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Response of Tannat (*Vitis vinifera* L.) to pre-flowering leaf removal in a humid climate

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ABSTRACT

The aim of this study was to test the effect of pre-flowering leaf removal (PFD) on grape sanitary status, yield, source-sink balance and berry composition. A completely random block design experiment was conducted over four seasons in a commercial vineyard of the Tannat/SO4 combination located in the south of Uruguay (34° 35´30 S, 56° 15´23 O). The PFD treatment was compared with a commercial defoliation (CC) comprising partial leaf removal between the fruit set and cluster closure stages (four leaves from the first node), a traditional practice carried out by winegrowers. Both treatments received equal phytosanitary management during the study. The results were conditioned by seasonal meteorological conditions, particularly rainfall, temperature and evapotranspiration. The seasonal effect was significant for yield components, berry composition and source-sink balance. The PFD treatment resulted in the lowest yields in all four years and modified the primary and secondary composition of berries. It also showed a higher anthocyanin potential in 2016, 2017 and 2019, related to a higher percentage of exposed clusters, lower yield and/or higher leaf to fruit ratio. In 2017, environmental conditions were more favourable for the development of bunch rot, and lower values of incidence (3 %) were recorded for the PFD treatment compared to CC (22 %), due to greater exposure of bunches and less compact bunches. The PFD vines showed a steady reduction in dry matter production capacity, mainly explained by the steady reduction in yields. The Tannat variety showed a differential response to PFD depending on the weather during the growth cycle. Under humid climate conditions, pre-flowering defoliation proved to be effective for yield control, by reducing bunch rot incidence, and for improving some attributes in terms of grape composition. This work provides useful information for grape growers in humid regions on how to improve the sanitary status and quality of their production.

KEYWORDS

leaf removal, season effect, yield, bunch rot, source-sink, grape composition

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INTRODUCTION

In viticultural regions with humid and sub-humid climate, canopy management is a determining factor for the quality and sanitary status of the harvest (Smart *et al.,* 1985; Zoecklein *et al.,* 1992). In this context, excessive vegetative growth can result in the generation of a microclimate within the bunch zone which can have a negative effect on the development of grape quality attributes. Dense canopies decrease light interception and modify its distribution, thus reducing photosynthetic activity and negatively impacting yield and grape composition (Smart and Robinson, 1991; Zoecklein *et al.,* 1992). In addition, high relative humidity and poor airflow within the canopy can increase the risk of bunch rot incidence, such as the grey mould caused by *Botrytis cinerea* (English *et al.,* 1989).

Leaf removal is a useful technique for ensuring good grape quality at harvest. In cases of excessive vigour, this technique can improve the ripening process while contributing to reducing rot incidence on clusters (Smart and Robinson, 1991; Molitor *et al.,* 2011). Timing and intensity are two important factors which influence the impact of leaf removal on plant response (Verdenal *et al.,* 2018). Early leaf removal has been proved to be a suitable technique for improving grape quality and reducing sanitary risks in many wine regions of the northern hemisphere (Poni *et al.,* 2006; Tardaguila *et al.,* 2010; Gatti *et al.,* 2012; Risco *et al.,* 2013).

Pre-flowering leaf removal (PFD) has been evaluated by several researchers for its potential as an alternative to cluster thinning for yield control and improving grape quality (Poni *et al.,* 2006; Tardaguila *et al.,* 2010; Sabbatini and Howell, 2010; Gatti *et al.,* 2012; Risco *et al.,* 2013; Verdenal *et al.,* 2017). Leaf removal at the blooming stage is associated with an increase in the abscission rate of flowers and/or reproductive structures likes ovules, thereby notoriously affecting fruit set, as well as yield (Candolfi-Vasconcelos *et al.,* 1994; Caspari and Lang, 1996). Moreover, undesirable effects can arise when leaf removal is practiced at an early stage of the growth cycle, including: sunburn, excessive yield reduction in the current and the following season, and plant vigour decline (Sabbatini and Howell, 2010; Risco *et al.,* 2013). Modifications on grape composition have been linked to higher leaf/fruit balance, higher skin/pulp ratio, modifications to the microclimate caused by a higher proportion of clusters exposed to sunlight, and greater photosynthetic efficiency of the remaining leaves (Poni *et al.,* 2006; Poni *et al.,* 2008; Palliotti *et al.,* 2011; Risco *et al.,* 2013).

Increased exposure of bunches to sunlight, together with the possible reduction of their compactness through pre-flowering leaf removal, creates an unfavourable microclimate for the development of rot-causing fungi, such as *Botrytis cinerea*, thereby improving the effectiveness of chemical control (Molitor *et al.,* 2015; Sternad Lemut *et al.,* 2015). Furthermore, the best sunlight exposition of the bunches could promote a modification to berry thickness and favour the biosynthesis of phytoalexins, which can lead to a better resistance to sunburn damage and infections caused by *B. cinerea* and other fungi (Percival *et al.,* 1994; Verdenal *et al.,* 2017).

Leaf removal induces modifications to the source-sink balance, which can result in important changes to the main physiological responses of vine in the short- and medium-term, thus affecting grape composition (Kliewer and Dokoozlian, 2005), reserves (Vaillant-Gaveau *et al.,* 2014), root growth (Hunter *et al.,* 1995), photosynthesis (Poni *et al.,* 2008; Palliotti *et al.,* 2011) and water use (Medrano *et al.,* 2007).

Tannat grafted on SO4 is the most implanted combination in Uruguayan viticulture (INAVI, 2019) – (http://www.inavi.com.uy/estadisticas/). Tannat is a red grape cultivar with medium-high vigour and high yield potential, which is susceptible to *Botrytis* rot (Ferrer *et al.,* 2009). From an oenological point of view, its grapes have a high sugar accumulation potential, good phenolic richness and moderate acidity levels (González-Neves *et al.,* 2004). According to the Köppen-Geiger classification, Uruguay has a template-humid and moderately rainy climate Cfa, with weather conditions of high interannual variability (Tiscornia *et al.,* 2016). Climate change scenarios have indicated a possible increase in this variability, as well as in rainfall during spring and summer (Giménez *et al.,* 2009). This could lead to an increase in phytosanitary risks, such as cluster rot incidence, which may limit yield and grape quality under Uruguayan conditions. In this context, pre-flowering leaf removal could be a useful viticultural technique for cluster rot management, yield control and additional improvements to grape composition. Therefore, the main objective of this work was to assess the PFD effects on the Tannat variety (*Vitis vinifera* L.) in terms of cluster rot incidence, yield, vegetative growth, grape composition and physiological balance indicators.

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MATERIALS AND METHODS

1.Plant material, soil and weather data

The experiment was carried out in a commercial non-irrigated vineyard in the Juanico locality (34° 35´30 S, 56° 15´23 W) from 2016 to 2019. The vineyard comprises a combination of the Tannat variety (clone 398) grafted onto SO4 rootstock (*V. berlandieri x V. riparia*), and trellised using vertical shoot positioning (VSP) and a double Guyot pruning system. The vineyard was 18 years old at the beginning of the experiment. The vines were spaced 1.25 m between plants and 2.5 m between rows and orientated towards the NW-SE. Spontaneous vegetation in the inter-row space was mechanically removed, while within-row vegetation was controlled by applying herbicide. Soil water availability was calculated on a monthly basis using a hydric balance according to MCC (Multicriterial Classification System) methodology adapted to Uruguay conditions (Ferrer *et al.,* 2007). The soil at this site is typical Argiudoll with a slope of less than 3 % and 110 mm of maximum water availability (Silva *et al.,* 2018). Both treatments received equal phytosanitary management during the study. Weather data were obtained from the INIA Gras platform (http://www.inia.uy/gras/) from INIA Las Brujas meteorological station located 11 km from the experimental site.

2. Experimental design and treatments

The randomised experimental design comprised three complete blocks with two treatments: commercial defoliation (CC), comprising the removal of 4 basal leaves between fruit set and bunch closure, and a pre-flowering leaf removal treatment (PFD) performed at stage 17 (Coombe, 1995). PFD was carried out by hand: 6-8 leaves were removed from the base to the top of the shoot, and the secondary shoot was also removed when present. Leaf removal in CC was carried out by the winegrower in all years of the evaluation. The treatments were replicated three times (blocks) on seven plants per experimental unit (n = 7). This experiment was carried out consecutively during the 2015-2016 (first) and 2016-2017 (second) seasons. For administrative purposes it was necessary to change the plants in 2018. Therefore, a new consecutive evaluation was carried out in the 2017-2018 (third) and 2018-2019 (fourth) seasons.

3. Canopy measurements and microclimate evaluation

Potential exposed leaf area (SA) was assessed at veraison according to Carbonneau (1995). Three plants per block and treatment were measured and photographed. The porosity of the canopy was estimated using the free software CobCal®. In addition, the percentage of exposed clusters and number of leaf layers was determined at veraison in the bunch zone on four plants per block (stage 35, Coombe, 1995) using the Point Quadrat method (Smart and Robinson, 1991). In 2016-2017, the microclimate was assessed using sensors. Photosynthetically active radiation (PAR) was calculated from light intensity recordings using a HOBO Pendant® 8K Data Logger UA-002-08; one sensor per treatment was placed in the bunch zone on a representative plant of the same experimental block. Hourly temperature and relative humidity within the canopy were recorded using a sensor HOBO® U23 ProV2 in each block. The average hourly readings of three sensors were calculated from bunch closure until harvest. Each sensor was positioned in the bunch zone, on one representative plant of each treatment and block.

4.Yield components, cluster compactness and cluster rot incidence

All plants from both treatments were harvested on the same date, which was chosen based on STT, acidity and pH values as proposed by González-Neves *et al.* (2004). At harvest, yield per vine (including rotten bunches), number of bunches per vine and berry weight were determined. Cluster rot incidence was evaluated based on the proportion of rotten bunches (%). Cluster compactness was assessed on ten clusters randomly sampled from each replication during pre-harvest. The density index of Ipach *et al.* (2005) was used to classify individual clusters according to their compactness. The average density index was calculated and compared between treatments. In addition, the mode of each treatment was calculated (DI. m). Bud fertility was evaluated the year after treatment by counting the number of inflorescences in principal shoot in all plants per block, this evaluation was made between 19-27 of Eichhorn y Lorenz modified system (Coombe, 1995).

5. Physiological indexes

In winter, the pruning weight was determined for all plants and the following physiological

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indices were calculated. Ravaz index (RI; Equation 1) (Ravaz, 1909). According to Ferrer *et al.* (1997), RI values from 7 to 10 are considered the equilibrium for the Tannat variety in Uruguayan environmental conditions. Leaf to fruit ratio (LF) is an important balance indicator for the relationship between leaf area and yield per vine. In our study we used exposed leaf area (SA, m^2/vine) to yield (kg/vine) as an indicator (Equation 2). According to Carbonneau and Cargnello (2003), the dry matter partitioning coefficients (DMPC) give a good estimation of the total renewable dry matter produced by the plant, as long as there is a good relationship between aboveground and belowground development. DMPC is therefore a good indicator of the plant's physiology and represents the basic level of metabolic activity achieved by the whole plant to ensure its development and consumption. DMPC is measured by the sum of the weight of pruning wood multiplied by 0.5, a coefficient that expresses the average percentage of dry matter in the wood, and the harvest weight multiplied by 0.2, a coefficient that expresses the average percentage of dry matter in the bunches. The relationship between the SA and DMPC (PB) is a good estimator of the plant's potential for investing energy in the primary and secondary metabolism processes during the crop cycle (Gómez del Campo *et al.,* 2002).

- RI = Yield/pruning weight
  [Equation Nº1]

- LF = SA (m^2)/Fruit weight (kg)
  [Equation Nº2]

- DMPC = Pruning weight (kg) * 0.5 + Yield (kg) *0.2
  [Equation Nº3]

- PB (m^2/kg dry matter) = SA (m^2)/DMPC (kg)
  [Equation Nº 4]

6. Grape composition

From veraison (stage 35 E and L modified) until harvest (stage 38 E and L modified), 250 berries per replication were collected on a weekly basis according to the protocol by Carbonneau *et al.* (1991). Juice from each sample was obtained with an electric blender (HR2290, Phillips, Amsterdam, The Netherlands). Following official methods (OIV, 2009), sugar content (TSS) was measured using a refractometer (Atago® N1, Atago®, Tokyo, Japan) and then transformed to sugar per litre of must using an equivalence table. The amount of sugar and anthocyanin was calculated on a per berry basis, according to Vila *et al.* (2010):

- g sugar per berry (S per berry): soluble solids (g/L) per berry weight (g)/(0.0046 x Brix + 0.9927)/1000
  [Equation Nº 5]

- g anthocyanin per berry (A*b): anthocyanin ApH1 (mg/L) per berry weight (g)/(0.0046 x Brix + 0.9927)/1000
  [Equation Nº 6]

pH was determined with a potentiometer (HI8521, Hanna Instruments®, Villafranca Padovana, Italy) and acidity was measured by titration, expressed as g tartaric/L of juice. Organic acids (tartaric and malic) were determined at harvest by high performance liquid chromatography (HPLC) using a Shimadzu L-10ADvp pump and photodiode array detector (DAD). After harvest, the phenolic potential of a 250-berry sample per replication was evaluated. Anthocyanin potential, phenolic richness and extractability were assessed according to Glories and Augustin, 1993. The dilution factor was calculated for each mash from the percentage of pulp in each sample according to González-Neves *et al.* (2004). A random sample of 50 berries per experimental unit from which the skin, pulp and seeds were separated (data not shown). The skin and seeds were weighed to obtain the pulp weight by difference, from that data the percentage of pulp was calculated. The must volume of each sample was considered for the concentration calculations of anthocyanin content. The must density was calculated using the Brix data. The anthocyanin content was determined by absorption spectrophotometry at 520 nm using an Unico®, S-2150 equipment (New Jersey, USA), and the values of concentration of pigments were expressed as milligram of equivalent of malvidin glucoside (EMG) per litre according to González-Neves *et al.* (2004).

- Dilution factor: (50 mL of solution + must volume)/must volume
  [Equation Nº 7]

- Must volume: (50 g of macerate* pulp percentage)/must density
  [Equation Nº 8]

7. Statistical analyses

One way ANOVA was carried out for the comparison of the treatment effect within the season. Two-way ANOVA was applied using

the treatment and year, and their interaction, as factors. The mean comparison was carried out with an LSD Fisher test at 5 % significance for the *post hoc* test. Factorial analyses were performed with three levels of significance: 0.1 (*); 0.05 (**); 0.01 (***). Tests were performed to demonstrate compliance with the assumptions of the model (linearity, homoscedasticity, independence and normality). Non-linear regression was used for modelling the relationship between anthocyanin content and agronomical variables, such as yield and leaf to fruit ratio. The average value of each block and all years was taken into account for non-linear and linear regression analyses. The T-test was performed at 5 % significance level for the parameters of the models. Statistical analyses were carried out using the InfoStat 2018® and Origin Pro Lab 2019 b® software.

RESULTS

1. Meteorological conditions

The amount of rainfall and its distribution showed high interannual variability. Thus, the second and fourth seasons were characterised by high rainfall and an atmospheric demand below the historical records. In both seasons, the soil water availability was high during all the months of the growth cycle (Figure 1). The highest rainfall was recorded during the ripening period (January and February) of the second season, and in December and January of the fourth season. Conversely, the lowest rainfall was recorded during the growth cycle of the first and third seasons (Table 1). The first season was the driest, which can be explained mostly by low rainfall in January and February and atmospheric demand higher than historic values.

TABLE 1. Weather conditions during the experimental period.

Year	Season	Month	ETP (mm)	Var H	Rainfall (mm)	Var H	Tm (ºC)	Var H	TM (ºC)	Var H	T min (ºC)	Var H
2015	1st	September	81	+8	19	-64	12.2	-0.8	17.7	-0.7	7.0	-1.1
2015		October	108	-2	59	-51	14.4	-1.6	18.1	-3.5	9.9	-0.9
2015		November	146	+5	72	-36	18.1	-0.6	24.1	-0.4	12.2	-0.8
2015		December	177	+4	121	+38	21.8	+0.5	27.9	+0.4	15.6	+0.5
2016		January	187	+7	11	-96	23.4	+0.3	29.8	+0.6	17.2	+0.1
2016		February	158	+27	71	-39	24.2	+2.0	30.5	+2.5	18.3	+1.3
2016	2nd	September	62	-11	64	-19	12.4	-0.7	17.5	-1.0	7.9	-0.1
2016		October	90	-19	84	-27	16.0	-0.1	21.2	-0.4	11.3	+0.5
2016		November	123	-19	80	-29	18.7	-0.0	24.9	+0.3	12.5	-0.5
2016		December	150	-23	104	+21	22.2	+0.9	28.9	+1.2	15.8	+0.7
2017		January	141	-39	148	+42	23.2	+0.1	29.7	+0.5	17.3	+0.3
2017		February	111	-20	100	-12	23.8	+1.6	29.9	+2.0	19.0	+2.0
2017	3rd	September	64	-9	130	47	14.7	+1.6	19.7	+1.2	10.5	+2.4
2017		October	107	-3	98	-13	16.1	+0.0	21.6	0.0	10.5	-0.3
2017		November	142	0	34	-74	17.7	-1.0	24.5	0.0	11.3	-1.7
2017		December	173	-1	76	-7	22.0	+1.0	28.7	+1.3	15.4	+0.3
2018		January	170	-11	76	-30	23.3	+0.3	30.2	+1.0	17.0	+0.0
2018		February	139	+8	30	-82	22.4	+0.3	29.4	+1.5	16.2	-0.7
2018	4th	September	72	-2	104	+21	15.3	+2.2	21.1	+2.6	10.8	+2.8
2018		October	101	-9	15	-96	15.6	-0.5	21.3	-0.3	9.7	-1.0
2018		November	131	-11	84	-24	19.3	+0.6	25.3	+0.7	13.8	+0.8
2018		December	146	-27	286	+202	20.0	-1.2	25.7	-1.7	14.4	-0.7
2019		January	138	-43	184	+77	22.9	-0.2	28.0	-1.1	17.9	+0.8
2019		February	122	-9	52	-60	21.7	-0.4	27.1	-0.1	15.9	-1.0

ETP = potential evapotranspiration (Penman), Tm = average air temperature. TM = average maximum air temperature. T min: average minimum air temperature. Period: 1st September to 28 February. Var H = variation with respect to historic records (1984-2014). Source: Plataforma INIA Gras

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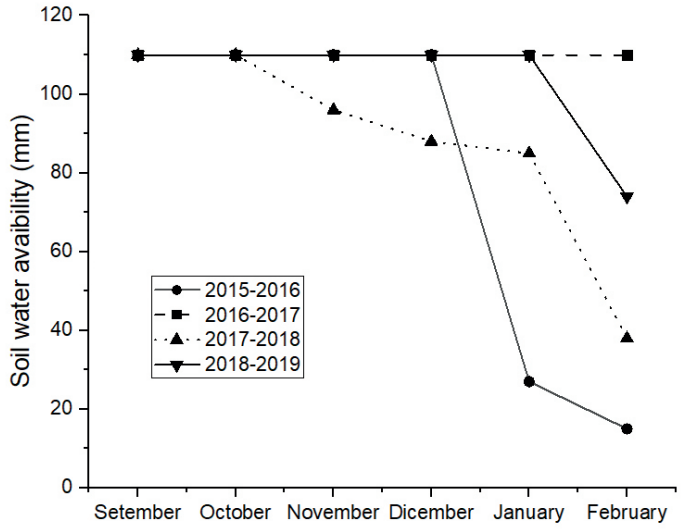


FIGURE 1. Estimation of monthly water storage in the soil according to season.
Period: 1st September to 28 February. Maximum water storage capacity: 110 mm. Soil depth: 120 cm (Silva *et al.*, 2018).

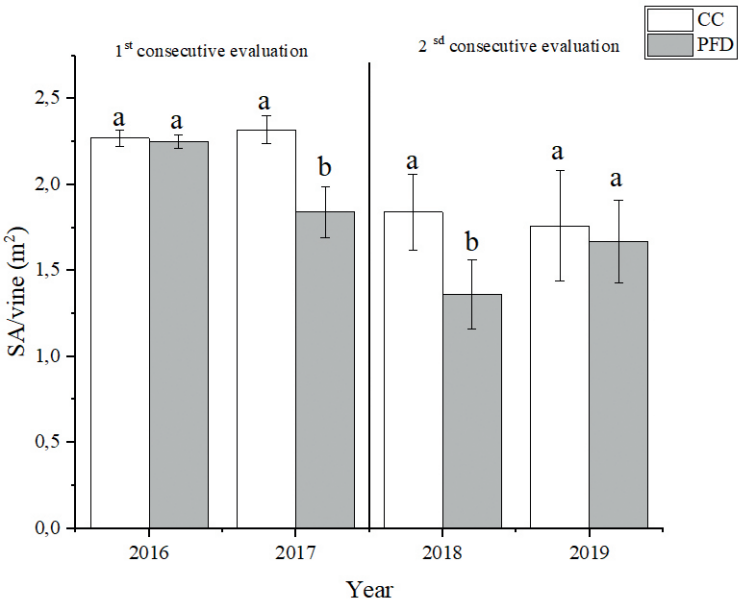


FIGURE 2. Potential exposed leaf area per vine (SA) according to treatment.

CC = Commercial control, PFD = Pre-flowering leaf removal in each year. Different letters on the columns for a given year indicate mean comparison by LSD Fisher for *post hoc* test (p-value ≤ 0.05). The vertical line indicates a change of plants. Each panel represents a consecutive evaluation on the same plants. Means ± S.E

This explains a large drop in soil water availability during the summer months as shown in Figure 1. The period 2017-2018 was dry too, with rainfall below historic records being recorded from October to January, causing a gradual decrease in water storage levels in the soil from October to harvest. In terms of temperature, with the exception of the fourth season, average air temperature was higher than historic values during the summer months, and the same trend was observed in terms of maximum average temperature.

2. Vegetative growth and microclimate conditions

During the first season in which PFD was carried out, the vines were able to recover leaf area as shown by exposed leaf area (SA) for each treatment and season (Figure 2). During the second season of consecutive leaf removal, PFD grapevines showed a significantly lower SA in comparison with CC vines. In the third season, there was lower SA on new plants subject to PFD than on the CC vines, and they did not recover leaf area.

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TABLE 2. Percentage of cluster exposure and number of leaf layers in bunch zone for control (CC) and pre-flowering leaf removal (PFD) Tannat grapevines in the 4 studied seasons.

Year	Treatment	Bunch exposure (%)	Number of leaf layers
2016	CC	75 **b**	1.7 **a**
	PFD	85 **a**	0.4 **b**
2017	CC	43 **b**	2,0 **a**
	PFD	88 **a**	0.5 **b**
2018	CC	52 **b**	2.8 **a**
	PFD	76 **a**	1.7 **b**
2019	CC	60 **b**	1.3 **a**
	PFD	81 **a**	1.5 **a**

Different letters for a given season indicate significant differences between treatments according to the LSD Fisher for *post hoc* test (p-value ≤ 0.05).

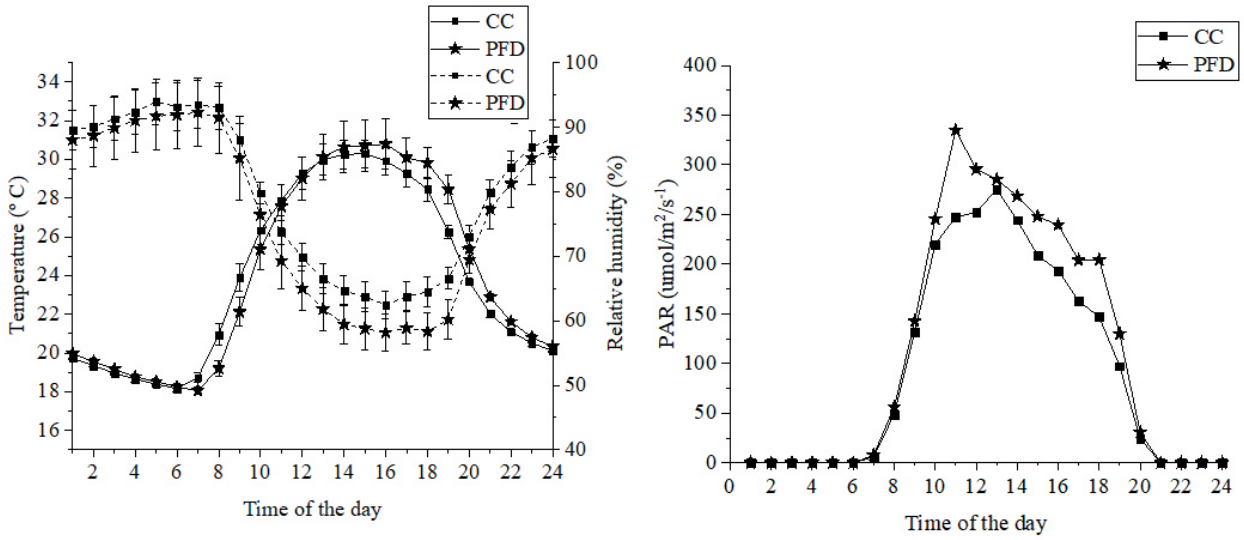


FIGURE 3. Microclimate conditions according to treatment during the 2016-2017 season.

A: Temperature (solid line) and relative humidity (dashed line) at bunch zone. B: Photosynthetic Active Radiation intercepted at bunch zone according to treatment, each dot represents average time of day. Each dot represents the hourly average from bunch closure to harvest. 90 days. Each dot represents hourly mean of three sensors ± D.E.

During the second consecutive application (2018-2019), PFD vines recovered leaf area. Cluster exposure was improved by PFD in all years of the study (Table 2) and PFD decreased the number of leaf layers in three out of the four years. During the second season, the microclimate was assessed and the highest temperature was recorded in the PFD canopy during early morning and afternoon hours. Relative humidity within the canopy decreased for PFD vines, while the light interception in the bunch zone increased as a result of early leaf removal (Figure 3).

2. Yield components, cluster rot incidence and cluster compactness

The lowest yields per vine were recorded for the PFD treatments in all four seasons of the study (Table 3). In the 2016 and 2017 vintages, the yield of PFD vines decreased significantly in comparison with CC: from 20 to 26 % respectively. In 2018 and 2019, the yields from PFD vines were 11 % (2018) to 39 % (2019) lower than those from CC vines. The PDF treatment mainly affected berry number per bunch, thus affecting bunch

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TABLE 3. Yield components, bud fertility and cluster compactness for Tannat grapevines subjected to control (CC) and pre-flowering leaf removal (PFD) in all seasons.

Year	Treatment	Yield (Kg/vine)	Bunch rot incidence (%)	Number of bunches	Bunch Weight (g)	Number of berries per bunch	Berry weight (g)	Density Index (average)	Density Index (mode)	Bud Fertility
2016	CC	7.5 **a**	0	21	403 **a**	233 **a**	1.7	3.8	3	1.4 **a**
	PFD	5.8 **b**	0	21	315 **b**	178 **b**	1.8	4.2	4	1.6 **b**
2017	CC	7.0 **a**	22.6 **a**	23	333 **a**	182 **a**	1.8	4.8 **a**	4	1.7
	PFD	5.2 **b**	3.2 b	24	245 **b**	130 **b**	1.9	3.8 **b**	3	1.7
Treatment		**	***	*ns*	***	***	*ns*	*ns*	-	**
Year		*ns*	**	**	***	***	*ns*	*ns*	-	*ns*
Y x T		*ns*	**	*ns*	*ns*	*ns*	*ns*	*	-	*ns*
2018	CC	5.3 **a**	3.9 **a**	19	254 **a**	145 **a**	1.8	3.5	4	1.7
	PFD	4.7 **b**	0 **b**	19	219 **b**	122 **b**	1.8	3.2	3	1.7
2019	CC	3.9 **a**	1,4	14	284 **a**	157 **a**	1.8 **a**	3.8	4	1.7 **b**
	PFD	2.3 **b**	0,8	12	201 **b**	101 **b**	2.0 **b**	3.6	3	1.9 **a**
Treatment		**	*ns*	*	**	**	*ns*	*ns*	-	*
Year		***	*ns*	***	*ns*	*ns*	*	*	-	*ns*
Y x T		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	-	*ns*

Density index: 1 (loose cluster) to 5 (high compactness of cluster) (Ipach *et al.,* 2005). Different letters for a given year indicate significant differences between treatments *post hoc* test by Fisher; no letters indicate absence of significance (p-value ≤ 0.05) Factorial analysis: treatment, year, T x Y: treatment per year at p-values ≤ 0.10 (*), 0,05 (**), 0.01 (***), *ns*: no significance. Different factorial ANOVA correspond to different vineyard sites.

weight, since berry weight only differed between treatments in 2019 and was greater for PFD. During the fourth season, a spring incidence of mildew caused by *Plasmopara viticola* was recorded (27 %), affecting the final number of clusters and, consequently, final yield per vine. With the exception of first year the mode value of the density index (D.I. m) observed for PFD plants was 3, whereas it was 4 for CC vines. Significant differences between treatments regarding the average values of the density index were only registered during the second season. According to the factorial analysis, the results on bunch compactness were not consistent. Bud fertility was increased by the PFD treatment in the first and fourth seasons (Table 3). The meteorological conditions of the 2017, 2018 and 2019 were favourable for grey mould incidence, with the highest absolute values of rot incidence being recorded during the 2017 vintage (Table 3). PDF decreased the bunch rot incidence in the second and third seasons. In the second season, the incidence level was negatively correlated with the percentage of cluster exposure (r = -0.81, p-value = 0.0002) and positively related to cluster compactness (r = 0.63, p-value = 0.08).

3. Physiological indexes

The PFD vines produced less dry mater (DMPC) than the CC vines in all the study years (Table 4). Taking into account the two pairs of seasons, DMPC decreased on average by 15 % in PFD vines compared to CC for the first leaf removal, while for the second it decreased by 29 %. Wood production was negatively affected by PDF. In 2017 and 2018, PFD vines showed lower pruning weight values than CC vines (Table 4), which was related to SA (r = 0.51, $p < 0.001$). Physiological behaviour (PB) was the highest for PDF plants in the two-consecutive evaluation. In the last two study years, the seasonal effect was significant for all the indices considered. In 2017-2018 the lowest vigour expression was found, as reflected by PW, RI and DMPC. The season per treatment interaction was significant for LF during second consecutive evaluation. Vines with a higher LF ratio showed a logarithmic response in terms of anthocyanin content per berry (R^2 = 0.77, p-value < 0.001), a particularly good association for PFD vines, which showed a highly significant linear relationship (R^2 = 0.81, p-value < 0.001).

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TABLE 4. Physiological indexes for the Tannat variety subjected to control (CC) and pre-flowering leaf removal (PFD) over all seasons.

Year	Treatment	Ravaz Index	Pruning weight (kg/vine)	Leaf to fruit ratio (m^2/kg)	Dry matter production coefficient (kg/vine)	Physiological behaviour (m^2 of SA/ kg of dry matter)
2016	CC	10.1	0.78	0.32 **b**	1.87 **a**	1.23 **b**
	PFD	8.9	0.73	0.40 **a**	1.58 **b**	1.52 **a**
2017	CC	8.8	1.00 **a**	0.37	2.17 **a**	1.18
	PFD	7.4	0.80 **b**	0.31	1.52 **b**	1.34
Treatment		*ns*	**	*	***	***
Year		**	***	*ns*	***	ns
T x Y		*ns*	*ns*	*ns*	*ns*	*ns*
2018	CC	11.7	0.49 **a**	0.33	1.32 **a**	1.31
	PFD	14.8	0.38 **b**	0.33	1.14 **b**	1.30
2019	CC	5.7	0.70	0.60 **b**	1.11 **a**	1.86 **b**
	PFD	4.2	0.65	0.86 **a**	0.81 **b**	2.36 **a**
Treatment		*ns*	**	*	***	***
Year		***	***	***	***	***
T x Y		*	*ns*	***	**	*ns*

Different letters for a given year indicate significant differences between treatments *post hoc* test by Fisher; no letters indicate absence of significance (p-value ≤ 0.05). Factorial analyse: treatment, year, T x Y: treatment per year at p-values ≤ 0.10 (*), 0,05 (**), 0.01 (***), *ns*: no significance. Different factorial ANOVA correspond to different vineyard sites.

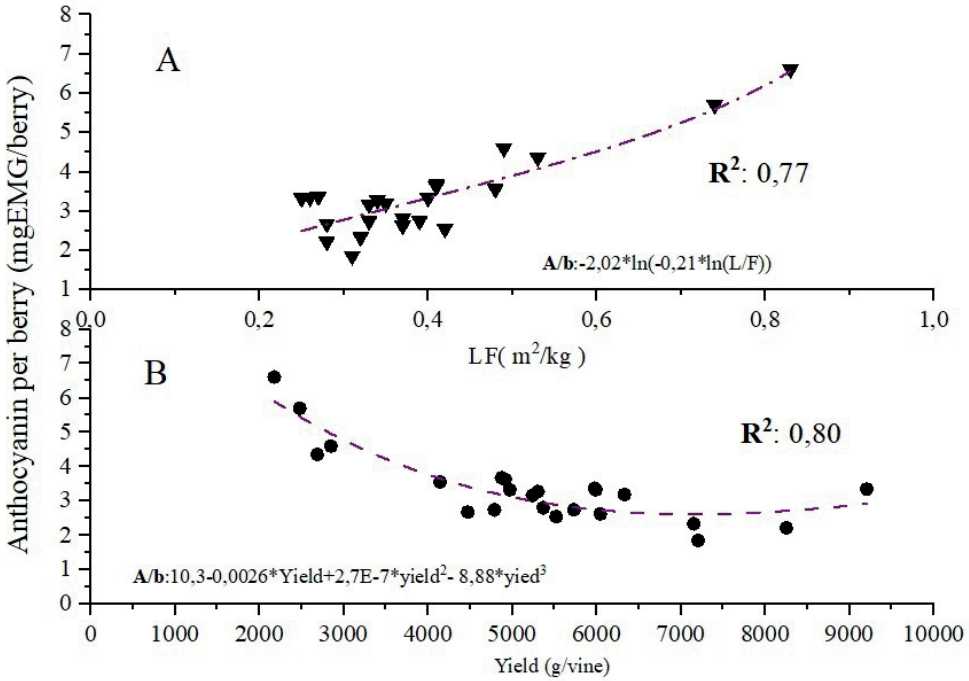


FIGURE 4. Anthocyanin grape potential (mg/berry) as a function of yield per vine and leaf to fruit ratio.

EMG: equivalent malvidin glucoside. Non-lineal-regression and linear polynomic model were fitted.

A: anthocyanin potential in berry (A/berry) as a function of leaf to fruit ratio.

B: anthocyanin potential in berry (A/berry) as a function of yield per vine

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TABLE 5. Composition of primary metabolites at harvest for the Tannat variety subjected to control (CC) and pre-flowering leaf removal (PFD) over all seasons.

Year	Treatment	Sugar per berry (mg/berry)	Total Soluble Solids (g/L)	Titratable acidity (g/L)	pH	Tartaric acid (g/L)	Malic acid (g/L)	M/T
2015	CC	331 **a**	226 **b**	6.4	3.60	4.89 **a**	7.70 **a**	1.57
	PFD	383 **b**	239 **a**	6.2	3.64	4.55 **b**	7.46 **b**	1.63
2017	CC	353	212 **b**	6.4	3.32	n/d	n/d	n/d
	PFD	391	232 **a**	6.2	3.30	n/d	n/d	n/d
Treatment		**	***	*ns*	*ns*	**	**	ns
Year		*ns*	*ns*	*ns*	***	-	-	-
T x Y		*ns*	*ns*	*ns*	*ns*	-	-	-
2018	CC	372	234	7.7	3.27	4.18	8.54	2.04 **a**
	PFD	381	235	8.1	3.34	4.50	8.45	1.87 **b**
2019	CC	357 **b**	217 **b**	7.7	3.32	3.32 **b**	7.76 **b**	2.33 **a**
	PFD	416 **a**	228 **a**	7.6	3.41	4.01 **a**	8.44 **a**	2.10 **b**
Treatment		**	*ns*	*ns*	**	***	*ns*	**
Year		*ns*	**	*ns*	**	***	**	**
T x Y		*ns*	*ns*	*ns*	*ns*	*ns*	**	*ns*

Tritable acidity = g of tartaric acid per litre, M/T = malic to tartaric acid ratio. Different letters for a given year indicate significant differences between treatments *post hoc* test by Fisher; no letters indicate absence of significance (p-value ≤ 0.05). Factorial analyse: treatment, season, T x Y : treatment per year at p-values ≤ 0.10 (*), 0,05 (**), 0.01 (***). *ns*: no significance. Different factorial ANOVA correspond to different vineyard sites.

TABLE 6. Composition of secondary metabolites at harvest for the Tannat variety subjected to control (CC) and pre-flowering leaf removal (PFD) over all seasons.

Year	Treatment	Anthocyanin Potential (mg EMG/L^{-1})	Anthocyanin Potential extractable (mg EMG/L)	Polyphenol Total Index (A280)	Extractability (%)
2016	CC	1339 **b**	661 **b**	44 **b**	50
	PFD	1638 **a**	830 **a**	54 **a**	52
2017	CC	1877 **b**	862 **b**	49 **b**	54 **a**
	PFD	1989 **a**	974 **a**	60 **a**	50 **b**
Treatment		***	***	**	*ns*
Year		***	***	**	*ns*
T x Y		*ns*	*ns*	*ns*	*
2018	CC	2059 **a**	948 **a**	62	53 **b**
	PFD	1792 **b**	681 **b**	62	59 **a**
2019	CC	1979 **b**	909 **b**	-	52
	PFD	3051 **a**	1361 **a**	-	55
Treatment		*ns*	*ns*	-	*
Year		***	**	-	**
T x Y		***	***	-	*ns*

EA (%) = extractability of anthocyanin. n/d = non data. Different letters for a given year indicate significant differences between treatments *post hoc* test by Fisher; no letters indicate absence of significance (p-value ≤ 0.05). Factorial analyses: treatment, year, T x Y: treatment per year at p-values ≤ 0.10 (*), 0,05 (**), 0.01 (***), *ns*: no significance. Different factorial ANOVA correspond to different vineyard sites.

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The relationships between evaluated physiological indices and other variables linked to primary metabolism like SST, pH and total acidity were not significant in our study (data not presented).

4. Grape composition at harvest

PFD always increased sugar content on a per berry basis (Table 5). The concentration of TSS in musts was improved by PDF in the first pair of years and in 2019. Titratable acidity was not affected by PDF and season. The tartaric and malic acid content were seasonal dependent. During 2016, CC obtained a higher malic and tartaric acid content, and the opposite response was observed for the 2019 harvest. In terms of anthocyanin concentration, the seasonal effect was clear, with a significant interaction between treatment and season being detected in the second consecutive evaluation (p-value < 0.01). In the first consecutive evaluation, PFD increased the phenolic richness and the anthocyanin potential of grapes. However, a different response was observed in the second consecutive evaluation. In 2018 harvest, the CC treatment obtained the highest value for anthocyanin potential and no difference in phenolic richness was detected. In 2019, PFD vines achieved higher anthocyanin potential (Table 6), and it was the year with the highest concentration of these compounds in the grape. Anthocyanin per berry content show a weak linear correlation with sugars per berry (R^2 : 0,09, p-value: 0.03). The potential of extractable anthocyanin was improved in 2017 and 2019. In 2017, the highest extractability (EA %) was detected in the PFD treatment, while the highest extractability was detected in 2018 in the CC treatment; the results of the factorial analysis did not show a consistent response in terms of this variable.

DISCUSSION

The weather conditions varied highly from year to year, which is common in Uruguay (Tiscornia *et al.,* 2016). The amount of rainfall during the cycle, and its distribution and thermal conditions during the different phenological periods, largely explain the yearly differences in yield, vegetative growth, sanitary status of harvest and grape composition (Ferrer *et al.,* 2020).

The highest levels in cluster rot incidence occurred in 2017, due to weather conditions (high rainfall during the ripening period) being favourable for grey mould development. During 2019, the largest amount of rainfall occurred between the 31st and 34th stages (Coombe, 1995), when the efficiency of the defence mechanisms of the berries was high, preventing *Botrytis cinerea* infections (Nair and Hill, 1992).

The incidence of bunch rot was notably reduced by PFD, in agreement with previous reports (Tardaguila *et al.,* 2010; Molitor *et al.,* 2011; Sternad Lemut *et al.,* 2015). The increased bunch exposure and decreased compactness in 2017 were negatively related to rot incidence levels, which is also in agreement with previous studies (Poni *et al.,* 2006; Molitor *et al.,* 2011). In general, decreasing bunch compactness caused by PFD was not consistent. According to Tello and Ibáñez (2018), the environmental conditions during the growth cycle are widely known to modulate this characteristic. A trend of increasing berry weight for the PFD treatment was observed, with differences only found in the last season; this could be due to an increase in bunch compactness and, consequently, in cluster rot susceptibility. Conversely, increased bunch exposure generated less favourable microclimatic conditions for the development of bunch rot (Table 2). Relative humidity levels were reduced during certain hours of the day; this was likely due to the higher incidence of solar radiation, resulting in an increase in temperature and in vapour pressure deficit within the bunch zone, thus decreasing the periods of wet tissue after a rain event (Figure 3). In addition, increased exposure improves airflow and the effectiveness of phytosanitary control (English *et al.,* 1989; Molitor *et al.,* 2015; Sternad Lemut *et al.,* 2015).

Rainfall amount and distribution determined vine vegetative growth capacity and recovery of leaf area losses caused by PFD (Figure 2), which occurred in the 2016 and 2019, as previously reported by Poni *et al.* (2006). Despite the conditions in the first season being the driest, the PFD vines were able to recover their leaf area. 74 % of the rainfall occurred during the first stages of vine development, a critical period for leaf area establishment (Champagnol, 1984). Furthermore, the water storage capacity of the soil was high until January, and it probably fulfilled the vine water requirements during the first stages of the growth cycle (Figure 1). In the second season, the absence of regrowth can be mainly explained by the continuity of PFD, the consecutive application inducing the carryover effect of a larger decrease in yield and low vigour, as confirmed by a decrease in DMPC and

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PW values (Table 4). In a regime of continuous PFD, it is possible that reserves are mobilised from perennial parts to make up for deficiencies caused by early leaf losses, leading to a significant decrease in yield and vigour across the seasons, as suggested by Candolfi-Vasconcelos *et al.* (1994) and Risco *et al.* (2013). Regarding the new plants in 2018, the absence of vegetative regrowth on PDF vines and lower vegetative expression can be explained by October to February rainfall being below historic values, affecting secondary shoot development. In the fourth season, low yields per plant and high water availability were both recorded from October until February, likely causing a shift in physiological balance towards the observed vegetative expression. In our study, the capacity for Tannat grapevines exposed to severe leaf removal in the early stages of the growth cycle to recover leaf area was conditioned by the interaction of prevailing environmental conditions during critical periods for leaf area establishment (particularly rainfall during the spring months) and vine capacity as expressed by the DMPC.

The drop in yield of PFD plants varied between 11 and 39 % when compared to control vines, which agrees with previous studies by Poni *et al.* (2006), Tardaguila *et al.* (2010), Risco *et al.* (2013) and Verdenal *et al.* (2018). The yield was mainly affected in terms of number of berries per cluster, thus having an impact on bunch weight (R^2= 0.88, *p*-value < 0.001); this result is in accordance with previous studies (Poni *et al.,* 2006; Palliotti *et al.,* 2011; Gatti *et al.,* 2012). According to the results of the factorial analysis, PFD did not differ in berry weight from CC; this variable only differed statistically in the fourth season, when it was greater than CC. This result is different from studies carried out in a semiarid climate where a decrease in berry weight was observed (Poni *et al.,* 2006; Risco *et al.,* 2013; Gatti *et al.,* 2012). The result observed in the fourth season could be due to a lower number of berries per bunch that promote cell division in the mesocarp during the herbaceous growth stage of berries, and when there is no water restriction (which was the case); this can result in compensatory growth of the berries during the ripening stage (Ojeda *et al.,* 2002). There was always a greater drop in yield from PFD vines after continuous PFD had been carried out for the second time; this can be explained by the carryover effect of removing leaves in the preceding season, which adds to the effect of PFD in the current season, as suggested by Risco *et al.* (2013). Reduction in leaf area due to PFD affects fruit set, yield and vine capacity expressed as DMPC. In such a situation, this could imply a re-translocation of carbohydrate reserves to other sink organs like berry during maturity, thus reducing available stocks for the next cycle (Candolfi-Vasconcelos *et al.,* 1994; Gómez del Campo *et al.,* 2002). PFD did not result in decreased bud fertility the following year (Table 3), which is consistent with Poni *et al.* (2006) and Verdenal *et al.* (2018), but contrasts with results of studies by Sabbatini and Howell (2010) and Risco *et al.* (2013). Slight increases in bud fruitfulness were recorded depending on the year, which can probably be explained by the impact of an increase in light intensity and temperature (known to be particularly important in induction and differentiation processes) on the first buds of the shoot (Figure 3), which could have resulted from the leaf removal in this variety at this stage (Buttrose, 1970; Collins *et al.,* 2020). Our results confirm the potential to use PFD as a yield management practice as an alternative to traditional bunch thinning (Poni *et al.,* 2006), the latter being a common practice in the production of quality wines in many viticultural regions, including in Uruguay. Manual cluster thinning is an expensive practice, which does not always have the desired results. In addition, pre-veraison cluster thinning in Tannat has been reported to result in yield compensation due to an increase in bunch weight, which also increases compactness and susceptibility to rot (Ferrer and González-Neves, 2002). PFD did not involve any yield compensation and it also improved the microclimate in the bunch zone, reducing humidity levels within the canopy (Table 2 and Figure 3) and improving the sanitary status of the harvest (Table 3). Regarding the interannual variability of the yield from both treatments, the most affected component was the number of bunches per vine, which is also influenced by the environmental conditions during floral induction in the previous season, mildew incidence in the current season, and level of vigour in vines (Vaillant-Gaveau *et al.,* 2014; Ferrer *et al.,* 2017).

The weather conditions contributed to modulating the physiological balance of the plants across the seasons, which is in agreement with Echeverría (2017). While the Ravaz index was different between years, similar values were obtained in the two first seasons, indicating equilibrium situations (Ferrer *et al.,* 1997). The third and fourth seasons, however, were completely different. In 2018, the balance shifted towards grape production, while in the fourth season

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it shifted towards wood production, due to the low yield and high-water availability during the whole growth cycle (Table 4 and Figure 1). The ranges of leaf to fruit ratio values recorded in this study (Table 4) are in agreement with those reported by Echeverría (2017), who studied the same variety in the same conditions. Without any restrictions on vegetative growth, higher leaf to fruit ratio values were obtained for the PDF vines, which is in accordance with previous studies (Poni *et al.,* 2006; Tardaguila *et al.,* 2010). PB (SA/dry matter) was also higher in PFD vines, explained by the lower fruit set and, consequently, the lower yield; when plants regained leaf area after early leaf removal this translated into a higher dry matter production capacity per leaf area unit. This could at least partially explain the improvement of some oenological attributes, such as sugar concentration in must and grape anthocyanin potential. Furthermore, PDF vines are likely to develop a more efficient canopy, due to having less internal leaves (Table 2) and younger leaves from secondary shoots, which contribute to improving microclimatic conditions for photosynthesis (Smart and Robinson, 1991; Poni *et al.,* 2006).

In terms of grape composition, the concentration of sugars in the must was higher for PFD vines in three out of the four years of evaluation. The decrease in yield observed in the present study produced a greater capacity for the remaining berries to accumulate sugars, as was suggested by Kliewer (1970); this is supported by the fact that the number of berries per bunch correlated negatively (R^2 = -0.53, *p*-value < 0.001) with the amount of sugar per berry. Despite the absence of differences in LF ratio in 2017, the early defoliated plants achieved a higher concentration of total soluble solids. This may be due to a lower number of berries per bunch (< 29 % when compared to CC), a greater exposure of bunches to sunlight (> 104 % when compared to CC), and differences in the canopy structure (Table 2), improving microclimatic conditions for photosynthesis and ripening (Figure 3). PFD did not induce any consistent changes in tritable acidity or pH, in accordance with Poni *et al.* (2006), Tardaguila *et al.* (2010), Gatti *et al.* (2012) and Mirás-Avalos *et al.* (2019). Lower M/T ratios in PFD must were recorded during the 2018 and 2019 vintages, and can be mostly explained by higher tartaric contents. The higher amount of light intercepted by a PFD canopy could lead to a large amount of CO_2 being used in tartaric biosynthesis (Kliewer and Schultz, 1964). The fact that there were fewer shaded adult leaves on PDF plants meant that a higher amount of tartaric acid was produced and subsequently exported to the berry. This process could mitigate the presumed increase in malic acid catabolism caused by the high sun exposition of bunches, keeping acidity levels stable, as suggested by Gatti *et al.* (2012). In 2019, the high-water availability during pre-veraison and post-veraison promoted vegetative growth at the expense of sugar concentration. The quantity of sugar per berry was higher in PFD, this variable was related to berry weight (R^2 = 0.67, *p*-value = 0.001), in accordance with previous studies (Dai *et al.,* 2011; Gil *et al.,* 2015; Mirás-Avalos *et al.,* 2019). The capacity for accumulating solutes was not restricted like in other studies under semi-arid conditions (Mirás-Avalos *et al.,* 2019). The increase in sugar concentration observed in PFD must during the "unfavourable" seasons (2017 and 2019) could result in winegrowers receiving a higher price for the grape.

Values for anthocyanin potential, extractable anthocyanin, phenolic richness and extractability in Tannat grapes were in agreement with those reported by González-Neves *et al.* (2004). Seasonal environmental conditions had an effect on how PFD influenced grape anthocyanin potential (Table 6). Anthocyanin concentration was not improved by PFD when both rainfall was lacking during the critical period for regaining leaf area after leaf removal and yield per vine was high (which occurred in 2018). LF explained a high proportion of variability in anthocyanin content per berry (Figure 4). Consequently, vine yield also influenced anthocyanin content per berry. However, is widely known that other factors during ripening, such as bunch light exposure, water status and temperature conditions, have an influence on the synthesis and degradation of these compounds (Smart and Robinson, 1991). The data collected here support other studies on the influence of yield and leaf to fruit ratio on the accumulation of secondary metabolites in the grape berry (Howell, 2001; Kliewer and Dokoozlian, 2005; Tardaguila *et al.,* 2010; Echeverría, 2017; Mirás-Avalos *et al.,* 2019). Despite the fourth season being unfavourable for the accumulation of sugars in the berry, the concentration of anthocyanins was exceptionally high. A lower linear relation between sugar quantity and anthocyanin quantity in the berries was found (R^2 = 0,09, *p*-value = 0,03), which indicate a multifactorial control of anthocyanin biosynthesis in grape.

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CONCLUSION

Carrying out PFD on the Tannat variety in a location with a humid climate had different effects depending on the seasonal weather conditions. PFD proved to be a suitable technique for yield control, where declines in yield were accumulative in a continuous application regime. Yield per vine was reduced by 11 to 39 % with respect to CC. In a year with favourable conditions for bunch rot, the technique showed a notable effectiveness for improving the sanitary status of the grapes and contributed to the improvement of some oenological attributes, such as sugar concentration, total polyphenols and anthocyanin content. However, these improvements were relative and appear to depend on the capacity for the recovery of vegetative growth, yield per vine, weather conditions and their partial interaction. PFD could be useful for correcting excess of vigour in grapevines. However, factors limiting vegetative growth, such as water supply during the critical stages of leaf area establishment, could lead to undesirable effects on grape composition and vine life. Despite these possible drawbacks, our study showed PFD to be a promising technique for vigorous grapevine varieties, like Tannat under humid climate conditions.

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