

IS EARLY DEFOLIATION A SUSTAINABLE MANAGEMENT PRACTICE FOR MEDITERRANEAN VINEYARDS? CASE STUDIES AT THE PORTUGUESE LISBON WINEGROWING REGION

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Abstract:

Context and purpose of the study - Recently early defoliation (ED) has been tested in several high-yielding grapevine varieties and sites aiming at reducing cluster compactness and hence, regulating yield and susceptibility to botrytis bunch rot infection. The reported results have been generally positive, encouraging growers to use this canopy management technique as an alternative for replacing the conventional time-consuming cluster thinning and, simultaneously, as a sustainable practice to reduce the use of fungicides. However, ED increases berry sunburn risks and/or can induce carry-over effects on vigor and node fruitfulness as shown in the two case studies reported in this work.

Material and methods- Two ED experiments were set up at a commercial vineyard located in the Lisbon winegrowing region with the varieties Aragonez, syn. Tempranillo (2013-2015) and Semillon (2018). In both experiments the ED treatment was compared with the non-defoliated (ND; control) using a randomized complete block design with 4 replicates per treatment. The ED treatment consisted of the removal of 5-6 basal leaves and any laterals at pre-flowering. Vegetative (leaf area and pruning weight) and reproductive components (%fruit-set, cluster number, cluster weight, yield) and berry composition were assessed.

Results - In the Aragonez experiment total leaf area at harvest and pruning weight were significantly lower in ED vines. The ED treatment presented also a significantly lower fruit-set, berry weight, cluster weight and compactness, as well as yield as compared to the control. In the third season ED presented a significantly lower cluster number indicating a negative carry-over effect on bud initiation induced by the early source limitation. Regarding berry composition, ED showed a higher Brix and a lower titratable acidity than the control but no significant differences were detected on skin anthocyanins. The incidence and severity of botrytis bunch rot infection was significantly higher in the control treatment. In the Semillon experiment the ED treatment showed also a significantly lower leaf area, fruit-set, berry weight, cluster weight, cluster compactness and yield. No significant differences were detected on berry composition except for Brix where ED showed a significantly higher value. Despite the low pressure of botrytis bunch rot infection the control presented the significantly highest incidence and severity. An heat event occurred during the first week of August induced a severe and significantly higher berry sunburn in the ED treatment.

Our results show that pre-bloom defoliation is a canopy management practice with high potential for regulating grape yield and with benefits for grape health. However, in Mediterranean climates, where water and heat stress can inhibit leaf area compensation, the negative effects observed either on berry sunburn and on node fruitfulness recommends to use this practice with care or even avoid it.

Keywords: early defoliation, fruit-set, grapevine, botrytis rot, berry sunburn.

1. Introduction

Recently, leaf removal at the fruit-zone before flowering (early defoliation - ED) has been tested in several high-yielding grapevine varieties and sites as a canopy management practice aiming at reducing cluster compactness and, hence, regulating yield and improving cluster tolerance to botrytis bunch rot (e.g. Poni *et al.*, 2006; Intrieri *et al.*, 2008, Tardaguila *et al.*, 2010, Diago *et al.*, 2012, Palliotti *et al.*, 2012; Acimov *et al.*, 2016; Alessandrini *et al.*, 2018). These effects were achieved by the negative physiological impact of a strong source limitation (6-8 basal leaves removed at pre-bloom) which induces a lower fruit set and hence, smaller and looser clusters that will promote a faster water evaporation, reducing the

spreading of bunch rot among berries. Furthermore, different reports showed the potential of ED to increase the skin-to-berry ratio, (Risco *et al.*, 2014), total soluble solids, as well as anthocyanin and phenol contents (Poni *et al.*, 2009; Gati *et al.*, 2012; Diago *et al.*, 2012). Almost all above cited reports showed no negative impacts of ED on plant reserves nor on node fruitfulness, results that were explained by the fast lateral leaf area recover which compensates the lost of primary leaf area.

The positive effects reported above have promoted the dissemination of the ED. However, in Mediterranean non-irrigated vineyards, this practice should be used with care, since the frequent water and heat stresses may reduce/inhibit leaf area compensation and, therefore, negatively impact plant reserves and induce carry-over effects on node fruitfulness. Furthermore, if lateral leaf area growth compensation at fruiting zone is small or absent, bunches will stay completely exposed during all summer, increasing the risks of berry sunburn.

This work aims to present two case studies where the above mentioned risks of ED were observed with strong negative impact on yield.

2. Material and Methods

Site and plant materials: This work presents data from two early defoliation experiments set up at a commercial non-irrigated vineyard located at Merceana, within the Alenquer appellation of Lisbon winegrowing region, West Portugal (39° 05' 40'' N, 9° 07' 56'' W). The climate is of the Mediterranean type with Atlantic influence and the soil presents a clay texture and a pH (H₂O) of 7.0. The first experiment was established in 2013 in a vineyard plot with 10 years-old grapevines of the red variety Aragonez (syn. Tempranillo), grafted onto SO4 rootstock and spaced 1 m within and 2.5 m between north-south oriented rows. An early defoliation treatment (ED), consisting of the removal of 6 basal leaves and any laterals developed within the 1-6 node shoot zone at pre-flowering from all shoots, was compared with an untreated control (C) during 3 seasons. However in this paper we will report only the third season results (2015). The second experiment was set up in 2018 in a close by vineyard plot with 5 years-old grapevines of the white variety Semillon, grafted onto SO4 rootstock and spaced 1.0 within and 2.5 m between East-West oriented rows. The same type of early defoliation (6 basal leaves and any laterals at pre-flowering) was compared with a non-defoliated control. In both plots the vines were spur-pruned with 12 nodes per vine on a unilateral cordon and trained to a VSP trellis system with two pairs of movable wires. With the exception of defoliation, standard cultural practices in the region were applied to all treatments. No fungicide sprays for botrytis control were applied.

Experimental Design - In both experiments the experimental design was a randomized complete block design with 4 replicates of 10 representative vines per treatment.

Measurements - Leaf area per shoot was assessed periodically in a sample of representative shoots per treatment from flowering onwards in a non-destructive way, according to Lopes and Pinto (2005). Leaf area per plant was calculated by multiplying the average leaf area per shoot by the average shoot number.

Percent fruit set was assessed in a sample of 48 clusters per treatment in Aragonez, as described in Lopes *et al.* (2014) and on 24 clusters per treatment in Semillon using the methodologies described in Alessandrini *et al.* (2018). The yield was monitored by recording the number of clusters and their total weight from 10 tagged vines per elemental plot (40 per treatment). Cluster compactness was calculated as the ratio between the number of berries and the rachis length. Botrytis bunch rot intensity (% bunches with visual symptoms) and severity (fraction of bunches with visual symptoms) were assessed in each tagged bunch. Furthermore, in the same tagged bunches, the fraction of sunburned berries was also assessed.

At harvest a sample of berries per treatment was collected from both sides of the canopy and berry fresh weight, total soluble solids and pH were assessed according to the International Organization of Vine and Wine (OIV, 1990) procedures. In the red variety experiment the total phenols and monomeric anthocyanin extractions from berries and skin berries respectively were performed adding 1 mL of methanol to 100 mg of fresh weight. Total phenols was quantified by the Folin Ciocalteu method with modification as described by Zarrouk *et al.* (2012). Total Monomeric anthocyanin was quantified by the pH-differential method as reported by Giusti and Wrolstad (2001).

Statistical analysis - ANOVAS were carried out in accordance with GLM procedures, from the SAS® program package (SAS Institute, Cary, NC, USA) and statistical differences between means were assessed by LSD test (P < 0.05).

3. Results and discussion

3.1. Leaf area

In both varieties ED induced a significant reduction of the primary leaf area per vine (measured at veraison). Lateral leaf area presented also lower values on ED treatment however the differences were not significant in the variety Aragonez. Consequently, in both experiments, total leaf area per vine was also significantly lower in ED as compared to the control (Fig. 1). These results corroborate those obtained by Tardaguila *et al.* (2010) with the varieties Graciano and Carignan at La Rioja, Spain, indicating a reduced lateral leaf growth compensation which, in our experiments, was more pronounced in Aragonez than in Semillon. However, our findings contrast with other reports where the growth response of the vines was sufficient to fully recover the reduction in leaf area (e.g. Poni *et al.*, 2006; Alessandrini *et al.*, 2018). The low lateral regrowth observed in the present investigation may be explained by the effects of the removal of all laterals presented in the basal nodes during the early defoliation combined with the high temperatures and mild water stress that was observed in the experimental fields. For example, 2015 was a very dry season with very low rainfall during winter and spring which have not enable the soil to refill and, consequently, have induced very low soil water availability during spring and summer with negative consequences on vegetative growth (Williams and Matthews, 1990).

3.2. Fruit set, yield and yield components

In both experiments, ED induced a significantly lower fruit set, cluster weight and yield as compared to the control (Table 1). These data are consistent with previous work (Poni *et al.* 2006; Intrieri *et al.* 2008; Tardaguila *et al.* 2010, Palliotti *et al.*, 2012) and are explained by the negative impacts on fruit set induced by a strong source limitation at pre-bloom. In Aragonez experiment, additionally to fruit set reduction, a significantly lower cluster number was also observed in ED treatment indicating a negative carry-over effect (2015 was the third consecutive season of the experiment) on bud initiation. This finding contrasts with most part of the above referred publications in which authors do not report any carry-over effect on next season's bud fruitfulness.

3.3. Berry composition and health

In Aragonez variety, ED induced a higher Brix and a lower titratable acidity than the control, however, no significant differences were detected on skin anthocyanins. The increase in sugars by ED treatment was also observed in previous reports with other varieties (Poni *et al.*, 2009, Palliotti *et al.*, 2011; Gatti *et al.*, 2015) and can be explained by the higher leaf area to fruit ratio as compared to the control (*vide* Table 1). The lower must titratable acidity observed in ED treatment was probably due to the enhanced degradation of malic acid caused by the increase in clusters' sun exposure and, consequently, in berry temperature (Esteban *et al.*, 1999). The results on berry skin anthocyanins were not expected as berry color enhancement is a recurrent reported response of red grape varieties subjected to early defoliation (e.g. Intrieri *et al.* 2008, Palliotti *et al.* 2012, Tardaguila *et al.* 2010, Diago *et al.*, 2012). This absence of response of berry skin anthocyanin content in ED treatment might have been caused by the excessive berry exposure and overheating which can inhibit anthocyanins synthesis and/or induce their degradation (Downey *et al.*, 2004; Spayd *et al.*, 2007; Zarrouk *et al.*, 2016).

In the Aragonez variety, despite the low pressure of botrytis bunch rot infection, the incidence and severity was significantly higher in the control treatment as compared to ED, where no infection was detected. This result confirm the early defoliation potentialities to enhance berry health as already reported by others (Poni *et al.*, 2006; Intrieri *et al.*, 2008; Tardaguila *et al.*, 2010; Palliotti *et al.*, 2012). In the variety Aragonez berry sunburn severity presented very low values in both treatments, with the control showing the highest berry damage (Table 2).

In the Semillon experiment, ED also induced higher °Brix than the control but no significant differences were detected on must titratable acidity and pH. The higher °Brix of ED can also be explained by higher leaf area to fruit ratio observed in ED as compared to the control (*vide* Table 1). Despite the low

pressure of botrytis bunch rot infection, almost all sampled Semillon bunches of the control treatment presented some infected berries while in ED no symptoms were detected (Table 2). Regarding the severity of Semillon berry sunburn, ED presented a significantly higher fraction of damaged berries in the ED treatment as compared to the control, which was almost unaffected (Table 2). These results were mainly due to the effects of a severe heatwave (three consecutive days with air temperatures above 40° C combined with very low air humidity) occurred during the first week of August 2018. The more exposed bunches of ED were not able to resist to the combined effect of high air temperature and irradiance, being the bunches located at the south side of the canopy the most damaged.

4. Conclusions

Our results show that pre-bloom defoliation is a canopy management practice with high potential for regulating grape yield and reduce cluster compactness with benefits for berry health. However, the carry-over effects observed on node fruitfulness recommend the use of this practice with care in Mediterranean non-irrigated vineyards, where water and heat stress can inhibit lateral leaf area compensation and a consequent excessive reduction in vine vigor and yield. Furthermore, in Mediterranean climates, where heat events are becoming more frequent, the high risk of berry sunburn recommends to avoid the use of canopy management techniques that excessively promote cluster sun exposure as is the case of ED. Further research on ED severity thresholds is needed in order to evaluate if a lower intensity of primary leaf removal and/or the retaining of the laterals would produce similar results on the reduction of bunch compactness, without inducing carry-over effect on bud fruitfulness while providing protection against berry sunburn and improve berry health.

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Litterature cited

- ACIMOV D., TOZZINI L., GREEN A., SIVILOTTI P., SABBATINI P.**, 2016. Identification of a defoliation severity threshold for changing fruitset, bunch morphology and fruit composition in Pinot Noir. *Australian Journal of Grape and Wine Research* 22, 399–408.
- ALESSANDRINI M., BATTISTA F., PANIGHEL A., FLAMINI R., TOMASI D.**, 2018. Effect of pre-bloom leaf removal on grape aroma composition and wine sensory profile of Semillon cultivar. *Journal of the Science Food and Agriculture*, 98, 1674–1684.
- DIAGO M.P., AYESTARÁN B., GUADALUPE Z., PONI S., TARDÁGUILA J.**, 2012. Impact of Prebloom and Fruit-Set Basal Leaf Removal on the Flavonol and Anthocyanin Composition of Tempranillo Grapes. *American Journal Enology and Viticulture* 63(3), 367-376.
- DOWNEY M.O., HARVEY J.S., ROBINSON S.P.**, 2004. The effect of bunch shading on berry development and flavonoid accumulation in shiraz grapes. *Australian Journal of Grape and Wine Research* 10, 55-73.
- ESTEBAN M.A., VILLANUEVA M.J., LISSARRAGUE J.R.**, 1999. Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organique acids and mineral elements. *American Journal Enology and Viticulture* 50, 418–433.
- GATTI M., GARAVANI A., CANTATORE A., PARISI M.G., BOBEICA N., MERLI M.C.**, 2015. Interactions of summer pruning techniques and vine performance in the white *Vitis vinifera* cv. Ortrugo. *Australian Journal of Grape and Wine Research* 21, 80–89.
- GIUSTI, M.M., WROLSTAD R.E.**, 2001. Anthocyanins: Characterization and measurement with UV-visible spectroscopy. In *Current protocols in food analytical chemistry*. Wrolstad R.E. (ed.), pp. F1.2.1–1.2.13. Wiley, New York.
- INTRIERI C., FILIPPETTI I., ALLEGRO G., CENTINARI M., PONI S.**, 2008. Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research* 14, 25-32.
- LOPES, C.M., AND P.A. PINTO**, 2005. Easy and accurate estimation of grapevine leaf area with simple mathematical models. *Vitis* 44(2), 55-61.

- LOPES C.M., MONTEIRO R., EGIPTO R., GENEBRA T., ZARROUK O., CHAVES M.M., 2014. Can early defoliation replace cluster thinning as a crop regulating technique? A case study with cv. Aragonez in a terroir of the Lisbon winegrowing region. Proceedings 10th Terroir Congress, 7-10 July, 2014, Tokaj, Hungary, 120-125.
- O.I.V., 1990. Recueil des méthodes internationales d'analyses des vins et des moûts. International Organisation of Vine and Wine, Paris.
- PALLIOTI A., GARDI T., BERRIOS J., CIVARDI S., PONI S., 2012. Early source limitation as a tool for yield control and wine quality improvement in a high-yielding red *Vitis vinifera* L. cultivar. *Scientia Horticulturae* 145, 10-16.
- PONI S., BERNIZZONI F., CIVARDI S., LIBELLI N., 2009. Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *Vitis vinifera* L. cultivars. *Australian Journal of Grape and Wine Research* 15, 185-193.
- PONI S., CASALINI L., BERNIZZONI F., CIVARDI S., INTRIERI C., 2006. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *American Journal Enology and Viticulture* 57, 397-407.
- RISCO D., PEREZ D., YEVES A., CASTEL J.R. INTRIGLIOLO D.S., 2014. Early defoliation in a temperate warm and semi-arid Tempranillo vineyard: vine performance and grape composition. *Australian Journal of Grape and Wine Research* 20, 111-122.
- SPAYD S.E., TARARA J.M., MEE D.L., FERGUSON J.C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal Enology and Viticulture* 53(3), 171-182.
- TARDAGUILA J., MARTINEZ DE TODA F., PONI S., DIAGO M.P., 2010. Early leaf removal impact on yield components, fruit, and wine composition of Graciano and Carignan (*Vitis vinifera* L.) grapevines. *American Journal Enology and Viticulture* 61, 320-332.
- WILLIAMS L.E., MATTHEWS M.A., 1990. Grapevine. In: Stewart, B.A., Nielsen, D.R. (Eds.), *Irrigation of Agricultural Crops. Series of Agronomy*, vol. 30. American Society of Agronomy, Madison, Wisconsin, USA, pp. 1019-1055.
- ZARROUK O., BRUNETTI C., EGIPTO R., PINHEIRO C., GENEBRA T., GORI A., LOPES C.M., TATTINI M., CHAVES M.M., 2016. Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. *Frontiers in Plant Science* 7, 1-18.
- ZARROUK O., FRANCISCO R., PINTO-MARIJUAN M., BROSSA R., SANTOS R., PINHEIRO C., COSTA J.M., LOPES C.M., CHAVES M.M., 2012. Impact of irrigation regime on berry development and flavonoids composition in Aragonez (Syn. Tempranillo) grapevine. *Agricultural Water Management* 114, 18-29.

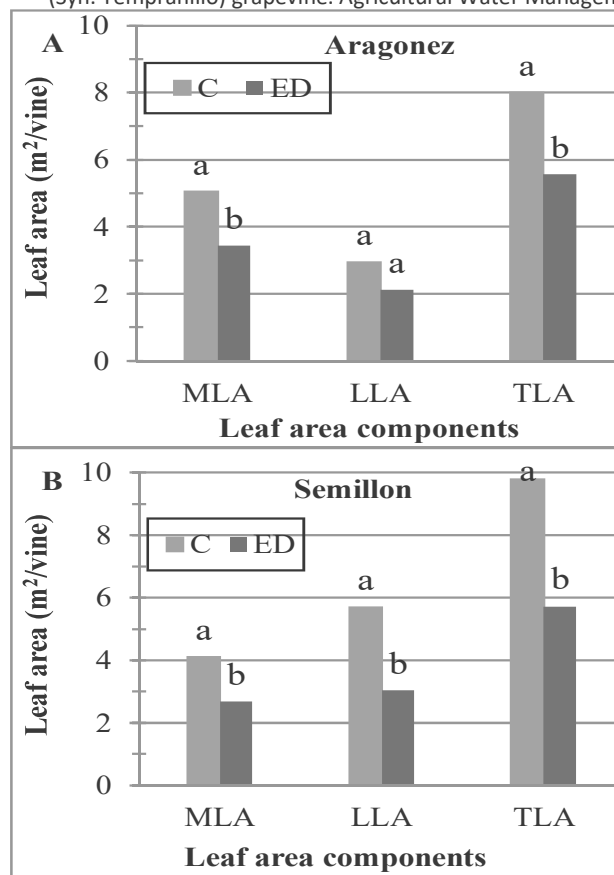


Figure 1: Effect of early defoliation at pre-flowering (ED) on leaf area components measured at veraison in Aragonez (A) and Semillon grapevines (B), at Quinta do Pinto, Merceana, Portugal. C: control non-defoliated. For each leaf area component different letter suffixes show statistically significant differences at $P < 0.05$.

Table 1. Influence of early defoliation (ED) on Aragonez and Semillon yield components and leaf to fruit ratio. C- control non-defoliated.

Variety	Treatment	Fruitset (%)	# Bunches /vine	Bunch weight (g)	Yield (Kg/ha)	Leaf area/yield (m ² /kg)
Aragonez	C	50.2	12.8	305.1	15600.0	2.1
	ED	32.1	8.7	118.0	4000.0	5.6
	<i>Sig.</i>	*	***	***	***	***
Semillon	C	46.0	20.9	226.0	19030.3	2.1
	ED	30.5	21.3	93.2	7907.6	2.9
	<i>Sig.</i>	**	ns	***	***	**

Sig: Statistical significance given by $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) and not significant (ns).

Table 2. Effect of early defoliation (ED) on Aragonez and Semillon grape composition, bunch compactness, botrytis infection and berry sunburn. C- control non-defoliated; TA – titratable acidity; Anthoc– skin anthocyanines.

Variety	Treat	Soluble solids (°Brix)	TA (g tart.)	pH	Anthoc. (mg malv.)	Bunch compactness ⁽¹⁾	Botrytis incidence (%)	Botrytis severity (%)	Sunburn Severity (%)
Aragonez	C	21.3	5.0	3.25	312.3	7.30	6.3	1.2	4.3
	ED	22.3	4.5	3.39	324.6	5.10	0.0	0.0	1.5
	<i>Sig.</i>	*	*	**	ns	*	*	*	*
Semillon	C	19.7	8.1	3.12	-	8.3	83.3	7.5	1.3
	ED	21.8	8.4	3.12	-	5.3	0.0	0.0	51.1
	<i>Sig.</i>	**	ns	ns	-		***	***	***

⁽¹⁾ Calculated as the number of berries per cm of main rachis length. Sig: Statistical significance given by $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) and not significant (ns).