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Research Note

Minimal Pruning as a Tool to Delay Fruit Maturity and to Improve Berry Composition under Climate Change

Wei Zheng,[1]* Vittorio del Galdo,[2] Jesús García,[1] Pedro Balda,[1] and Fernando Martínez de Toda[1]

[1]Instituto de ciencias de la vid y del vino, ICVV (Universidad de La Rioja, CSIC, Gobierno de La Rioja), Finca La Grajera, Autovía del Camino de Santiago LO-20 Salida 13, 26007 Logroño, La Rioja, Spain; and [2]Montpellier SupAgro, 2 place Pierre Viala, 34060 Montpellier, France.

*Corresponding author (zheng1987wei@gmail.com; tel: +34-625893463; fax +34941299721)

Wei Zheng is Master in Agronomy, Phd candidate in Viticulture; Vittorio del Galdo is Master in Horticulture; Jesús García is Master in Agronomy; Pedro Balda is Phd in Oenology; Fernando Martínez de Toda is Phd in viticulture and Full Professor of Viticulture.

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Abstract: Minimal pruning (MP) is considered a viable technique to reduce labor costs and produce high quality wine grapes. To evaluate its effects on grapes cultivated in warm areas, a long-term study on Tempranillo (*Vitis vinifera* L.) was conducted in Badarán (La Rioja, Spain). For each vintage between 1999 and 2013, grapes from MP vines and those conventionally hand pruned (CHP) were evaluated for yield and total soluble solids (TSS). On this basis, from 2014, a further study was initiated in which grapes were analyzed at the same TSS to verify the effects of MP on fruit maturation and to determine the effects of MP on fruit quality. The long-term study showed that MP increased yield by 56% and reduced TSS by 9% compared to CHP. Results from 2014 and 2015 demonstrated that MP delayed fruit maturity (22 Brix) by ≈ 17 days. At the same TSS level (22 Brix), MP had lower berry weight by 24% and cluster weight by 57%, and increased yield by 51%. Must from MP fruit had higher total anthocyanin concentration

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(+17% in 2014 and +21% in 2015). However, this improvement in potential wine color was more likely due to smaller berry size rather than higher anthocyanin synthesis per unit area of berry skin. The study indicates that MP can effectively delay berry ripening and help to improve potential wine color.

Key words: minimal pruning, ripening delay, anthocyanin, climate change, berry quality

Introduction

Climate change models predict an average warming in global wine producing regions of 2°C in the next 50 yr (Jones et al. 2005). Under this trend, the greatest problems faced by the wine industry are a decoupling of phenolic and technological maturities of grapes, and excessively high alcohol contents in wine, especially in warm areas such as in Spain (Martínez de Toda et al. 2013).

Anthocyanins are an important component of red wine grape quality. In most cases, factors that favor carbohydrate accumulation also contribute to anthocyanin synthesis, especially in the first 5 weeks after veraison when this correlation is high (Pirie and Mullins 1977). However, high temperatures during berry development can delay the onset of anthocyanin accumulation ultimately leading to low levels at harvest (Sadras and Moran 2012). During berry maturation high temperatures can also cause inhibition of some key biosynthesis enzymes as well as anthocyanin degradation (Mori et al. 2005, 2007). In addition, high temperatures can accelerate grapevine phenological stages (Keller 2010) leading to a decoupling of phenolic and technological maturities (i.e. sugar concentration, titratable acidity and pH of the grape juice). While sugar accumulation becomes earlier and more rapid during a warmer period of the

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growing season phenolic accumulation is inhibited and berry anthocyanin concentration may not reach a desirable level at harvest. The combination of high TSS and low acidity can produce high-alcohol, unbalanced wine.

For an established vineyard, the negative effects of global warming on fruit maturation could be mitigated by adopting cultural techniques that delay maturation, such as shoot trimming (Martinez de Toda et al. 2013, Palliotti et al. 2014), post-veraison distal leaf removal (Palliotti et al. 2013), late winter pruning (Palliotti et al. 2014), double pruning (Gu et al. 2012), and minimal pruning (MP). Research over 30 years in Australia showed that traditional severe pruning was unnecessary in some viticultural regions and can produce wines of low quality due to the development of shaded, tight clusters with large berries, and difficulties in the control of pests and diseases (Clingeleffer 2010). MP, in most cases, produces higher yields than does hand pruning (Martinez de Toda and Sancha 1998, Morris and Cawthon 1981, Reynolds 1988, Schultz and Weyand 2005), and improves canopy light and vine health conditions by reducing vine vigor (Archer and van Schalkwyk 2007, Clingeleffer 2010). Low bud fruitfulness, small clusters and low berry weight are yield components associated with MP (Bates and Walter-Peterson 2008). While MP can save labor and reduce management costs, it does not perform well for some late ripening cultivars, especially in cool and high-rainfall areas (Schwab 2005). Without crop adjustment, MP tends to over-crop leading to delayed or insufficient ripening (Bates and Morris 2009, Morris and Cawthon 1981), although this effect can be lessened by trimming low-hanging fruiting canes or by applying mechanical crop thinning 20-30 days after bloom (Poni et al. 2000). The propensity for MP to delay maturation could, on the other hand, make it effective for counteracting the effects of climate warming, leading to enhanced accumulation of berry

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anthocyanins and acidity maintenance. Archer and van Schalkwyk (2007) found that MP resulted in better color in berry skins and in wines with a similar alcoholic level. Holt et al. (2008) found that grapes from mechanically-pruned vines consistently had higher anthocyanin concentration and content than those from cane or spur pruned vines. Based on 30 years of experience in Australia, Clingeleffer (2010) concluded that grapes from minimally pruned vines generally produce greater wine color. In contrast, Morris and Cawthon (1981) found that continuous mechanical pruning led to low TSS and poor color. Similarly Rousseau et al. (2012) found a lower color intensity in wines from MP vines than traditionally-pruned vines.

The main goal of this study was to evaluate the effects of MP on delaying grape maturity under the conditions of the La Rioja Valley in northern Spain. Another goal was to assess the effects of MP on fruit quality including the relationship between berry anthocyanin and TSS.

Materials and Methods

Plant material and growth conditions. The study was conducted in a commercial vineyard of *Vitis vinifera* cv. Tempranillo located in Badarán (42°22′4.4″N, 2°48′33.2″W, 620 m.a.s.l.), La Rioja in northern Spain. The vineyard was planted in 1986, on 41-B rootstock. Spacing was 1.1 x 2.6 m (vine x row) in north-south oriented rows with a density of 3500 vines/ha. The minimal pruning (MP) treatment was applied to vines that originally had a spur pruned free-horizontal cordon (without shoot positioning) at a height of 150 cm, but had not been pruned since 1996. Every 3 or 4 yr, MP vines were subjected to a regular shape maintenance by mechanical trimming to prevent shoots from contacting to the ground and from excessive extension. The most recent trimming was carried out in the summer of 2015. The control

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(conventional hand pruning, CHP) vines were trained in the traditional gobelet (2-3 arms per vine) and were pruned to 12 buds per vine. The vineyard was subjected to the common viticultural practices in the region. Original climatic data was provided by the nearest meteorological station situated in Villar de Torre. Mean monthly temperatures from 2005 to 2013 were calculated and served as normal monthly average temperatures.

Experimental design and measurement of variables. The experiment was conducted in two rows that accommodated a completely randomized design consisting of three replicates of 10-vine plots per pruning treatment which included conventionally hand pruning (CHP) and MP. From 1999 (3 yr after the establishment of MP), for each vintage, CHP and MP grapes were harvested at the same time. Yield and berry juice soluble solids (SS) were measured each year.

In 2014 and 2015, grapes of the two treatments were analyzed at the same TSS level (22 Brix). Veraison date was recorded when 50% the berries began to show color. The maturity was monitored during the entire ripening phase. To estimate leaf area per shoot, the method based on leaf disc sampling was used (Smart and Robinson 1991). 15 shoots per treatment were taken for the measurement. For each of them, the weight of all the leaves (without leaf petioles) and the weight of 100 3.80-cm2 discs were used to estimate leaf area (cm2) per shoot as their quotient X 380. The fruit was harvested when TSS averaged 22 Brix. Yield, clusters per vine and shoots per vine were determined on five vines per plot (15 vines per treatment). Cluster weight was measured on five clusters per treatment replicate. Berry weight was measured on 200 berries per replicate sampled randomly from the harvested fruit. Subsequently, each 200- berry sample was crushed manually to obtain juice for chemical analysis. TSS, pH, titratable acidity (TA), tartaric acid and malic acid were analyzed by standard methods (OIV 2013). Total anthocyanins were

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determined at 22 Brix according to Iland et al. (2004). Total anthocyanins were expressed by concentration (mg/g berry fresh mass) as well as by anthocyanin "density" (mg anthocyanins /cm^2 grape skin surface); the former value indicates the potential wine color while the latter one reflects the anthocyanin synthesis capacity of the grape skins.

Yield and TSS data of the long-term trial were analyzed with a paired-samples t-test (p=0.05). Data of 2014 and 2015 was tested for homogeneity of variance using Levene's test and were subjected to two-way (pruning method x year) analysis of variance (ANOVA), using the general linear model and F-test; since interaction between treatments and years was observed for some of the parameters, pruning systems were also analyzed as one way ANOVA for each year. The statistical analysis was performed using statistical package SPSS 16.0 (SPSS Inc., Chicago, US) for Windows.

Results and Discussion

Long-term observations. From 1999 to 2013, yield was higher in response to MP than to CHP (15300 kg/ha vs 9800 kg/ha), which is in agreement with a 10-year MP experiment with Riesling in Geisenheim, Germany (Schultz and Weyand 2005), in which MP led to 25%-75% higher yield. Since the grapes were always harvested at the same time, compared with CHP (20.2 Brix on average), MP grapes had lower TSS (18.4 Brix on average), which would produce a wine with a potential alcohol content of 10.8%. This level was too low for most winemakers 10 yr ago, however, it has become acceptable nowadays with a growing demand of low-alcohol wines. This long-term observation is mostly consistent with a previous study performed in the same region with Grenache (Martinez de Toda and Sancha 1998). It can be concluded that MP is

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viable as a labor-saving growing technique for certain cultivars under the viticultural conditions of the Rioja wine region.

Weather conditions. The 2014 vintage had an unusually warm September and October when the grapes matured (Figure 1). In comparison, the weather in 2015 was unusually hot from May through July however September and October were relatively cool.

Yield components. MP effectively delayed veraison by 1-2 weeks (Table 1). In 2014, MP increased yield by 77% as compared to CHP, whereas yield per vine was not affected by the pruning treatments in 2015. MP vines had 10-11 times more shoots but only 20%-40% of them bore fruit compared with 100% of shoots on CHP vines. Berry weight was 12% to 35% lower and the number of berries per cluster was 47% to 53% lower in response to MP compared with CHP. These effects of MP on yield components are consistent with previous studies (Bates and Walter-Peterson 2008, Poni et al. 2000, Schultz and Weyand 2005).

Often the ratio of leaf area to fruit production (LA/P) is used to assess potential berry maturation and quality. Normally, the LA/P required during maturation should range from 0.8 to 1.2 m^2/kg (Kliewer and Dokoozlian 2005), according to which, in both years, MP had enough LA to support fruit ripening. However, almost all the expected attributes of MP (delayed veraison, delayed TSS accumulation, lower berry weight, fewer berries per cluster, etc.) were found. Champagnol (1984) found that clusters are mainly supported by leaves on the same shoot, although nutrient transfer from other shoots occurs during maturation. In this study, as is typical, MP vines had many non-fruiting shoots whose leaves could contribute only indirectly to berry composition. In addition, due to more retained buds and earlier budburst, MP vines develop

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canopies more quickly than do conventionally pruned vines (Lakso, 1993). In this study shoots had fewer leaves on MP vines (10, on average) than on CHP vines (> 15). Final canopy size was attained earlier by MP vines than CHP vines which continued to generate new leaves and lateral shoots. Poni et al. (1994) reported that leaves normally reached maximum photosynthetic capacity at 30-35 days of age. From about 50 days, it started to decline persistently and 4-month-old leaves retained 45% of the maximum photosynthesis capacity. Hence it is not difficult to infer that during the ripening phase, the "source" of MP vines are all "old leaves" while CHP vines still possess enough high-efficiency leaves. All these above factors contribute to a low "source to fruit" ratio for MP in most of the time.

Must composition. For both seasons, grapes of both treatments were analyzed at the same TSS level (Table 2). MP delayed maturation by ≈ 17 days. In 2014, grapes from MP vines had higher TA and better organic acid composition (i.e. higher tartaric acid and lower malic acid concentration), which is consistent with Clingeleffer (2010). The pH of berries was surprisingly high considering their high TA. In 2015, berry TA and pH were both lower in response to MP than CHP. These results indicate that further study of MP effects on berry acidity is warranted.

At 22 Brix, compared with CHP, MP produced grapes with higher total anthocyanin concentration (mg/g) for both years. Regarding anthocyanin synthesis capacity (mg anthocyanins / cm^2 skin surface), there was no difference between the pruning treatments, which suggests that wines made from MP grapes would be more intensely coloured mainly as a result of smaller berry size rather than enhanced anthocyanin synthesis per unit area of berry skin. The lack of a response to pruning treatments by skin anthocyanin content is surprising given that it has been reported that light pruning to increase fruit exposure can enhance anthocyanin biosynthesis

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independent of berry size (Holt et al 2008), and that the canopy of MP vines can be more porous thus enhancing fruit exposure (Lakso 1993, Reynolds 1988). Our results may indicate that there was no pruning effect on fruit exposure but we did not evaluate treatment effects on canopy density and fruit exposure. Ambient temperatures may also have influenced our results. The studies of Mori et al. (2007) and Mori et al. (2005) observed that higher temperatures during the day or night led to a decrease in anthocyanin accumulation. However, their experiments were conducted under greenhouse conditions and the difference in temperature between control and high temperature groups was substantial (ΔT = 10°C or 15°C). In this experiment, from veraison to harvest maturity (22 Brix), daily mean air temperatures for CHP and MP were 17.8°C and 16.9°C in 2014, 17.6°C and 17.1°C in 2015, respectively. These differences were unlikely to affect anthocyanin synthesis. Martínez de Toda et al. (2014) reported an increase in anthocyanins : sugars ratio for Grenache in response to severe trimming compared with non-trimming treatment; and with a corresponding mean temperature difference during maturation of 2.3°C. Therefore, delaying maturation by creating cooler conditions during ripening might be an effective manner to restore anthocyanin : sugars ratio, but the difference in temperature must be considerable.

Conclusions

MP produced moderately higher yields and delayed berry development under the study conditions of La Rioja Valley. Berry ripening was achieved under MP, and the higher anthocyanin concentrations in MP compared with CHP fruit resulted from smaller berries rather than anthocyanin synthesis capacity. The slightly cooler ripening conditions caused by MP seem insufficient to enhance anthocyanin accumulation. Further studies should be done to confirm and

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evaluate the delayed maturation caused by MP compared with CHP and its effect on fruit quality in other varieties and under different climatic conditions.

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Table 1 Effects of minimal pruning (MP) and conventional hand pruning (CHP) on yield components, vine leaf area and leaf area to production ratio for Tempranillo vines in 2014 and 2015.

Pruning treatment	Veraison date	Shoots/ vine	Production (P) (kg/ha)	Clusters/ vine	Clusters/ shoot	Cluster weight (g)	Berries/ cluster	Berry weight (g)	Leaf area (LA) (m^2/vine)	LA/P (m^2/kg)
2014										
CHP	15 Aug	11	8900	10	0.91	263	156	2.19	5.91	2.34
MP	31 Aug	108	15700	40	0.37	105	74	1.42	7.09	1.58
Significance level[a]		***	**	***	***	***	**	**	ns	***
2015										
CHP	13 Aug	8	6100	9	1.13	219	131	1.67	4.67	2.69
MP	20 Aug	92	7700	21	0.23	104	70	1.47	6.98	3.19
Significance level		***	ns	***	***	***	**	ns	ns	ns

[a]The difference between treatments was assessed with independent-samples t-test; **, ***, ns: significant at $p \leq 0.01$, $p \leq 0.001$, or not significant, respectively.

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Table 2 The effects of minimal pruning (MP) and conventional hand pruning (CHP) on must composition and berry anthocyanins for Tempranillo vines in 2014 and 2015.

Pruning treatments	Date of fruit maturation (22 Brix)	Titratable acidity (g/L)	pH	Tartaric acid (g/L)	Malic acid (g/L)	Total Anthocyanins (mg/g)[a]	Anthocyanins (mg/cm^2 skin surface)[a]
2014							
CHP	1-Oct	3.85	3.41	4.4	3.1	1.31	0.37
MP	21-Oct	5.30	3.60	4.9	2.7	1.53	0.39
Significance level[b]		**	***	***	**	*	ns
2015							
CHP	15-Sep	7.55	3.39	---[c]	3.2	0.96	0.25
MP	28-Sep	6.34	3.23	5.3	3.5	1.16	0.25
Significance level		***	***	---	ns	*	ns

[a]Measured at 22 Brix.
[b]The difference between treatments was assessed with independent-samples t-test; **, ***, ns: significant at $p \leq 0.01$, $p \leq 0.001$, or not significant, respectively.
[c]Missing data.

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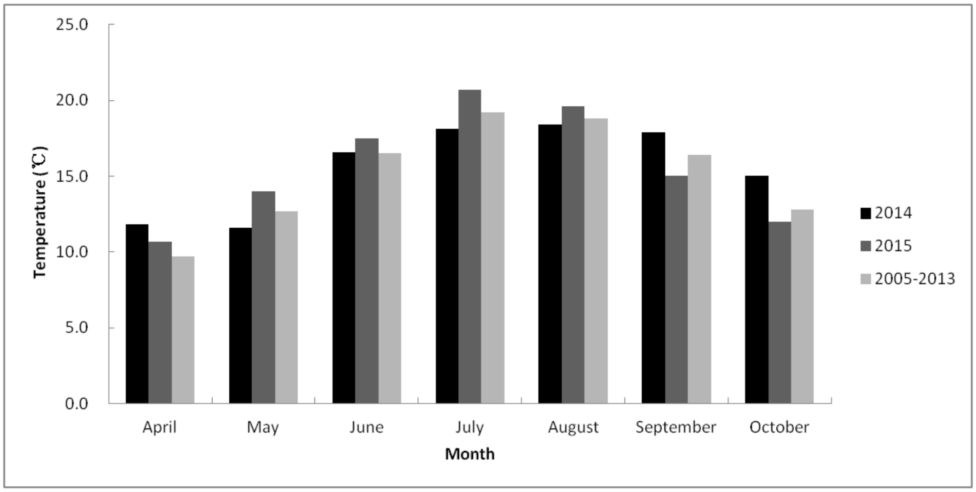

Figure 1 Mean monthly temperatures during growing season (Villar de Torre, La Rioja, Spain).