CAN EARLY DEFOLIATION IMPROVE FRUIT COMPOSITION OF TEMPRANILLO GRAPEVINES IN THE SEMI-ARID TERROIR OF UTIEL-REQUENA, SPAIN?

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ABSTRACT

Early defoliation has been found a useful tool to reduce cluster compactness and to improve fruit composition in vigorous sites of different viticultural areas. Our objective was to test the usefulness of this technique under the semi-arid climatic conditions of the Utiel- Requena D. O. (Valencia, south-east Spain) with the cv. Tempranillo. In deficit drip irrigated vines, planted in north- south oriented rows with vertical shoot positioning, four treatments were applied during 2008 and 2009. Control (C), non-defoliated vines, were compared with defoliation performed either just before anthesis (phenological stage H, treatment ED), or at fruit set (phenological stage J, treatment LD). In both defoliation treatments leaves from the first 6 nodes, including laterals, were removed. In a fourth treatment, only the leaves facing east from the first 8 nodes were removed at phenological stage H (EED). Sixteen vines per treatment were randomly selected within the vineyard. In the first season, as an average for all defoliation treatments yield was reduced 21% by leaf pulling. In the second season, there was a heavier reduction in yield (41%). Flower and berry number per cluster were similar in 2008, but in 2009 flower number decreased for ED, and berry number for ED and LD. In both years, lower berry weights at harvest were obtained in all defoliation treatments. Fruit composition was also modified by early-defoliation. For grapes harvested at the same date, treatment LD increased sugar content in 2009.The malic acid concentration decreased for all defoliation treatments in 2008, and the tartaric acid levels increased for all defoliation treatments in both years. Total acidity decreased for ED and LD in both years, though significantly only in 2008, when it decreased in proportion to the intensity of defoliation. Both ED, and particularly LD, improved berry composition, increasing the concentration of total phenolics, anthocyanins and tannins. The synthesis of phenolic substances was instead not increased by leaf pulling. Overall results indicate that defoliation carried out at fruit set is the most effective treatment to improve berry phenolic concentration and soluble solids. However, growers should take into account the important yield penalty due to defoliation, particularly in the mid-term. In addition, a decrease in malic acid concentration could be also detrimental during wine-making. This indicates that the early defoliation technique needs to be used with caution in the semi-arid and warm terroirs.

KEYWORD

Fruit set–yield–total soluble solids–malic acid–phenolics

INTRODUCTION

An excessive vegetative growth and high yield of grapevines is relatively frequent in many vine growing regions around the world. This is due to different reasons like improvements in cultural practices through soil management, irrigation, cultivation and pest and disease control (Hunter 1995).

The defoliation practice consists on removing all or part of the leaves of the basal zone of the shoots to improve grape exposure to sun-light and the air circulation. Defoliation has been used since a long time and it is still practiced in vineyards manually harvested. After last advances on mechanization, this practice has turned economically feasible for the majority of the winegrape growing areas.

Defoliation with the aim of improving grape composition is normally employed from fruit set to veraison in high density canopies, with substantial benefits in terms of pigmentation and tolerance to diseases in many instances (Reynolds *et al.,* 1996). However, vegetative growth and yield are adversely affected when the partial defoliation is imposed at the first stages of development (between budbreak and fruit set) (Hunter, Le Roux, 1992). Moreover, fruit well exposed to solar light have in general higher total soluble solids, anthocyanins, phenolic compounds and lower juice titratable acidity, malate, pH and grape weight in comparison to shaded fruits or not exposed (Bergqvist *et al.,* 2001).

The aim of this study was to test the effects of early defoliation carried out at two different stages around flowering and at two severity levels on fruit set, yield and fruit composition of cv. Tempranillo in a semi-arid terroir.

MATERIALS AND METHODS

Site description and experimental design. The experiment was carried out during 2008 and 2009 in a *Vitis vinifera* L. (cv. Tempranillo) vineyard planted in 1991 on 161-49 rootstock at a spacing of 2.45 by 2.45m (1666 vines/ha). The vineyard is located near Requena (39º, 29' N; 1º, 13' W; elevation 750m), Valencia, Spain. The vines were drip- irrigated since year 2000 and trained to a vertical trellis on a bilateral cordon system oriented north-south. Canopy management practices, all manually performed, included shoot thinning and shoot-tip cutting. All treatments were fertilized at a rate of 30–20–60–16 kg ha^{-1} of N, P, K, and Mg, respectively. The soil is a Typic Calciothird, with a clay loam to light clay texture, highly calcareous, and of low fertility (0.66% organic matter, 0.04% nitrogen). The soil is deep (>2m), available water capacity is around 200 mm m^{-1} and bulk density 1.43 to 1.55 t m^{-3}.

Budbreak for Tempranillo in the region usually occurs by mid-April and flowering by early June; veraison is reached by early August, with harvest during late September and leaf fall at the beginning of November. Climate is continental and semiarid with average annual rainfall of 430 mm, of which around 65% falls during the dormant period. The vineyard was drip deficit irrigated to replace only 75% of the grapevine potential water needs. Weather conditions were measured with an automated meteorological station located in the plot.

Defoliation treatments. Sixteen vines per treatment were randomly selected within the vineyard and treatments applied were:
- C: Control, un-defoliated.
- ED: Early defoliation. Leaf pulling was carried out just before flowering (phenological stage H, Baggiolini 1952). All the leaves of the first six nodes were removed, including leaves from lateral shoots.
- LD: Late defoliation. Leaf pulling was carried out at fruit set (phenological stage J, Baggiolini 1952). All the leaves of the first six nodes were removed, including leaves from the lateral shoots.
- EED: East early defoliation. Leaf pulling was carried out just before flowering (stage H). Only the leaves facing east of the eight first nodes were removed, including lateral shoots.

Flowering, fruit set and yield determinations. In all experimental vines four clusters were selected and photographed against dark background with a digital camera held perpendicular to the inflorescence just before anthesis. A regression between actual flower number (obtained by destructive counting on 30 inflorescences taken from guard vines) and the number of flowers counted on photoprints was then established. The resulting linear relationship (Nºflowers = 2.098 * Nºflowers photo; R^2 = 0.96) was then used to estimate the actual flower number per cluster. These same selected clusters were harvested one week before vintage and the number of berries per cluster were counted in order to obtain the fruit set rate and weighted to obtain the cluster and berry fresh weight. At harvest, the remaining clusters per vine were accounted and weighted separately to obtain the total yield per vine.

Berry composition. Samples of sixteen clusters per treatment were collected in the field and from them four replicated sub-samples of 150 g per treatment were obtained. After gentle crushing using a Thermomix (F6, 30seconds) juice components were analyzed. Total soluble solids (ºBrix) were determined with a digital refractometer (Atago). The titratable acidity (with NaOH 0.1N to an end point of pH = 8.2) and pH were measured using an automatic titrator (785 DMP Titrino) and the concentration in malic and tartaric acids using an enzymatic and colorimetric method, respectively (Multianalyzer Systea Easychem).

Phenolic compounds were analyzed in another four independent replicates per treatment (150 g) crushed with seeds (Ultraturrax, at 16000 rpm) until homogenization. Then 10 ml of ethanol (1:1 v/v) were added to 1 g of homogenate and after 1 hour of gentle agitation samples were centrifuged at 6000 *g* (Centromix) for 10 min. Later the supernatants were frozen at -20ºC until analysis. The concentration of total polyphenols and anthocyanins was determined following procedures described by Iland *et al.,* (2004) and the analysis of tannins done by the method of methyl cellulose precipitation with the modifications proposed by Sarneckis *et al.,* (2006). The results presented are the average of two sampling dates in both years, collected respectively about 1 to 2 weeks before harvest and at harvest each year.

Statystical analysis. Analysis of variance was performed using the MIXED procedure of the SAS statistical package (version 8.2; SAS Institute, Cary, NC). Data from each season were analysed separately. Differences between treatments means were assessed by Dunnett's *t* test against the control vines and by designated contrasts for timing of defoliation (ED-LD) and for defoliation intensity (ED-EED). In order to check if differences in fruit composition due to leaf pulling were related with the existing differences in berry fresh weight among treatments, the effects of defoliation on the fruit composition variables were also assessed including as a covariate berry fresh weight.

RESULTS AND DISCUSSION

Meteorological data. The annual average temperature was 12.3ºC in 2008 and 13.0ºC in 2009, and the differences between seasons were greater from April to June in agreement with the first stages of vine development (Fig 1). The vintage was advanced four weeks (1-Sept) in 2009 with respect to 2008 (29-Sept). The precipitations were greater in 2008 (584 mm) than in 2009 (395.6 mm) and around flowering were plentiful (Fig 1).

Flowering, fruit set and yield. There were no differences in the flower number per cluster in 2008 but in 2009 the ED treatment had lower flower number than C, and there were also differences due to timing of defoliation. Percent fruit set was similar in all treatments in 2008 but lower for LD than for ED and C in 2009. The ED and LD treatment had lower berry number per cluster in 2009 and differences between defoliation intensity levels were also statistically significant (Table 1). The carry over effects of defoliation on flowering intensity in 2009, can be explained considering that the clusters start to develop at the beginning of the flowering period of the previous season (Shaulis *et al.,* 1965). This period is then critical, not only for fruit production in the current year, but also for the harvest of the next season (Candolfi-Vasconcelos, Koblet, 1990).

In both years, the defoliation treatments decreased yield. In 2008, differences respect to C were only significant for LD, but on average for all defoliation treatments they were 21% lower. In 2009, defoliation produced an even higher yield reduction, around 48% for ED and

LD and around 27% for EED (Table 1). This yield reduction was both due to lower cluster number, thought differences were not statistically significant, and also to lower cluster weight. Besides cluster weight decreased significantly with intensity of defoliation in both years (Table 1). This severe yield decrease due to early leaf pulling has not been observed in other varieties like Sangiovese or Trebbiano (Poni *et al.,* 2006).

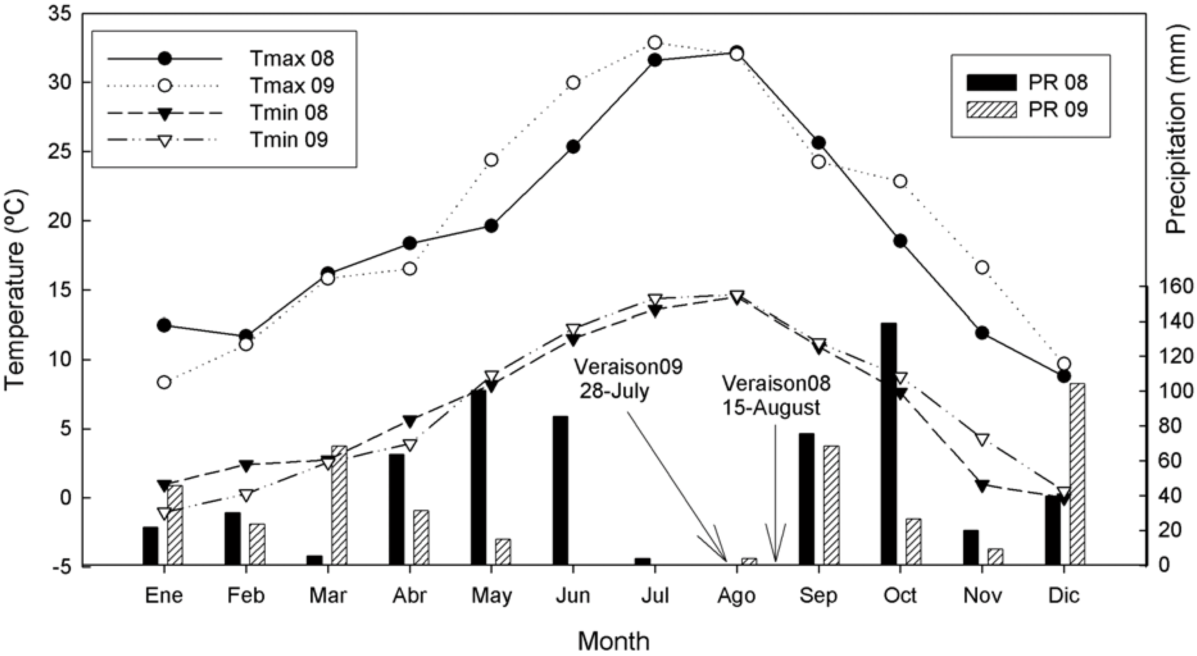

Figure 1. Meteorological data in Requena (Spain) for 2008 and 2009.

Berry weight decreased in all defoliation treatments in both years (Table 1). These findings agree with Candolfi-Vasconcelos and Koblet (1990), who showed that the removal of the main leaves during the early development period implies eliminating the only available source organ, and may therefore lead to a yield reduction at harvest due to the abscission of flowers and lower fruit set. Moreover, Harris *et al.,* (1968) stated that if the stage I of berry growth is altered, then the rate of dry mass accumulation will be reduced and the final berry size will be lower.

Table 1. Flowering, fruit set and yield parameters. * indicates significant differences against C; x indicates significant differences between ED and LD; y indicates significant differences between ED and EED, all at P<0.05.

	Flower (n)		Berry (n)		Fruit set (%)		Cluster (n)		Yield (kg)		Cluster weight (g)		Berry weight (g)	
Treatm.	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
C	379	307	156	182	43	70	25	18	6.9	5.8	316.8	360.9	2.0	1.9
ED	353	236* x	140	122* y	41	64	23	13	5.4	2.9*	261.8* y	215.2* y	1.8*	1.7*
LD	361	312	144	129*	42	49* x	23	15	5.1*	3.1*	255.9*	225.4*	1.7* x	1.6* y
EED	400	277	164	168	44	67	22	14	5.8	4.2	324.2	303.3*	1.9*	1.8*

Fruit composition. Higher total soluble solids (ºBrix) in juice were observed in LD in both years, but differences respect to C were only significant in 2009, when there were also significant differences due to timing of defoliation (Table 2). In 2009, Brix values were in general higher than in 2008, probably because of a faster sugar accumulation due to higher temperatures (Fig. 1).

Total acidity decreased for ED and LD in both years, though significantly only in 2008, when it decreased in proportion to the intensity of defoliation (Table 2). pH was unaffected by defoliation in both years. Tartaric acid concentration was increased by all defoliation treatments in both years. Malic acid concentration decreased for all defoliation treatments in 2008, but was unaffected in 2009 (Table 2). Increasing cluster exposure to direct sunligth probably increased berry temperature (Smart, Sinclair, 1976), what favoured the malic acid degradation. The lack of effect of defoliation on total acidity and malic acid concentration in 2009 could be explained at least in part by the earlier vintage (four weeks before) what presumably reduced malic acid combustion by fruit respiration.

Table 2. Technological berry parameters. * indicates significant differences against C; x indicates significant differences between ED and LD; y indicates significant differences between ED and EED, all at $P<0.05$.

	ºBrix		Total Ac.(g/l)		pH		Tartaric acid (g/l)		Malic acid (g/l)	
Treatm.	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
C	22.5	23.9	4.7	4.3	3.77	3.94	4.36	4.79	2.89	2.66
ED	22.3	24.1	4.4*^{y}	4.1	3.83	3.94	4.80*	5.53*^{y}	2.29*	2.48
LD	23.0	25.6*^{x}	4.3*	4.0	3.85	4.01	4.93*	6.17*^{x}	1.82* [illegible]	2.46
EED	21.8	23.8	4.6	4.2	3.79	4.02	5.04*	5.88*	2.02*	2.70

In both years, the total phenolic concentration respect to C was higher for ED and LD. In 2008 it was higher for ED than for LD and in 2009, ED was higher than EED. The anthocyanins concentration was higher in the LD in both years and only in 2009 for ED. These results are in agreement with previous findings by Poni *et al.,* (2009) who also showed that early defoliation increased berry phenolic concentration.

The tannin concentration was increased in ED and EED in 2008 and in all defoliation treatments in 2009 (Table 3). Tannin concentration was significantly affected in both years by timing of defoliation. These changes of phenolic substances concentration in the berries produced by defoliation could not be attributed to the effects on the berry size as indicated by the non-significance of this parameter when used as covariate in the statistical analysis.

The trends described in terms of concentration do not exactly match those observed in terms of synthesis (e. g. expressed in g/vine) in 2008 and especially in 2009, when a decrease in the synthesis by all defoliation treatments of practically all the compounds can be clearly observed (Table 3).

Table 3. Phenolic berry parameters. * indicates significant differences against C; x indicates significant differences between ED and LD; y indicates significant differences between ED and EED, all at $P<0.05$.

	IPT (mg/g)		IPT (g/vine)		Anthoc.(mg/g)		Anthoc.(g/vine)		Tannins (mg/g)		Tannins (g/vine)	
Treatm.	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
C	23.8	25.9	162	149.4	1.30	1.18	8.83	6.78	8.4	12.6	57.2	72.4
ED	26.8*^{y}	29.5*^{y}	144	84.3*^{y}	1.44*	1.29^{y}	7.73	3.66*^{y}	10.8	15*	58.1	43*^{y}
LD	28.5*^{x}	30.3*	155	94.8*	1.50*	1.34*	7.66	4.18*	13.1*^{x}	16.4*^{x}	66.2	51.4*
EED	25	26.1	143	109*	1.36	1.17	7.85	4.88*	11.1*	14.9*	64.1	62.5

CONCLUSIONS

Although the early defoliation practice has given satisfactory results in high yielding varieties or cooler and more humid climates where vines are usually vigorous, under our experimental conditions, the severe yield penalty found mainly in the second year of the study make the early defoliation technique unadvisable for the semi-arid terroir of Requena. Under our environmental conditions, it could be interesting to test this technique by removing a lower number of leaves (e. g. only 3-4 nodes) or avoiding the shoot thinning and shoot-tip cutting practices in order to alleviate the source limitation due to leaf removal.

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