Australian Journal of Grape and Wine Research 26, 290–299, 202

Carry-over effects on bud fertility makes early defoliation a risky crop-regulating practice in Mediterranean vineyards

C.M. LOPES[1], R. EGIPTO[2], O. ZARROUK[3] and M.M. CHAVES[3]

[1] Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal; [2] Instituto Nacional de Investigação Agrária e Veterinária, Dois Portos, Portugal; [3] Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade NOVA de Lisboa, Oeiras, Portugal

Corresponding author: Professor Carlos M. A. Lopes, email carlosmlopes@isa.ulisboa.pt

Abstract

Background and Aims: Recently, early defoliation (ED) has been used widely to regulate yield and reduce bunch compactness to improve tolerance to bunch rot. The aim of this study was to test ED, as an alternative to the conventional crop thinning (CT), to regulate yield and improve tolerance to bunch rot of the *Vitis vinifera* L. cv. Aragonez.

Methods and Results: Early defoliation and CT were compared with an untreated Control over three consecutive seasons in a commercial vineyard in Portugal. Early defoliation had little effect on tolerance to bunch rot and led to progressive loss of yield reducing fruitset, bunch number, berry number and mass, compared to Control vines. Reduced bunch number is likely to be a function of reduced bud fertility. Crop thinning also reduced yield but, unlike ED, the yield reduction was less and remained constant over the seasons studied. Both ED and CT had little effect on berry composition.

Conclusions: Among the two crop-regulating techniques, CT appears more suitable than ED to control grape yields in regions where yield limitations are imposed. The progressive yield decline caused by ED is likely to be not economically viable in the region studied.

Significance of the Study: Early defoliation reduces bud fertility in unirrigated vineyards under Mediterranean conditions. Therefore, this practice should be avoided under those conditions.

Keywords: *bunch rot, crop thinning, fruitset,* Vitis vinifera L.*, yield components*

Introduction

The grapevine (*Vitis vinifera* L.) red cultivar Aragonez (synonym Tempranillo) is the most widely planted red cultivar in Portugal accounting for 11% (about 21 000 ha) of the Portuguese vineyard area (Instituto da Vinha e do Vinho 2018). This Iberian cultivar is planted in most of the Portuguese winegrowing regions, from the north (e.g. Douro and Dão), to the south (e.g. Lisboa and Alentejo). One reason for its popularity is its high-yielding capacity. The high yield potential of Aragonez results from medium bud fertility combined with large to medium tight bunches (Instituto da Vinha e do Vinho 2011). These traits are common in vigorous vineyards across the appellation Alenquer (Lisboa sub-region), which is characterised by deep clay calcareous soils (Silva 2010). This high yield potential is not compatible with the yield limitation imposed to the production of protected designation of origin (PDO) wines in this appellation [80 hL/ha (Instituto da Vinha e do Vinho 2018)]. In response, growers often use conventional crop thinning (CT: removal of the distal bunch from shoots with two intact bunches at the onset of veraison) in order to increase the leaf-to-fruit ratio and, hence, to prevent over-cropping, while improving berry composition (Petrie and Clingeleffer 2006, Tardaguila et al. 2008, Zhuang et al. 2014). The efficacy of CT, however, as a crop-regulating practice is questionable. In addition to being a time-consuming and expensive practice (Smith and Centinari 2019), when done manually, it may produce unsatisfactory results by inducing compensatory berry growth and progressive positive carry-over effects on vigour and bud fertility (Gatti et al. 2012).

The maritime influence in the Lisboa winegrowing region creates favourable climate conditions for fungal diseases [mild temperature, high RH and high frequency of morning dew (Reis and Gonçalves 1981)], including botrytis bunch rot (*Botrytis cinerea*), particularly in cultivars with compact bunches. The application of synthetic fungicides is therefore a common practice in these regions, in spite of their economic cost and potentially negative impact on the environment. In order to reduce susceptibility to fungal diseases, defoliation at the bunch zone is often used in these vineyards to reduce canopy density and improve bunch zone ventilation and light microclimate. In order to prevent berry sunburn, however, defoliation is conducted only on the side of the canopy, which is exposed in the morning.

For the last two decades, pre-flowering leaf removal in the fruit-zone [early defoliation (ED)] has been tested in several high-yielding grapevine cultivars and sites, to reduce bunch compactness and to regulate yield [e.g. Poni et al. (2006, 2009), Intrieri et al. (2008), Diago et al. (2010), Tardaguila et al. (2010, 2012), Palliotti et al. (2011, 2012), Acimov et al. (2016), Alessandrini et al. (2018)]. Early defoliation induces a strong carbon source limitation (six to eight basal leaves removed at pre-flowering) which could reduce fruitset. In the resulting looser bunches, less contact between adjacent berries should impair the spread of bunch

doi: 10.1111/ajgw.12437

rot related fungal infections between berries. Also looser bunches will promote better within-bunch air circulation and, therefore, faster water evaporation (Hed et al. 2009). Nevertheless, a recent report showed that these benefits of ED might be balanced by the shortening of the bunchstem because a carry-over effect of the strong source limitation (Hed and Centenari 2018). Early defoliation might increase the skin-to-berry ratio and the seed-to-berry ratio (Tardaguila et al. 2010, Risco et al. 2014), hence, influencing berry composition by increasing the concentration of anthocyanin and phenolic substances [e.g. Poni et al. (2009), Diago et al. (2012), Gatti et al. (2012)]. Furthermore, the enhancement of bunch light exposure promoted by ED can also enhance the flavonoid concentration of grapes (Hunter et al. 1991, Dokoozlian and Kliewer 1996, Spayd et al. 2002).

Taking into account the role of basal leaves as the main contributors of plant assimilates between the period of flowering and fruitset (Vasconcelos and Koblet 1990, Poni et al. 2008, Palliotti et al. 2011), a drastic reduction of leaf area due to ED could negatively impact on carbon balance and allocation to sinks and on reproductive activity (Holzapfel et al. 2010), which may negatively affect plant carbohydrate reserve replenishment (Bennett et al. 2005, Smith and Holzapfel 2009, Vasconcelos et al. 2009, Frioni et al. 2018). This may reduce inflorescence primordia initiation and size in latent buds (Noyce et al. 2016) and, in turn reduce bud fertility for the following season (May 2000). In several ED studies, however, no negative impacts on bud fertility for the following season were noticed. The authors postulate that the positive effects are related to prompt development leading to increased lateral leaf area [e.g. Poni et al. (2006), Intrieri et al. (2008), Tardaguila et al. (2010), Palliotti et al. (2012)] or by the positive effects on bud differentiation promoted by the improved light microclimate within the renewal zone of the canopy (Sanchez and Dokoozlian 2005).

A likely inhibition of bunch compaction and reduction in grape yield following ED could make this practice a valuable alternative to conventional CT techniques. In some ecological conditions, however, such as the Mediterranean unirrigated vineyards, the frequent water and heat stress can reduce or even inhibit the lateral leaf area development. This will prevent compensation for the reduced leaf area by defoliation and, hence, may impact negatively bud fertility by lowering plant carbohydrate reserves (Risco et al. 2014). Furthermore, the combination of a reduction in lateral leaf area development with the defoliation of basal leaves promotes bunch overexposure during the summer, increasing vulnerability to berry sunburn (Lopes et al. 2019). Additionally, during hot days, the risk of compromising berry composition increases because of the negative impact of high temperature on malic acid degradation rate (Bergqvist et al. 2001) and to a reduction in anthocyanin accumulation (Bonada et al. 2015, Zarrouk et al. 2016).

The aim of this study was to test the effectiveness of manual leaf removal pre-flowering as an alternative to CT for limiting crop yield and for promoting indirect tolerance to bunch rot of the red Aragonez (Tempranillo) under unirrigated and humid growing conditions in west Portugal. The study was designed to evaluate the effect of ED and CT on vegetative growth, yield components and berry health and composition over three seasons.

Material and methods

Experimental details

The experiment was conducted over three consecutive growing seasons (2013–2015) in a commercial rain-fed vineyard, located near Merceana, within the Alenquer appellation of the Lisbon winegrowing region (39°05′40″N, 9°07′56″W). Ten-year-old *Vitis vinifera* L. cv. Aragonez (synonym Tempranillo) grapevines, grafted onto SO4 rootstock with rows oriented north–south and row and vine spacing of 2.5 m × 1 m, respectively, were used for the study. Vines were spur-pruned on a unilateral royat cordon with a bud load of approximately 12 nodes per vine (six two-node spurs) and trained to a vertically shoot positioned trellis system with two pairs of movable wires forming a vertical canopy wall with an approximate height of 1.4 m above the cordon. During spring [stage E-L 12, modified Eichhorn and Lorenz system (Coombe 1995)], shoot thinning was applied to adjust shoot number to the number of count nodes retained at winter pruning. The canopy was manually trimmed once a season when the shoots were around 25 cm above the top foliage wire (between the stages of berry pea size and bunch closure). Aside from defoliation and CT, standard local commercial vineyard growing practices were applied to all treatments. No fungicide sprays for botrytis bunch rot control were applied. The climate is Mediterranean type with an Atlantic influence. Weather data were recorded by an automatic weather station located within the experimental vineyard. The soil has a clay loam texture, a pH (H_2O) of 6.8 and 1.7% organic matter.

The experimental design was a randomised complete block with three treatments and four replicates per treatment. An ED or CT treatment was compared to an untreated Control (C). The ED treatment entailed the removal of six primary basal leaves and any presented laterals within the first six nodes from the base of each primary shoot before flowering (stage E-L 19). The CT treatment entailed the removal of the distal bunch from shoots with two intact bunches at the onset of veraison, therefore, leaving each shoot with the basal bunch only. As standard practice used by the grower the CT treatment also comprised a low intensity defoliation (within one to three node shoot zone) at the east side of the canopy, performed at bunch closure (stage E-L 32).

Each replicate consisted of three contiguous rows of 50 m length, where the treatments were assigned randomly, one per each row. From each row, ten representative vines were tagged (40 vines per treatment) and used for measurement. Treatments were applied on the same vines over the three seasons.

Vegetative growth and canopy density

In each season, leaf area per shoot was assessed periodically, by measuring 32 representative shoots (fruitful shoots of average length) per treatment (eight per replicate) from flowering onwards non-destructively after Lopes and Pinto (2005). In order to estimate the leaf area removed by canopy management practices, leaf area was assessed just prior and immediately after performing the practice. Leaf area per vine was estimated by multiplying the average leaf area per shoot by the average shoot number. Canopy density was assessed in the 2014 and 2015 seasons, at full veraison (stage E-L 36) by point quadrat analysis (Smart and Robinson 1991), using 80 insertions per treatment (20 per replicate) in the bunch zone. In winter, shoot number and fresh 1-year-old pruning mass were recorded on a per vine basis.

Reproductive traits

Bud fertility, expressed as the number of inflorescences per burst node, was assessed before flowering on the tagged vines by counting the number of inflorescences on the shoots emerging from the count nodes retained with winter pruning. This assessment was also performed during the spring of 2016 (year 4 after the start of the experiment) with the aim to evaluate potential carry-over effects on bud fertility. Fruitset was assessed for eight shoots across four vines per replicate. Four of the eight shoots contained one inflorescence while the rest contained two inflorescences. To assess the number of flowers, the inflorescences were tagged and enclosed with fine mesh bags before flowering which were removed after fruitset to allow the counting of flower caps, as described by Keller et al. (2001).

At harvest tagged bunches were individually picked, weighed and destemmed. From each bunch, live green ovaries were discarded and the berries were separated into three classes, according to the main type of berries found: 'normal berries' (healthy, all size berries), dried berries (dehydrated/sunburned berries) and rotten berries. For each class, berry number and berry fresh mass were recorded. Fruitset was calculated as the ratio between total number of berries (sum of the three classes) and the number of flowers estimated from the number of caps collected in the mesh bags and expressed as a proportion (Dry et al. 2010). Bunch compactness was estimated on the same bunches, as the number of berries per cm of main rachis length (Hed et al. 2009). At harvest, the yield was measured on the tagged vines by recording the number of bunches and their total fresh mass.

Berry composition and bunch rot infection

For analysis of berry composition, at harvest, a sample of 200 berries per replicate was collected from both sides of the canopy and from the top, front, back and bottom of bunches located in positions across the entire fruiting zone. Berry fresh mass, TSS and pH were assessed according to the Organisation Internationale de la Vigne et du Vin procedures (Organisation Internationale de la Vigne et du Vin 1990). Total phenols and total anthocyanins were extracted from whole berries and berry skins, respectively. Briefly, 1 mL of methanol was added to 100 mg of the fresh ground tissue, gently agitated in the dark and at room temperature for 2 h, before being centrifuged at 4°C for 15 min at 16 100 *g*. The supernatant was removed and one additional extraction for 30 min was conducted. The two supernatants were then mixed. Total phenols were quantified by the Folin Ciocalteau method with modification as described by Zarrouk et al. (2012) and expressed as gallic acid equivalents. Total monomeric anthocyanin was quantified by the pH-differential method as reported by Giusti and Wrolstad (2001) and expressed as malvidin equivalents.

At harvest, botrytis bunch rot incidence (% infected bunches) and severity (% of infected berries per bunch) were assessed in each tagged bunch used for assessment of fruitset.

Data analysis

Each season ANOVA was done separately using the GLM procedures from the SAS program package (SAS Institute, Cary, NC, USA). Treatment was included as a fixed effect and block as a random effect. Statistical differences between means were assessed by Tukey HSD test at $P < 0.05$. Data from botrytis bunch rot incidence and severity (expressed as a proportion) were subjected to a square root transformation prior to analysis (Gomez and Gomez 1984).

Results

Weather conditions and phenology

Budburst (50%) occurred at the end of March in 2014 and 2015 and in the beginning of April in 2013 (Table 1). Flowering (50%) occurred during late May in 2014 and in 2015, and at the beginning of June in 2013. Veraison (50%) was recorded by the end of July in 2013 and in 2014, whereas it occurred a few weeks earlier during the 2015 season. Commercial harvest occurred during the second half of September for the three seasons (Table 1).

Growing degree days (GDD) varied little across seasons while accumulated annual rainfall (1 October to 30 September) varied among seasons, with 2014 experiencing the highest annual rainfall and 2013 and 2015 having a similar amount (Table 1). Rainfall between budburst and fruit maturity accounted for 15–37% of the annual rainfall per season. The seasons 2013 and 2015 were the driest growing seasons and 2014 was the wettest, with 51 mm (21%) occurring in September, before harvest (Table 1).

Vegetative growth and canopy density

Removing the first six main leaves reduced ED primary shoot leaf area (PLA) by 78, 73 and 74% in 2013, 2014 and 2015, respectively, as compared to that of the Control. In the three seasons, from the day after leaf removal onwards, ED vines had significantly lower PLA (Figure 1a–c). From flowering to defoliation and trimming dates (pea size to

Table 1 Date and day of the year of main phenological stages, growing degree days and rainfall for the three seasons of the experiment (2013–2015).

Season	Budburst (E-L 3)†	Flowering (E-L 19)	Veraison (E-L 23)	Harvest (E-L 38)	GDD (°C)	Rainfall (mm) Annual‡	Rainfall (mm) Growing season§
2013	3 April (93)	3 June (154)	28 July (209)	26 September (269)	1552.6	584.1	150.5
2014	24 March (83)	25 May (145)	28 July (209)	29 September (272)	1517.8	648.3	239.1
2015	26 March (85)	20 May (140)	9 July (190)	23 September (266)	1558.1	574.4	88.3

†Modified Eichhorn and Lorenz phenological stage (Coombe 1995); ‡Annual accumulated rainfall (October–September); §Accumulated rainfall from budburst to harvest. GDD, growing degree days–active heat summation calculated using a baseline of 10°C from budburst to harvest.

berry closure stages) CT PLA did not differ from that of the Control. After trimming and defoliation CT showed slightly lower PLA than the Control in 2013 (Figure 1a–c).

In all treatments and for the 2014 and 2015 seasons lateral leaf area (LLA) increased from flowering to veraison and plateaued thereafter. In 2013 LLA increased until mid-ripening (Figure 1d–f). In the three seasons, LLA for ED was lower only at bunch closure (2013 and 2014) and during ripening in 2015 (Figure 1d–f).

In all treatments and seasons, total leaf area (TLA) increased from flowering to the bunch closure stage and, thereafter, a slight decrease was observed after trimming (and after defoliation in CT). Total leaf area increased again until veraison and a plateau was attained thereafter, except in 2013 where it continued to increase until mid-ripening in the Control and ED treatments (Figure 1d–f). Due to the lack of LLA compensation in ED, the relative difference in TLA among treatments mirrored those described for PLA.

Because of the shoot thinning applied in spring for adjusting shoot number to the number of count nodes retained at winter pruning, shoot number per vine was similar for all treatments during all three seasons. Therefore, when analysing LA on a per vine basis, similar results to the ones reported for shoot LA were obtained (Table 2). For example, the vine TLA values at mid-ripening were significantly lower in ED vines as compared to the other two treatments. Shoot LA for CT vines was not significantly different from the Control. Despite the differences reported above in LLA, no significant difference was obtained between treatments when evaluated as a proportion of the TLA (Table 2).

Average pruning mass was lowest in 2013 and highest in 2014 (Table 2). With the exception of the first season, ED induced a significant reduction in vine pruning mass as compared to the other two treatments. As shoot number per vine presented similar values in all treatments, changes in individual cane mass mirrored those reported for vine pruning mass (Table 2).

As expected, defoliation induced a significant difference in canopy density at the bunch zone in 2014 and 2015 (data from 2013 are not available), with the value of the leaf layer number being lowest for ED vines and highest for the Control vines (Table 2).

Yield and yield components

As compared to the other two treatments, ED had a lower per cent fruitset in 2013 and 2015, but not in 2014 (P value = 0.191; Table 3). No significant difference in bud fertility was detected in the first season, however, in the following two seasons, ED bud fertility was significantly lower than the other treatments (Table 3). This pattern was maintained during 2016 (year 4 after experiment began), where the ED selected vines presented a significantly (P value = 0.017) lower fertility than the Control (1.0 vs 1.6 inflorescences/count node, respectively).

Berry number per bunch varied with season and was generally lower for ED (Table 3). In the three seasons, ED treatment reduced berry and bunch mass (Table 3).

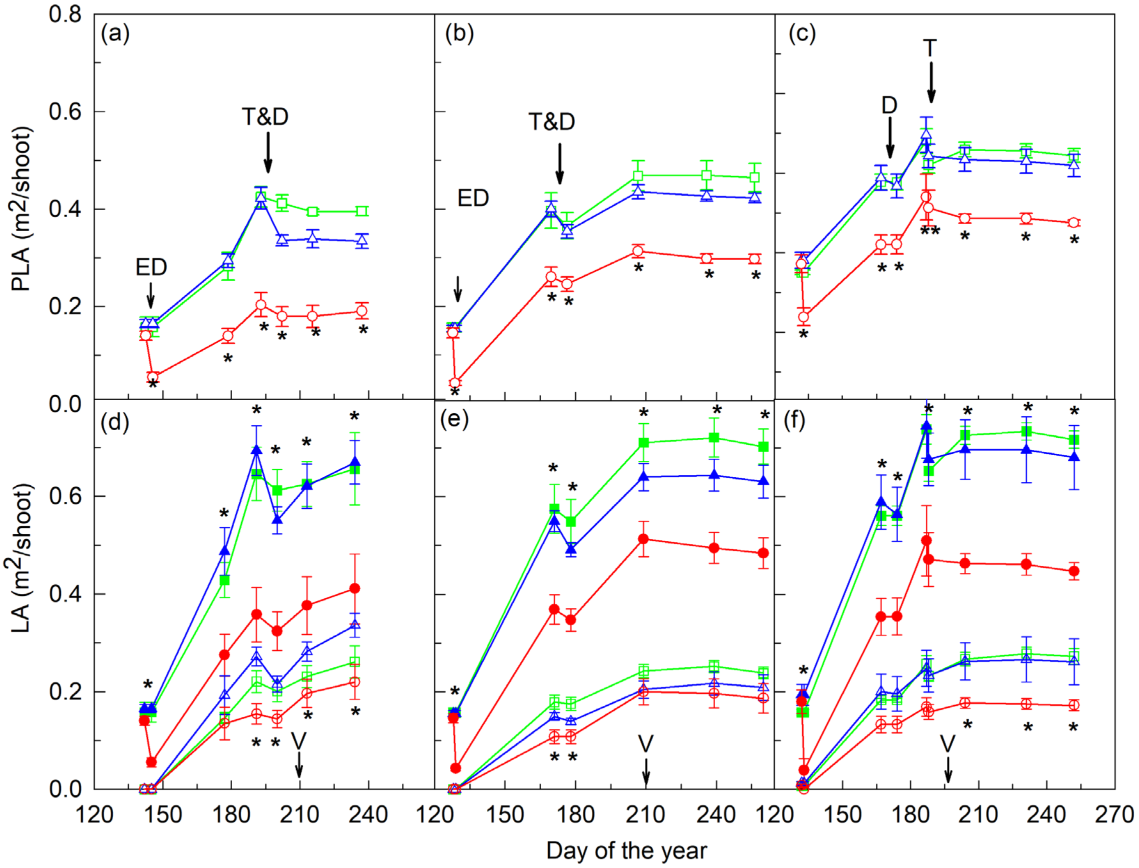

Figure 1. (a–c) Primary (PLA), (d–f) total (TLA) (●, ▲, ■) and lateral (LLA), (○, △, □) shoot leaf area (LA) evolution throughout the three growing seasons [(a,d) 2013; (b,e) 2014 and (c,f) 2015]. C, control untreated (■, □); CT, crop thinning (▲, △); ED, early defoliation (●, ○); D, defoliation (1–3 node shoot zone) at the east side of the canopy; T, trimming; V, veraison. Vertical bars indicate SE ($n = 32$) and * indicates significance at $P < 0.05$. (d,e,f) At the bottom panels the asterisks above the markers refer to TLA while those below the markers refer to LLA.

Table 2 Influence of early defoliation at pre-flowering and crop thinning on vegetative growth and leaf layer number assessed at full veraison at bunch zone for cv. Aragonez.

Year/ treatment	Shoots/ vine	Primary LA† (m^2/vine)	Total LA† (m^2/vine)	Lateral LA† (%)	LLN‡	Pruning mass (kg/vine)	Cane mass (g)
2013							
C	11.3	4.5a	7.4a	37.1	nd	0.80	70.7
CT	11.6	3.5b	6.4ab	44.3	nd	0.79	68.6
ED	11.6	2.2c	4.8b	48.9	nd	0.74	63.4
P-value	0.682	<0.001	0.049	0.448	—	0.388	0.361
2014							
C	11.6	5.4a	8.3a	34.0	2.5a	1.07a	92.7a
CT	11.3	5.0a	7.5a	32.8	1.6b	1.04a	92.3a
ED	11.6	3.5b	5.7b	37.0	0.5c	0.91b	78.2b
P-value	0.631	<0.001	0.015	0.549	<0.001	0.038	0.049
2015							
C	11.6	5.3a	8.5a	37.9	2.7a	0.98a	84.5a
CT	11.2	4.8a	7.8a	37.6	1.7b	0.93a	84.1a
ED	12.5	3.6b	5.8b	37.9	0.7c	0.81b	65.3b
P-value	0.062	0.017	0.022	0.987	0.001	0.025	0.013

In each column and per each year, values with a different letter show a statistically significant difference at $P < 0.05$ by Tukey HSD test; nd, no data. †Assessed on a per vine basis (n = 16); ‡Assessed by point quadrat methodology (Smart and Robinson 1991). C, Control untreated; CT, crop thinning; ED, early defoliation at pre-flowering; LA, leaf area assessed at mid-ripening; LLN, leaf layer number.

Table 3 Influence of early defoliation at pre-flowering and crop thinning on cv. Aragonez per cent fruitset, yield components, yield and leaf to fruit ratio.

Year/ treatment	Fruit set† (%)	BF (bunch/ shoot)	Berries/ bunch†	Berry FM† (g)	Bunch FM‡ (g)	Bunch/ vine‡	Yield‡ (kg/vine)	LA/yield§ (m^2/kg)
2013								
C	42.8a	1.3	147.8	1.90a	255.7a	16.7a	4.2a	1.8b
CT	44.1a	1.4	159.5	1.67a	240.7a	10.2b	2.4b	2.6a
ED	24.2b	1.3	109.9	1.30b	155.0b	15.6a	2.4b	2.0b
P-value	0.011	0.932	0.425	0.015	0.002	<0.001	<0.001	0.033
2014								
C	52.1	1.2a	136.9a	2.75a	275.0a	13.4a	3.7a	2.2b
CT	50.6	1.1a	120.3ab	2.52a	286.0a	8.5b	2.4b	3.1ab
ED	42.7	0.9b	102.3b	1.97b	136.7b	10.2b	1.4c	4.3a
P-value	0.191	0.017	0.049	0.002	<0.001	0.004	<0.001	0.049
2015								
C	50.2a	1.2a	141.3a	2.43a	307.1a	12.8a	3.9a	2.2c
CT	46.2a	1.1a	143.7a	2.37a	282.4a	8.4b	2.4b	3.4b
ED	32.1b	0.7b	96.3b	1.88b	118.6b	8.7b	1.0c	5.7a
P-value	0.005	0.001	0.012	0.014	<0.001	<0.001	<0.001	<0.001

In each column and per each year, values with a different letter show a statistically significant difference at $P < 0.05$ by Tukey HSD test. †Assessed on a shoot basis (n = 32); ‡Assessed on a per vine basis (n = 40); §Leaf area assessed at mid-ripening. BF, bud fertility; C, control untreated; CT, crop thinning; ED, early defoliation at pre-flowering; FM, fresh mass; LA, leaf area.

Compared to the Control, ED showed a significantly lower bunch number, except for the first season. In the three seasons, CT reduced bunch number compared to Control vines. Bunch numbers for CT vines were similar to those for ED vines, except in 2013, where bunch number per vine was higher for ED vines (Table 3).

Across all seasons, the Control had the highest yield (15.7 t/ha, 3 year mean). The yield of the other two treatments was similar in the first season (2013), however, in the subsequent seasons, ED vines yielded lower than CT vines (Table 3). In the first season, the leaf area to yield ratio showed a significantly higher value in the CT treatment as compared to the other two treatments. In the following two seasons, however, ED vines exhibited significantly the highest leaf area to yield ratio while the Control vines had the lowest leaf area to yield ratio (Table 3).

Berry composition, bunch compactness and botrytis infection

At harvest, TSS was similar among treatments except for 2015, where TSS for ED was higher (+0.7 to 1.0°Brix) than that of the other two treatments (Table 4). In all seasons, TA was significantly higher in the Control compared to that of the ED treatment, which had the lowest value. In all seasons, the pH of the ED must was higher than that of the Control (not significant in 2014; *P* value = 0.073), while that of the CT was intermediate in value except for 2013, where it was significantly higher than the Control (Table 4). Total berry skin anthocyanin and total phenols were similar for treatments, except for 2013 where CT vines had higher total phenols than other vines (Table 4).

Bunch compactness was always lower in ED vines in 2014 and 2015 (Table 4). In 2013 and 2015 the pressure of botrytis bunch rot infection was low, with the Control vines having the highest botrytis incidence and severity while the other two treatments had no (2013) or few symptoms (2015). In 2014, because of the heavy rains that occurred during the second half of the ripening period (51 mm in the first 3 weeks of September, just before harvest) the incidence and severity of botrytis bunch rot infection was high in all treatments (Table 4).

Table 4 Influence of early defoliation at pre-flowering and crop thinning on cv. Aragonez grape composition, bunch compactness and botrytis infection at harvest.

Year/ treatment	TSS (°Brix)	TA (g tartaric acid/L)	pH	Anthocyanins (mg malvidin equiv./g skin FM)	Total phenols (g GAE/g FM)	Bunch compactness†	Botrytis	
							Incidence (%)†	Severity (%)‡
2013								
C	22.0	5.76a	3.34b	313.3	4.0b	9.3	1.13a	19.5a
CT	22.5	4.75b	3.46a	344.6	5.4a	8.7	0.0b	0.0b
ED	22.5	4.77b	3.41ab	352.4	3.9b	7.2	0.0b	0.0b
P-value	0.251	0.015	0.015	0.275	0.048	0.527	0.458	0.017
2014								
C	22.7	5.63a	3.25	247.9	3.1	10.8a	85.1	47.4
CT	23.5	5.34ab	3.33	222.9	3.4	9.8ab	84.7	43.7
ED	23.6	5.18b	3.38	252.4	3.7	8.5b	77.2	36.4
P-value	0.585	0.046	0.073	0.373	0.336	0.020	0.577	0.191
2015								
C	21.3b	4.95a	3.25b	312.3	2.7	10.2a	6.25a	1.18a
CT	21.6b	4.91a	3.32b	nd	nd	10.2a	4.12a	0.78a
ED	22.3a	4.46b	3.39a	324.6	2.8	7.4b	0.00b	0.00b
P-value	0.023	0.040	0.002	0.498	0.824	0.001	0.024	0.022

In each column and per each year, values with a different letter show a statistically significant difference at $P < 0.05$ by Tukey HSD test; nd, no data. †Incidence expressed as a proportion of infected bunches; ‡Severity expressed as a proportion of berries with visual symptoms. C, control untreated; CT, crop thinning; ED, early defoliation at pre-flowering; FM, fresh mass; GAE, gallic acid equivalents.

Discussion

Vegetative growth

At the time of leaf removal, pulling the first six primary leaves and any laterals induced a drastic reduction in actual leaf area per shoot (~70%, 3 years mean). Unlike other defoliation studies [e.g. Poni et al. (2006), Alessandrini et al. (2018)], in our experiment the post-flowering lateral formation was insufficient to compensate the amount of LA removed by the defoliation. Hence, ripeness was achieved with a significantly lower TLA in ED vines as compared to that in the other treatments. Similar results were reported by Tardaguila et al. (2010), who observed no apparent compensation of leaf area in an ED experiment with the cvs Graciano and Carignan at La Rioja, Spain. Also, Bubola et al. (2017) and Smith and Centinari (2019), in similar experiments with the *V. vinifera* cvs Teran and Grüner Veltliner reported that canopy growth for ED treated vines did not compensate for the leaf area removed. Our results might be explained by the low lateral leaf area growth observed in ED shoots, presumably because of the removal of all presented laterals in the six basal nodes, combined with the likely negative effects of the mild water stress (Table 1) on the formation and growth of new lateral shoots. Indeed, the years 2013 and 2015 were considered dry with low rainfall during winter and spring not allowing the full refilling of soil water (data not shown). Low soil water availability during spring and summer is known to reduce, or even inhibit, vine vegetative growth (Williams and Matthews 1990).

Despite the likely increased photosynthetic rate of the remaining leaves (Vasconcelos and Koblet 1990, Poni et al. 2006, Palliotti et al. 2011), the absence of LA compensation in ED vines indicates that the intensity of applied defoliation might have been too severe, suggesting the need to test a lower defoliation intensity.

The optimal threshold for defoliation intensity to regulate fruitset appears to be also cultivar dependent. In most of the ED experiments, the severity of defoliation ranged from six to eight basal leaves [e.g. Poni et al. (2006, 2009) Intrieri et al. (2008) Tardaguila et al. (2010, 2012) Palliotti et al. (2011, 2012)], an intensity that has induced an adequate carbon source limitation to trigger a significant reduction in fruitset. Acimov et al. (2016) tested five intensities of defoliation in the cv. Pinot Noir under cool climate conditions and identified the defoliation of eight basal nodes as the threshold to induce a significantly lower fruitset. Lower defoliation intensities were also tested, but not all were successful in reducing fruitset. While Alessandrini et al. (2018) were able to obtain a significant reduction of fruitset by removing only five basal leaves in the cv. Semillon, in another experiment using different defoliation intensities Sabbatini and Howell (2010) observed that four leaves appeared insufficient to induce a source limitation stress to significantly reduce fruitset in Pinot Noir.

In our study, in the CT treatment, as the defoliation was done only on one side of the canopy and with low intensity, the effect on LA was not significant as compared to Control vines (with the exception of 2013), indicating a minimal effect on source size. Furthermore, the plateau reached by LA during the ripening period (Figure 1) combined with the absence of pruning mass differences relatively to the Control, showed that no vegetative growth compensation occurred in response to CT.

In the ED treatment, dormant cane mass, one of the best indicators of vine vigour, was also significantly reduced in the last two seasons, ranging from 15% in 2014 to 17% in 2015 relative to that of the Control. These results are likely because of the negative impact of the lower leaf area of ED on canopy photosynthesis causing lower carbohydrate reserves in grapevine woody canes at the end of the growing season (Candolfi-Vasconcelos et al. 1994, Holzapfel et al. 2010, Silva et al. 2017). Despite this reduction in vigour, however, the values of ED cane mass are still above the threshold (20–40 g) proposed by Smart et al. (1990) for optimal vigour.

In the ED vines, canopy density at the bunch zone (assessed by leaf layer number during berry ripening), was significantly reduced as compared to the Control, because of the strong defoliation intensity and the lower post-flowering formation and growth of lateral shoots. These results are in line with the majority of the reports on defoliation [e.g. Smart et al. (1990), Hunter et al. (1991), Dokoozlian and Kliewer (1996), Spayd et al. (2002), Poni et al. (2009), Diago et al. (2012), Gatti et al. (2012), Alessandrini et al. (2018)], showing that basal defoliation is a strong tool to manipulate canopy microclimate and, therefore, improve bunch zone light microclimate and aeration.

Reproductive growth

The yield of the Control vines was the highest and was stable across the three seasons and higher than the limit imposed by the appellation rules for PDO red wines [80 hL/ha (Instituto da Vinha e do Vinho 2018)]. In the three seasons, the ED treatment promoted a strong source limitation at pre-flowering, which significantly reduced fruitset, berry number and mass, bunch mass and yield, as compared to the other two treatments (Table 3). These results are consistent with previous studies at different sites and with different cultivars [e.g. Poni et al. (2006, 2009) Intrieri et al. (2008) Tardaguila et al. (2010)] and are explained by the known negative impacts on fruitset induced by carbon starvation promoted by the defoliation of the most photosynthetically active leaves (Poni et al. 2008). In all treatments, fruitset was variable among bunches (CV = 49, 29 and 40% for pooled data of 2013–2015, respectively). Besides the natural variability of this trait (May 2004), bunch position on the shoot may have also contributed to this variability as, in general, the basal bunch showed lower fruitset as compared to the distal one (data not shown), as also reported by Eltom et al. (2017). Also, the magnitude of the fruitset reduction induced by ED relatively to the Control was quite variable among seasons, ranging from 18% (2013 and 2015) to 9% (2014). This reduction in fruitset observed in the ED vines, resulted in fewer berries per bunch and consequently, lower bunch mass as no compensation effects on berry mass were observed. Indeed, in the three seasons, ED berry mass also showed lower values than the other two treatments, results that can be attributed to the negative effect of the early removal of mature basal leaves on berry size, as reported previously [e.g. Poni et al. (2006, 2008) Intrieri et al. (2008) Tardaguila et al. (2010)].

Both crop-regulating techniques raised the leaf area-to-yield ratio when compared to the Control. Furthermore, ED also reduced bud fertility (except for the first season) indicating a carry-over effect likely promoted by reduced availability of carbohydrate during the initiation and differentiation of inflorescence primordia (Hunter and Visser 1990, Vasconcelos and Koblet 1990) caused by source limitation. This negative carry-over effect was also observed in other ED experiments, mainly when using a more severe defoliation intensity [e.g. Sabbatini and Howell (2010), Risco et al. (2014), Acimov et al. (2016), Noyce et al. (2016), Verdenal et al. (2018)].

In our experiment, it appears that the negative impact of the source limitation of the ED treatment was not fully offset by the likely positive effects of the improved light microclimate on the fertility of the basal buds retained at winter pruning for next season's crop (Intrieri et al. 2008). The negative effect of ED on bud fertility progressed with the season, ranging from −24% in 2014 to −32% in 2015, relative to the Control. Carry-over effects were even stronger in the fourth season, where a 42% reduction was observed. Low bunch number, combined with the reduction in berry number and mass, led to a progressively greater yield reduction for ED vines over time (−43, −62 and −74% relative to the Control in 2013, 2014 and 2015, respectively), indicating that the defoliation intensity applied was extremely severe. A lower intensity of primary leaf removal combined with the retention of the laterals on the basal nodes should be tested in order to achieve the optimal level of source limitation to induce an adequate decrease in fruitset and bunch compactness without impacting negatively bud fertility.

Crop thinning of the CT treatment induced a quite stable yield reduction (57–64% of the Control) over the three seasons, meeting the legal limitations of 80 hL/ha imposed by the appellation rules (Instituto da Vinha e do Vinho 2018). The thinning of all distal bunches induced a decline in bunch number at harvest, ranging from 34 (2015) to 39% (2013) relative to the unthinned Control, without any compensation on other bunch components. Indeed, the absence of a significant difference of berry and bunch mass relative to the Control shows that the yield compensation mechanisms (heavier retained bunches with larger berries and lower skin-to-pulp ratio), expected from CT (Morris et al. 1987, Keller et al. 2005, Petrie and Clingeleffer 2006, Gatti et al. 2012), did not occur in this study. This absence of effects on CT yield components, which might be explained by the above mentioned impact of the mild water stress on vegetative and reproductive growth, suggests that CT is a more effective yield-regulating technique under the study conditions. As CT did not affect bunch mass, the yield decline relative to the Control (−39% 3 year mean) mirrored that of the decline in bunch number (Table 1). This response is consistent with other studies [e.g. Keller et al. (2005), Petrie and Clingeleffer (2006)], indicating that CT was an effective practice in reducing grape yield. As the yield loss, however, was not compensated by significant improvements in berry composition there is no financial reward for the growers.

When comparing the effect on the yield of the two crop-regulating techniques applied in our experiment, only in the first season (2013) did both techniques produce a similar reduction in yield (57% of the Control). In the following seasons, while the yield reduction in the CT vines was maintained (65 and 62% of the Control, respectively, in 2014 and 2015), in ED vines a trend of continuous decline was observed, with yield being 38 and 26% of the Control, respectively, in 2014 and 2015. The yield components responsible for the differences among ED and CT treatments in the last two seasons were berry number and berry mass, which were lower in ED vines. These results suggest that, between the two crop-regulating techniques tested, and for the intensity of the ED and CT techniques used in our study, CT is a better approach to achieving the legal yield limitations imposed for PDO wines in the Alenquer appellation. The large yield reduction seen in 2014 and 2015 for ED treated vines is economically unacceptable.

Berry composition and botrytis infection

Compared to the Control, the effects of ED and CT on berry composition were minimal. These results are likely because of the high leaf area-to-yield ratio achieved in all the three treatments, which were all above the threshold required for maximum level of TSS, berry mass, and berry colouration at harvest for single-canopy type trellis systems [0.8–1.2 m^2/kg (Kliewer and Dokoozlian 2005)] (Table 3). Must TSS was significantly higher in ED vines only in 2015, however, the low absolute difference between treatments (+ 0.7° and

1.0°Brix for CT and C, respectively) appears too small to be taken into account. The TA and pH were the must parameters that consistently were significantly different in ED vines throughout the three seasons, respectively, recording lower and higher values relative to the Control, results that can be explained by the enhancement of malic acid degradation rate caused by the high bunch sun exposure (Esteban et al. 1999, Bergqvist et al. 2001). This significant decrease in TA of the ED must can be considered a negative outcome of this crop regulating technique for our Mediterranean climate conditions, where maintenance of sufficient acid concentration is becoming more difficult because of global warming (Schultz and Stoll 2010).

The enhancement of anthocyanins and total phenols in berry skin has been reported for red grape cultivars subjected either to ED [e.g. Intrieri et al. (2008), Palliotti et al. (2012), Tardaguila et al. (2010), Diago et al. (2012), Gatti et al. (2012)] or to CT (Keller et al. 2005, Petrie and Clingeleffer 2006). In our experiment, however, despite the significant yield reduction and the lower leaf layer number in the bunch zone, we were unable to detect any significant enhancement in the concentration of total phenols of ED or CT berries at harvest. The absence of response of berry total anthocyanins in ED vines, also reported by Acimov et al. (2016), could be attributed to the likely detrimental effect of excessive berry exposure and high temperature on the anthocyanin biosynthetic pathway (Spayd et al. 2002, Downey et al. 2004, Zarrouk et al. 2016). Also, the higher leaf to fruit-ratio observed either in ED and CT vines did not improve the concentration of berry skin total anthocyanins and total phenols. Therefore, without any enhancement of fruit composition there is little incentive for growers to apply those techniques.

Due to the lower per cent fruitset, bunch compactness of ED was the lowest for all treatments (a 3 year mean reduction of 24% relative to the Control). Lower bunch compactness is often associated with less susceptibility to bunch rot (Hed et al. 2009). These likely effects could justify the absence of botrytis bunch rot infection in ED bunches during the dry harvest seasons of 2013 and 2015. In contrast to these seasons, the unusually wet and hot September (harvest month) in 2014 induced a high incidence and severity of botrytis bunch rot infection in all treatments (Table 4). These results suggest that ED enhances berry health (Poni et al. 2006, Intrieri et al. 2008, Tardaguila et al. 2010, Palliotti et al. 2012) but only when the weather conditions are not too favourable for the disease development. Indeed, when the ripening period was wet and the temperature was adequate to promote the fungi development, the advantages of the less compact and more exposed bunches of ED were ineffective in lowering bunch rot development as compared with Control vines.

Conclusions

In this study two crop regulating techniques, namely ED and CT, were compared with an untreated Control throughout three growing seasons in order to evaluate ED as an alternative to CT for regulating crop yield while improving tolerance to bunch rot of the *V. vinifera* L. cv. Aragonez (Tempranillo).

The results indicate that, under the Alenquer conditions, and for the treatment intensities used in the study, ED should not be considered as viable alternative to the costly and time-consuming conventional CT. Furthermore, as both crop-regulating techniques have only minimally improved fruit composition, there is no other incentive for growers to use any of the crop regulating techniques at the intensity tested in our experiment, besides the achievement of the legal yield limitations imposed for PDO wines in this appellation.

Acknowledgements

This research received funding from the European Community's Seventh Framework Programme (FP7/2007–2013), grant agreement n° 311775, Project Innovine. Dr Olfa Zarrouk was supported by postdoctoral fellowships from INNOVINE and Fundação para a Ciência e a Tecnologia (SFRH/BPD/111693/2015). Part of this work was supported by Fundação para a Ciência e a Tecnologia', through R&D Unit, UID/Multi/04551/2013 (GreenIT). We also thank the estate Quinta do Pinto for the experimental vineyard facilities.

References

Acimov, D., Tozzini, L., Green, A., Sivilotti, P. and 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. and 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.

Bennett, J., Javis, P., Creasy, G.L. and Trought, M.C.T. (2005) Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature Chardonnay grapevines. American Journal of Enology and Viticulture **56**, 386–393.

Bergqvist, J., Dokoozlian, N. and Ebisuda, N. (2001) Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. American Journal of Enology and Viticulture **52**, 1–7.

Bonada, M., Jeffrey, D.W., Petrie, P.R., Moran, M.A. and Sadras, V. O. (2015) Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. Australian Journal of Grape and Wine Research **21**, 240–253.

Bubola, M., Sivilotti, P., Janjanin, D. and Poni, S. (2017) Early leaf removal has larger effect than cluster thinning on cv. Teran grape phenolic composition. American Journal of Enology and Viticulture **68**, 234–242.

Candolfi-Vasconcelos, M.C., Candolfi, M.P. and Koblet, W. (1994) Retranslocation of carbon reserves from the woody storage tissues into the fruit as a response to defoliation stress during the ripening period in *Vitis vinifera* L. Planta **192**, 567–573.

Coombe, B.G. (1995) Adoption of a system for identifying grapevine growth stages. Australian Journal of Grape and Wine Research **1**, 104–110.

Diago, M.P., Vilanova, M. and Tardáguila, J. (2010) Effects of timing of manual and mechanical early defoliation on the aroma of *Vitis vinifera* L. Tempranillo wine. American Journal of Enology and Viticulture **61**, 382–391.

Diago, M.P., Ayestarán, B., Guadalupe, Z., Poni, S. and Tardáguila, J. (2012) Impact of prebloom and fruit set basal leaf removal on the flavonol and anthocyanin composition of Tempranillo grapes. American Journal of Enology and Viticulture **63**, 367–376.

Dokoozlian, N.K. and Kliewer, W.M. (1996) Influence of light on grape berry growth and composition varies during fruit development. Journal of the American Society for Horticultural Science **121**, 869–874.

Downey, M.O., Harvey, J.S. and 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.

Dry, P.R., Longbottom, M.L., McLoughlin, S., Johnson, T.E. and Collins, C. (2010) Classification of reproductive performance of ten winegrape varieties. Australian Journal of Grape and Wine Research **16**, 47–55.

Eltom, M., Trought, M.C.T., Agnew, R., Parker, A. and Winefield, C.S. (2017) Pre-budburst temperature influences the inner and outer arm morphology, phenology, flower number, fruitset, TSS accumulation and variability of *Vitis vinifera* L. Sauvignon Blanc bunches. Australian Journal of Grape and Wine Research **23**, 280–286.

Esteban, M.A., Villanueva, M.J. and Lissarrague, J.R. (1999) Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids and mineral elements. American Journal of Enology and Viticulture **50**, 418–433.

Frioni, T., Acimovic, D., Tombesi, S., Sivilotti, P., Palliotti, A., Poni, S. and Sabbatini, P. (2018) Changes in within-shoot carbon partitioning in Pinot Noir grapevines subjected to early basal leaf removal. Frontiers in Plant Science **9**, 1–11.

Gatti, M., Bernizzoni, F., Civardi, S. and Poni, S. (2012) Effects of cluster thinning and pre-flowering leaf removal on growth and grape composition in cv. Sangiovese. American Journal of Enology and Viticulture **63**, 325–332.

Giusti, M.M. and Wrolstad, R.E. (2001) Anthocyanins: characterization and measurement with UV–visible spectroscopy. Wrolstad, R. E., ed. Current protocols in food analytical chemistry (Wiley Publishers: New York, NY, USA) pp. F1.2.1–1.2.13.

Gomez, K.A. and Gomez, A.A. (1984) Statistical procedures for agricultural research (John Wiley: New York, NY, USA).

Hed, B. and Centenari, M. (2018) Hand and mechanical fruit-zone leaf removal at prebloom and fruitset was more effective in reducing crop yield than reducing bunch rot in 'Riesling' grapevines. HorTechnology **28**, 296–303.

Hed, B., Ngugi, H.K. and Travis, J.W. (2009) Relationship between cluster compactness and bunch rot in Vignoles grapes. Plant Disease **93**, 1195–1201.

Holzapfel, B.P., Smith, J.P., Field, S.K. and Hardie, W.J. (2010) Dynamics of carbohydrate reserves in cultivated grapevines and vine growth. Horticultural Reviews **37**, 143–211.

Hunter, J.J. and Visser, J.E. (1990) The effect of partial defoliation on growth characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon II. Reproductive growth. South African Journal of Enology and Viticulture **11**, 26–32.

Hunter, J.J., De Villiers, O.T. and Watts, J.E. (1991) The effect of partial defoliation on quality characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. II. Skin color, skin sugar, and wine quality. American Journal of Enology and Viticulture **42**, 13–18.

Instituto da Vinha e do Vinho (2011) Catálogo das castas para vinho cultivadas em Portugal (Instituto da Vinha e do Vinho: Lisboa, Portugal).

Instituto da Vinha e do Vinho (2018) Anuário 2017. Vinhos e Aguardentes de Portugal (Instituto da Vinha e do Vinho: Lisboa, Portugal).

Intrieri, C., Filippetti, I., Allegro, G., Centinari, M. and 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.

Keller, M., Kummer, M. and Vasconcelos, M.C. (2001) Reproductive growth of grapevines in response to nitrogen supply and rootstock. Australian Journal of Grape and Wine Research **7**, 12–18.

Keller, M., Mills, L.J., Wample, R.L. and Spayd, S.E. (2005) Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. American Journal of Enology and Viticulture **56**, 91–103.

Kliewer, W.M. and Dokoozlian, N.K. (2005) Leaf areal crop weight ratios of grapevines: influence on fruit composition and wine quality. American Journal of Enology and Viticulture **56**, 170–181.

Lopes, C.M. and Pinto, P.A. (2005) Easy and accurate estimation of grapevine leaf area with simple mathematical models. Vitis **44**, 55–61.

Lopes, C.M., Vendeiro, M., Egipto, R., Zarrouk, O. and Chaves, M.M. (2019) Is early defoliation a sustainable management practice for Mediterranean vineyards? Case studies at the Portuguese Lisbon winegrowing region. Koundouras, S., ed. Proceedings 21st GiESCO International meeting: a multidisciplinary vision towards sustainable viticulture; 23–28 June 2019; Thessaloniki, Greece (Group of international Experts of Vitivinicultural Systems for Co-operation: Montpellier, France) pp. 462–467.

May, P. (2000) From bud to berry, with special reference to inflorescence and bunch morphology in *Vitis vinifera* L. Australian Journal of Grape and Wine Research **6**, 82–98.

May, P. (2004) Flowering and fruitset in grapevines (Lythrum Press: Adelaide, SA, Australia).

Morris, J.R., Sims, C.A., Striegler, R.K., Cackler, S.D. and Donley, R. A. (1987) Effects of cultivar, maturity, bunch thinning, and excessive potassium fertilization on yield and quality of Arkansas wine grapes. American Journal of Enology and Viticulture **38**, 260–264.

Noyce, P.W., Steel, C.C., Harper, J.D.I. and Wood, R.M. (2016) The basis of defoliation effects on reproductive parameters in *Vitis vinifera* L. cv. Chardonnay lies in the latent bud. American Journal of Enology and Viticulture **67**, 199–205.

Organisation Internationale de la Vigne et du Vin (1990) Recueil des méthodes internationales d'analyses des vins et des moûts (Organisation Internationale de la Vigne et du Vin: Paris, France).

Palliotti, A., Gatti, M. and Poni, S. (2011) Early leaf removal to improve vineyard efficiency: gas exchange, source-to-sink balance, and reserve storage responses. American Journal of Enology and Viticulture **62**, 219–228.

Palliotti, A., Gardi, T., Berrios, J., Civardi, S. and 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.

Petrie, P.R. and Clingeleffer, P.R. (2006) Crop thinning (hand *versus* mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet Sauvignon (*Vitis vinifera* L.) in a warm climate. Australian Journal of Grape and Wine Research **12**, 21–29.

Poni, S., Bernizzoni, F. and Civardi, S. (2008) The effect of early leaf removal on whole-canopy gas-exchange and vine performance of *Vitis vinifera* L. 'Sangiovese'. Vitis **47**, 1–6.

Poni, S., Bernizzoni, F., Civardi, S. and 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. and Intrieri, C. (2006) Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. American Journal of Enology and Viticulture **57**, 397–407.

Reis, R.M.M. and Gonçalves, M.Z. (1981) O clima de Portugal. Caracterização climática da região do Ribatejo e Oeste (Instituto Nacional de Meteorologia e Geofísica: Lisboa, Portugal).

Risco, D., Perez, D., Yeves, A., Castel, J.R. and 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.

Sabbatini, P. and Howell, G.S. (2010) Effects of early defoliation on yield, fruit composition, and harvest season cluster rot complex of grapevines. HortScience **45**, 1804–1808.

Sanchez, L.A. and Dokoozlian, N.K. (2005) Bud microclimate and fruitfulness in *Vitis vinifera* L. American Journal of Enology and Viticulture **56**, 319–329.

Schultz, H.R. and Stoll, M. (2010) Some critical issues in environmental physiology of grapevines: future challenges and current limitations. Australian Journal of Grape and Wine Research **16**, 4–24.

Silva, M.J.N.C.G. (2010) Yield control on the grapevine variety Aragonez. MSc thesis, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal.

Silva, A., Noronha, H., Dai, Z., Delrot, S. and Gerós, H. (2017) Low source–sink ratio reduces reserve starch in grapevine woody canes and modulates sugar transport and metabolism at transcriptional and enzyme activity levels. Planta **246**, 525–535.

Smart, R.E. and Robinson, M. (1991) Sunlight into wine. A handbook for winegrape canopy management (Winetitles: Adelaide, SA, Australia).

Smart, R.E., Dick, J.K., Gravett, I.M. and Fisher, B.M. (1990) Canopy management to improve grape yield and wine quality – principles and practices. South African Journal of Enology and Viticulture **11**, 3–17.

Smith, M.S. and Centinari, M. (2019) Impacts of early leaf removal and cluster thinning on Grüner Veltliner production, fruit composition, and vine health. American Journal of Enology and Viticulture **70**, 308–317.

Smith, J.P. and Holzapfel, B.P. (2009) Cumulative responses of Semillon grapevines to late season perturbation of carbohydrate reserve status. American Journal of Enology and Viticulture **60**, 461–470.

Spayd, S.E., Tarara, J.M., Mee, D.L. and Ferguson, J.C. (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. American Journal of Enology and Viticulture **53**, 171–182.

Tardáguila, J., Blanco, J.A., Poni, S. and Diago, M.P. (2012) Mechanical yield regulation in winegrapes: comparison of early leaf removal and crop thinning. Australian Journal of Grape and Wine Research **18**, 344–352.

Tardáguila, J., de Toda, F.M., Poni, S. and Diago, M.P. (2010) Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. American Journal of Enology and Viticulture **61**, 372–381.

Tardáguila, J., Petrie, P.R., Poni, S., Diago, M.P. and de Toda, F.M. (2008) Effects of mechanical thinning on yield and fruit composition of Tempranillo and Grenache grapes trained to a vertical shoot-positioned canopy. American Journal of Enology and Viticulture **59**, 412–417.

Vasconcelos, M.C. and Koblet, W. (1990) Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera*. Evidence of compensation and stress recovering. Vitis **29**, 199–221.

Vasconcelos, M.C., Greven, M., Winefield, C.S., Trought, M.C.T. and Raw, V. (2009) The flowering process of *Vitis vinifera*: a review. American Journal of Enology and Viticulture **60**, 411–433.

Verdenal, T., Zufferey, V., Dienes-Nagy, A., Belcher, S., Lorenzini, F., Rösti, J., Koestel, C., Gindro, K. and Spring, J.L. (2018) Intensity and timing of defoliation on white cultivar Chasselas under the temperate climate of Switzerland. Oeno One **52**, 93–104.

Williams, L.E. and Matthews, M.A. (1990) Grapevine. Stewart, B.A. and Nielsen, D.R., eds. Irrigation of agricultural crops (American Society of Agronomy: Madison, WI, USA) pp. 1019–1055.

Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., Lopes, C.M., Tattini, M. and 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. and 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.

Zhuang, S., Tozzini, L., Green, A., Acimovic, A., Howell, G.S., Castellarin, S.D. and Sabbatini, P. (2014) Impact of cluster thinning and basal leaf removal on fruit quality of Cabernet Franc (*Vitis vinifera* L.) grapevines grown in cool climate conditions. HortScience **49**, 750–756.

Manuscript received: 19 January 2020
Revised manuscript received: 31 March 2020
Accepted: 10 April 2020