winter pruning), and 2 forcing dates (F1 and F2) with four replicates per treatment (experimental plots of 4 rows and 18 vines each). A random block experimental design was used. Crop forcing consisted of hedging the growing shoots to seven nodes and removing all the summer laterals, leaves and primary clusters with scissors to force the budbreak of the first bud developed in the current season. Forcing was applied in 2017 three days after anthesis in F1 (May 18) and 22 d after anthesis (June 6) in F2.

The Control treatment was irrigated replacing 100 % of the ETc using the Kc recommended by FAO for this latitude and depending on the phenology of the vine. The F1 and F2 treatments were also irrigated replacing 100 % of the ETc, but in this case the ETc was calculated using a weighing lysimeter integrated in one of the four replicates of treatment F1.

Vine phenology, yield and berry components: Phenology monitoring was performed weekly according to the modified E-L system (Coombe 1995). Starting from mid-March ("cotton bud" stage), a visual inspection was made of ten plants per plot to determine the most representative growth stage (the stage shown by at least 50 % of plants) as well as the most backward and the most advanced stages in the sample.

In each treatment, the vines were manually harvested when the must concentration of total soluble solids (TSS) reached 23-24 °Brix (a common harvesting criterion for this variety in this area), considering the average of the four elementary plots. Control vines were harvested on August 22, F1 on September 12 and F2 on October 17 in 2017. All the clusters of 10 control vines per experimental plot were counted and weighed and 5 clusters per plot were collected at random to establish the number of berries per cluster. To characterize berry morphology, a sample of 20 berries per experimental plot (80 berries per treatment) were frozen (-80 °C). To determine the relative distribution of berry dry mass components, the berries were subsequently separated into pulp, skin, seeds and pedicel by measuring the dry weight of each part after drying at 60 °C in a dry oven to constant weight.

Grape composition: Samples of 300 g of berries per plot were collected randomly each week from veraison to harvest. In the laboratory, the grapes were destemmed and then crushed and homogenised in a TM-31 Thermomix blender (Vorwerk, Wuppertal, Germany) at speed setting 3 for 1 min. An aliquot of the mash (pulp, juice, skins and seeds) obtained was filtered and used to determine technological parameters. The TSS (°Brix) was determined by refractometry (ATR ST plus, Schmidt + Hansch, Berlin, Germany), and pH and titratable acidity (TA, $g \cdot L^{-1}$) (Crimson Micro pH-metre, Barcelona, Spain) according to ECC formal methods (ECC, 1990). Tartaric and malic acid content ($g \cdot L^{-1}$), ($g \cdot L^{-1}$) were enzymatically analyzed according to ECC formal methods (ECC 1990) using an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

Phenolic compounds were extracted following the methodology previously described by Kontoudakis *et al.* (2010) with some modifications. A second aliquot of homogenate per repetition (25 g) was macerated for 16 h at 22-24 °C in oxalic acid buffer 0.3 M (pH 1.0; 25 mL). The macerated samples were centrifuged at 4°C for 10 min (Allegra 25R, Beckman Coulter). The extraction process was carried out in triplicate. Total polyphenol content (TPP) was determined according to Singleton and Rossi (1965), and total anthocyanin (TAN) content was quantified by the pH differential method (Lee *et al.* 2005). All determinations were carried out using an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

Microvinifications and wine analysis: At harvest, 60 kg of clusters from the central vines of each elementary plot were destemmed and crushed. A 50 L steel tank was filled to two thirds with this mash, which was then fermented at 22-24 °C. Total SO_2 was added to the mash (50 mg·kg^{-1}), which was inoculated with a commercial yeast strain of *Saccharomyces cerevisiae* (Viniferm 3D, Agrovin, Spain) at 25 g·hL^{-1}. Fermentation was monitored daily, measuring density and total phenolic index (TPI) by spectrophotometric absorbance at 280 nm (UV/visible UV-1700 spectrophotometer, Shimadzu, Shimadzu Corporation, Kyoto, Japan). The must was racked when the increase in TPI levelled off. Once fermentation was completed, the wines were settled at 4 °C and sulphur content was then added to the wine to achieve 30 mg·L^{-1} of free SO_2. Finally, the wines were bottled and stored at 15 °C until analysis.

Wine analysis was carried out four months after bottling. Wine alcohol content (% v/v) was analysed according to ECC methods (ECC 1990), and pH, titratable acidity, and tartaric and malic acid content were analysed as in the must determinations. Wine TPP content was determined according to Singleton and Rossi (1965) and TAN content was quantified by the pH differential method (Lee *et al.* 2005) using an autoanalyzer (Y15, Biosystems, Barcelona, Spain).

HPLC separation, identification and quantification of anthocyanins were performed on an Agilent 1200 LC system (Agilent Technologies, Palo Alto, CA) equipped with a degasser, quaternary pump, column oven, 1290 infinity autosampler, UV-Vis diode-array detector (DAD) and the Chemstation software package for LC 3D systems (Agilent Technologies) to control the instrument and for data acquisition and analysis. Separation was performed in a Kromasil® 100-5-C18 (250 x 4.6 mm) column (AkzoNobel, Bohus, Sweden). The analysis was carried out as described in Pereira Natividade *et al.* (2013) with slight modifications to improve peak resolution. For the analysis of anthocyanins, a 10 mL extract was injected directly into the HPLC and the column was maintained at 40 °C. The mobile phase consisted of a gradient mixture of a solvent A (0.85 % phosphoric acid solution) and solvent B (acetonitrile), with a flow rate of 1 mL·min^{-1}. The gradient was started with 100 % of solvent A and adjusted for 90 % of solvent A and 10 % of solvent B at 10 min; 85 % of solvent A and 15 % of solvent B at 20 min; 80 % of solvent A and 20 % of solvent B at 30 min; 67 % of solvent A and 33 % of solvent B at 40 min; 65 % of solvent A and 35 % of solvent B at 45 min; and 100 % of solvent B at 55 min. Absorbance at 520 nm was measured by the DAD detector for identification of anthocyanins by their elution order and by comparison to the retention times of commercially available standards (malvidin-3-glucoside, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside and peonidin-3-O-glucoside (Extrasynthese, Genay, France)). All measures were expressed in mg malvidin glucoside·L^{-1}.

The anthocyanins present in extracts were identified as the monoglucoside forms (3-glu) of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv); the acetylglucoside forms (DpA, CyA, PtA, PnA, and MvA), and the p-coumaroyl-glucoside forms (DpC, CyC, PtC, PnC, and MvC).

Statistical data analysis: The data were statistical analysed by one-factor ANOVA (IBM SPSS 20) and the Tukey-b test. Differences between means were considered statistically significant when $p < 0.05$.

Results

Vine phenology, yield and berry components: Natural bud break occurred on April 3 for all treatments, while the onset of anthesis occurred on May 16 in C and, after forcing, on June 26 in F1 and July 13 in F2. Veraison occurred on July 6 in C, and on August 9 and September 13 in F1 and F2, respectively. The two forcing treatments shifted and shortened the full vegetative cycle (from the regrowth of buds to harvest in F1 and F2) compared to the Control vine cycle (bud break to harvest; Fig. 1). In F1, the harvest was delayed 23 d compared to C, while in F2 the delay was 58 d. This displacement situated berry ripening in a period of lower temperature. The average temperatures recorded from bud break to veraison were 22.6 °C, 25.2 °C and 25.6 °C for C, F1 and F2 respectively, and 26.0 °C, 24.9 °C and 21.0 °C from veraison to harvest. The pre-veraison stage was consequently shortened from 94 d in C to 83 d in F1 and the post-veraison stage from

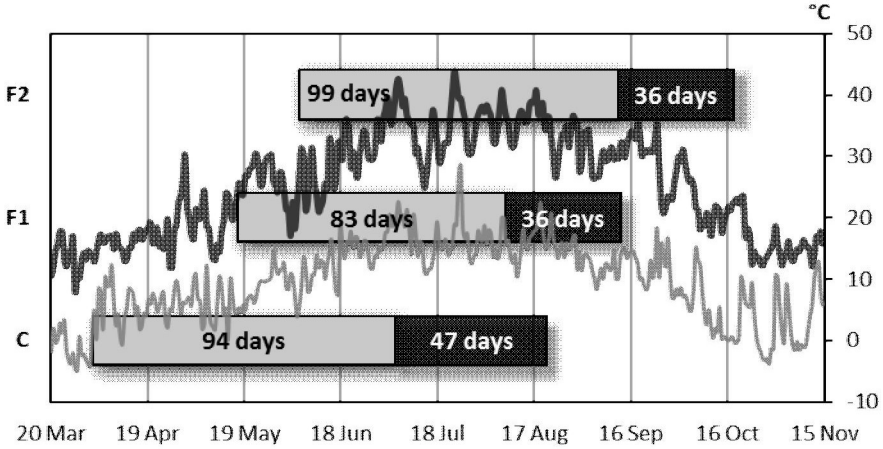

Fig. 1: Duration of the vegetative cycle in the different treatments: from natural bud break to harvest in the Control and post-forcing bud break to harvest in the F1 and F2 treatments. Grey bars from bud break to veraison and black bars from veraison to harvest. Black line corresponds to maximum and grey line to minimum daily temperature.

Table 1

Yield and its components and relative distribution of berry dry mass: pulp, skin, seeds and pedicel

Treatment	Clusters per vine	Cluster weight (g)	Berry weight (g)	Yield (kg·ha^{-1})	% Pulp	% Skin	% Seeds	% Pedicel
C	16.25 c	240.63 a	1.86 a	12952.50 a	73.42 a	15.01	10.34 b	1.23 b
F1	24.90 b	81.07 b	1.06 b	6471.70 c	73.43 a	14.09	10.60 b	1.88 a
F2	36.38 a	77.07 b	1.06 b	9216.67 b	68.72 b	15.57	13.65 a	2.06 a

Statistical analysis: one-factor ANOVA and Tukey-b test (both $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments.

47 d in C to 36 d in both F treatments. Average berry and cluster weight were significantly higher in C and similar between F1 and F2 with berry weight of 1.06 g and cluster weight lower than 100 g (Tab. 1). The number of clusters per vine was higher in F1 (24.90) and, especially, F2 (36.38) compared to C which had only 16.25 clusters per vine. Yield was lower in F1 and F2 compared to Control and yield in F2 was higher than in F1.

Tab. 1 shows the effect of bud forcing on yield, yield components and berry relative distribution of dry mass. F1 had a lower berry weight than C, but with a similar weight distribution of pulp, skin and seeds. However, in F2, with a berry weight similar to F1, the proportion of pulp was lower and the proportion of seeds higher than in C and F1.

Grape composition: The evolution of total anthocyanin (TAN) content and TSS throughout ripening is shown in Fig. 2. The highest values of TAN (at phenolic maturity) in the grapes of the forcing treatments (F1 and F2) were reached at lower TSS values than in C, and remained more stable after that.

Tab. 2 shows grape composition at harvest for each treatment. Grape TSS content was similar in all three treatments. No effects of the forcing treatments were observed on berry pH or malic acid concentration, but F2 showed higher titratable acidity and tartaric acid concentration. The

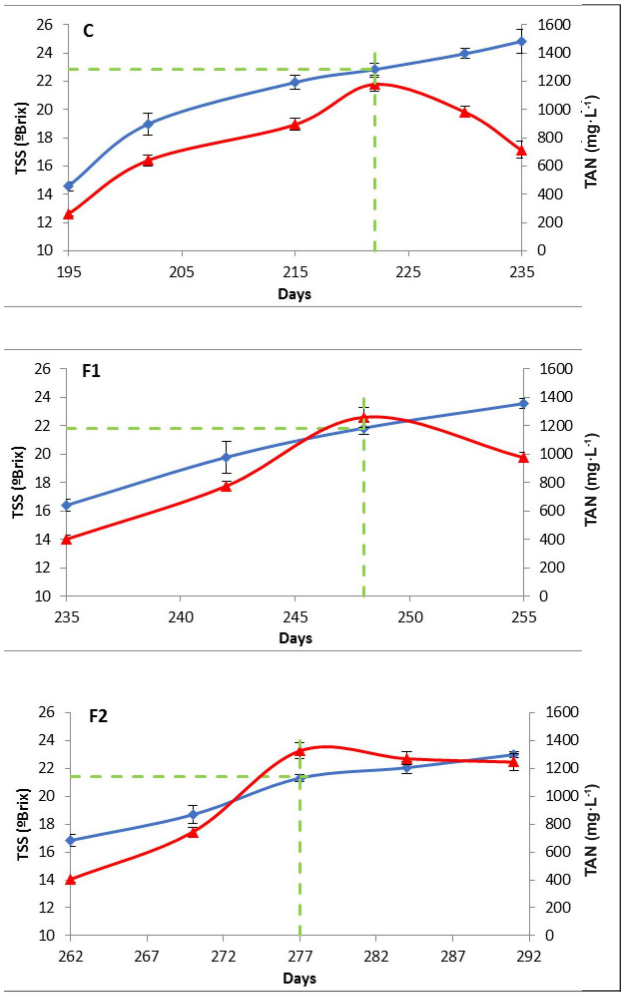

Fig. 2: Post-veraison evolution of TSS (blue) and TAN (red) in grapes of C, F1 and F2 treatments. Each point represents the average of 4 experimental plots and the bars represent the standard error of the mean.

Table 2

Effect of forcing on grape composition at harvest and phenolic maturity

Date of sampling	Parameter	Treatment C	F1	F2
Harvest	Day	235	255	291
	TSS [a] (°Brix)	24.8	24	23.8
	pH	3.9	3.9	3.6
	TA [b] (g·L^{-1})	4.4 b	4.3 b	5.6 a
	Malic ac. (g·L^{-1})	2.0	2.6	2.6
	Tartaric ac. (g·L^{-1})	3.9 ab	3.5 b	4.4 a
	TPP [c] (mg·L^{-1})	2141.8 b	2117.7 b	2882.5 a
	$TPP_{d.s.}$ (mg·g^{-1} dry skin)	48.8 b	56.0 b	74.4 a
	TAN [d] (mg·L^{-1})	713.8 c	974.7 b	1247.2 a
	$TAN_{d.s.}$ (mg·g^{-1} dry skin)	16.3 c	25.8 b	32.2 a
Phenolic maturity	Day	222	248	277
	TSS [a] (°Brix)	22.8 a	21.8 ab	21.3 b
	pH	3.8 a	3.6 b	3.4 c
	TA [b] (g·L^{-1})	5.5 b	6.1 b	7.9 a
	Malic ac. (g·L^{-1})	3.0	3.1	3.8
	Tartaric ac. (g·L^{-1})	5.3	4.5	5.1
	TPP [c] (mg·L^{-1})	2957.5 ab	2707.0 b	3280.5 a
	TAN [d] (mg·L^{-1})	1178.7	1257.8	1327.5

Statistical analysis: one-factor ANOVA and Tukey-b test (both $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments. (a) TSS: total soluble solids; (b) TA: titratable acidity; (c) TPP: total polyphenols; (d) TAN: total anthocyanins; $TPP_{d.s.}$ total polyphenols referred to dry skin; $TAN_{d.s.}$ referred to dry skin.

Table 3

Effect of forcing vine regrowth on wine composition

Treatment	Alcohol content (% v/v)	pH	Volatile acidity ($g \cdot L^{-1}$)	Malic acid ($g \cdot L^{-1}$)	Tartaric acid ($g \cdot L^{-1}$)	TPP [a] ($mg \cdot L^{-1}$)	TAN [b] ($mg \cdot L^{-1}$)	Catechins ($mg \cdot L^{-1}$)	Tannins ($mg \cdot L^{-1}$)
C	14.8	4.1	0.4 a	2.0 b	1.8	1581.9 b	324.1 b	995.7 b	1330.7 b
F1	14.3	4.0	0.2 b	2.9 a	2.4	2013.9 a	467.9 a	1448.7 ab	1720.9 ab
F2	13.8	4.0	0.2 b	2.8 a	2.4	2212.0 a	481.1 a	1752.1 a	1995.9 a

Statistical analysis: one-factor ANOVA and Tukey-b test (both $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments. (a) TPP: total polyphenols; (b) TAN: total anthocyanins.

must TAN and TPP contents in F1 and F2 were significantly higher than in C, and TPP content was significantly higher in F2 than in F1 and C. These increases were due to a higher synthesis of these compounds as evidenced by the higher values of polyphenols and anthocyanins from the dry skins ($TPP_{d.s.}$ and $TAN_{d.s.}$).

Tab. 3 shows the effects of forcing on wine composition. There were major differences between the Control and F1 and F2 wines. The wines from the forcing treatments had higher TPP and TAN concentrations, as well as higher catechin, tannin and malic acid values, while volatile acidity was lower. The other parameters showed no statistically significant differences in the FI and F2 treatments compared to C. The F1 and F2 treatments showed similar wine composition, although with tendencies to higher values of TPP, TAN, catechins and tannins in F2. The different polyphenolic composition of the wines had an impact on their sensory characteristics, since the tasters defined F1 and F2 wine as more astringent and of higher intensity of colour (data not shown) and were defined as "greener wines".

Tab. 4 shows that the distribution of the different anthocyanidins did not vary for the three treatments, with malvidin 3-glucoside (Mv) the highest anthocyanidin in all treatments. Nevertheless, F1 and F2 had higher anthocyanin content than C. Moreover, F1 had the highest acetylated anthocyanin and the lowest rutinoside content of the three treatments.

Table 4

Effect of forcing vine regrowth on anthocyanin profile of 'Tempranillo' wines

Compound* ($mg \cdot L^{-1}$)	Treatment		
	C	F1	F2
Mv	88.34 b	122.37 a	115.93 a
Pt	13.79 b	21.60 a	24.27 a
Dp	8.21 c	13.71b	19.84 a
Pn	3.86 c	5.82 b	9.06 a
Cy	0.22 c	0.83 b	1.80 a
∑Monoglucoside	114.02 b	163.93 a	170.54 a
MvC	20.84 a	21.55 a	14.31 b
PtC	3.33 b	4.92 a	3.47 b
PnC	2.05 b	2.86 a	2.00 b
CyC	1.06 b	1.69 a	0.96 b
∑Coumaroyglucoside	26.99 b	30.73 a	20.44 b
MvA	2.85 b	4.04 a	2.86 b
PtA	0.36 c	1.88 a	1.32 b
DpA	0.95 b	1.35 a	1.04 b
PnA	0.50 b	0.93 a	0.63 b
CyA	0.48	0.53	0.49
∑Acetylglucoside	4.74 b	8.33 a	5.95 b
PnR	2.19 a	1.26 b	2.17 a
CyR	0.32	0.36	0.37
∑Rutinoside	2.42 a	1.53 b	2.44 a
∑Total anthocyanin	147.87 b	204.22 a	199.08 a

Statistical analysis: one-factor ANOVA and Tukey-b test (both $p < 0.05$). Different letters indicate the existence of statistically significant differences between treatments. *Mv: malvidin-3-glucoside; Pt: petunidin-3-glucoside; Dp: delphidin-3-glucoside; Pn: peonidin-3-glucoside; Cy: cyanidin-3-glucoside; MvC: malvidin-3-glucoside coumarate: PtC: petunidin-3-glucoside coumarate; PnC: peonidin-3-glucoside coumarate; CyC: cyanidin-3-glucoside coumarate; MvA: malvidin-3-glucoside acetate; PtA: petunidin-3-glucoside acetate; DpA: delphidin-3-glucoside acetate; PnA: peonidin-3-glucoside acetate; CyA: cyanidin-3-glucoside acetate; PnR: peonidin-3-rutinoside; CyR: cyanidin-3-rutinoside.

Discussion

Forcing could be a useful tool in warm vineyard areas to delay the maturation cycle of the vineyard to a period in which the temperatures are more favourable for fruit ripening (Gu *et al.* 2012, Martinez de Toda *et al.* 2019). However, the delay in ripening to milder temperatures depends on the forcing date. In this study, F1 delayed ripening for 23 d but no decrease in temperature was observed during ripening compared to the control. The lowest temperature was attained during the grape ripening for the second forcing date (F2). The results in this study indicate that choosing the correct date on which forcing is to be applied is essential to promote more favourable environmental conditions for grape ripening; much, however, will depend on the climatic conditions of the year.

The number of clusters per vine increased when the forcing treatment was applied compared to the Control treatment. Between both forced treatments, the number of clusters per vine increased in F2 probably because the earlier forced (F1), induced the regrowth of the less fertile prompt buds, instead of the regrowth of dormant buds. In F2 the lateral shoots from prompt buds were eliminated and the number of dormant buds (more fertile) re-grown were greater than in F1. An increase in the number of clusters

was also observed in some forcing treatments in the second year of the trial undertaken by Gu *et al.* (2012) for 'Cabernet Sauvignon'. However, Martinez de Toda *et al.* (2019) in 'Tempranillo' had a similar or lower number of clusters per vine in the forcing treatments with respect to conventional pruning. These differences observed with the same variety were probably due to the fact that in the latter study forcing was done leaving 2-3 buds per shoot whereas in this experiment shoots were forced to 7 nodes.

The few previous studies indicate a decrease in yield with this technique, similar to this experiment, due to a lower number of berries per cluster and smaller berries (lighter clusters) compared to the Control. The loss of yield in F1 versus F2 could be due to the reduction in bud fertility when the forcing technique is applied at a date close to flowering. This was also observed by Gu *et al.* (2012) and Martinez de Toda *et al.* (2019). This adverse effect on yield could be interesting in vigorous varieties subject to regulation with yield limitation. In our study, F2 productivity was close to the limit established under the AOC Ribera del Guadiana certification system for red grapes (10,000 kg·ha^{-1}). The effect of forcing on yield components was similar to that found in previous works: lower berry weight, lower cluster weight and higher number of clusters (Gu *et al.* 2012. Martínez de Toda *et al.* 2019).

According to the results of Martínez de Toda *et al.* (2019) and Gu *et al.* (2012), and those presented in this paper, the date (or rather the phenological state) on which the forcing takes place plays an important role in the results obtained and is also variable between years. Although only two forcing dates were tested in this study, which may seem few given the importance that the date can have on the results, a total of 5 forcing dates were tested in the same vineyard in a parallel work (data not published). These dates ranged from one week before flowering until July 18, and it was observed that the first dates produced a reduced delay in the date of harvest and that applying the forcing treatment after June 26 resulted in the grapes not reaching maturity. The lack of ripening in the later forcing dates was a response that was also observed in the previously mentioned articles.

Application of the forcing technique entails an increase in production costs and the results show that yield is reduced. It is therefore clear that application of this technique must be supported by a considerable and positive change in grape characteristics and its oenological potential. After forcing, the ripening period was completed in a fewer number of days; however, a decrease was observed in average temperature in this period of 1.1 °C in F1 and 5 °C in F2 compared to control. The lower temperatures increased the biosynthesis and accumulation of anthocyanin compounds and, consequently, anthocyanin content was highest in F2. The F1 treatment also had a higher anthocyanin content than the control (Tab. 2). Tian and Gu (2019) also found higher anthocyanin content in grapes of forced vine regrowth treatments for 'Cabernet Sauvignon' cultivated in California. The increase in total polyphenol and anthocyanin content was due to higher synthesis, since the proportion of berry skin was similar in all treatments (Tab. 1), and not to the smaller berry size in F1 and F2 (Ojeda 2002). These pigments accumulate in berries in response to environmental factors such as temperature or light (Buttrose *et al.* 1971, Kliewer 1977). It seems that the meteorological conditions during the ripening of F1 and F2 improved their synthesis, accumulation and stability. The forcing technique also modified the dynamics of anthocyanin accumulation in the berry, with greater stability in anthocyanin content observed after reaching its maximum concentration. This difference may be due to the displacement of the harvest dates to a period of more favourable temperatures in F2 (Mori *et al.* 2007, Mira de Orduña 2010, Sadras and Moran 2012). Fig. 2 shows that the decoupling effect of anthocyanin and sugar accumulation observed in the control treatment decreased in the case of F1 and F2. When the maximum polyphenol content was reached in these latter two treatments (phenolic maturity) the TSS concentration was lower than in the Control (Tab. 2). Wine composition was also modified by the techniques applied; the chemical composition of the grape was reflected and, in some aspects, increased in the wines.

Global warming is causing an increase in alcohol content in wines (Palliotti *et al.* 2014), but application of the forcing technique used in the present study can help to obtain wines with lower alcohol content, high polyphenol and anthocyanin content, and higher malic and tartaric acid concentrations (Tab. 3).

The control wines are similar to the 'Tempranillo' wines described in previous works, from the same (Valdés *et al.* 2009, Gamero *et al.* 2014) and other areas (Intrigliolo and Castel 2010). Both berry and wine composition were modified by forcing vine regrowth. Differences were found between the control wines (elaborated from Control grapes) and the F1 and F2 wines (elaborated from F1 and F2 grapes, respectively). Compared to the control treatment, volatile acidity was lower and malic acid content higher in F1 and F2 (Tab. 3). As expected, the general trend was for a higher phenolic compound content in F1 and F2 than in the control. Catechin and, especially, tannin contents were notably higher in the F1 and F2 wines. The presence of these compounds is necessary for the aging of wines. Since flavan-3-ols compounds like catechins and tannins are distributed principally in seeds and to some extent in the skin, their higher content in the F2 wines (Tab. 3) could be explained by the higher seed % in the F2 berries (Tab. 1).

In an interesting study, Monagas *et al.* 2006 showed that chromatic attributes of red wines could be predicted by their phenolic profile using polynomial regression techniques. The substances which provided the best fitting model in that study were the anthocyanin compounds. Therefore, it is interesting to analyse the effect of this technique on the anthocyanin profile of the wines. Tab. 4 shows that, as previously found by Tian and Gu (2019) in 'Cabernet Sauvignon' grapes from California, the response of anthocyanin compounds was likely linked with their acylation status and the associated anthocyanidin since the response to the forcing treatments had a higher repercussion in the non-acetylated than in the acetylated anthocyanins. The coumaroyl and acetyl glucoside compounds also increased in the F1 treatment compared to the control and F2 treatments. The increase in coumarate forms is particularly important, as these are more stable than the monoglucosides and offer greater stability to wine colour (Heller *et al.* 1996,

Degenhardt *et al.* 2000, Ribéreau-Gayon *et al.* 2000). The relevance of these findings can also be seen in the study by Gamero *et al.* (2018), who reported that the colour intensity of 'Tempranillo' wines from this area was high and positively correlated with the presence of monoglucosides, coumaroyl derivatives and delphinidin and petunidin compounds. Indeed, in our study, the F1 and F2 wines showed a higher colour intensity than that of the control wines (5.3, 5.8 and 3.7, respectively: data not shown). Finally, the increased total anthocyanin and tannin content in F1 and F2 compared to Control will have increased polymerization reactions, improving anthocyanin stabilization.

It should be noted that this preliminary paper does not consider certain key aspects that may determine the practical interest of this technique. Such aspects include, among others, the most appropriate training system, mechanization of the process to reduce costs, the incidence of pests and diseases, and the medium- and long-term effect on the vineyard. The main focus of this study was to evaluate the analytical and productive results of one year's application of the technique, since it is the oenological aspects which will undoubtedly determine its future applicability and the potential benefits of any future studies.

Conclusions

The forcing technique was effective in delaying the grape ripening period, but only in the case of the latter of the two tested forcing dates did this delay lead to a lower average temperature during ripening. The suitability of the forced date seems very dependent on the meteorological characteristics of the current season and longer-term studies will be necessary. The application of this technique meant a significant loss of production and it will be necessary to increase the bud number per vine to reduce this effect. At harvest, crop forcing resulted in changes of berry characteristics, with a higher phenolic compound content in F1 and F2 than in the control that were accentuated in the wines with positive effects on acidity, polyphenolic and anthocyanin compounds and tannins. With respect to the anthocyanins profile of the wines, the response to the forcing treatments had a higher repercussion in the non-acetylated than in the acetylated anthocyanins. Finally, the intensity of colour of crop forcing wines was higher than control wine.

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