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DEFOLIATION AND WATER LEAF POTENTIAL EFFECTS ON OENOLOGICAL PROPERTIES OF MERLOT (*Vitis vinifera* L.) GRAPE MUST

Serkan Candar^{®⊠1}, Elman Bahar^{®2}, İlknur Korkutal^{®2}, Fatma Betül Aktaş^{®3}

¹ Tekirdağ Viticulture Research Institute, 59200, Tekirdağ, Turkey

² Tekirdağ Namık Kemal University, Faculty of Agriculture, Horticulture Department, 59100 Tekirdağ, Turkey

³ Tekirdağ Namık Kemal University, Institute of Natural and Applied Sciences, 59100 Tekirdağ, Turkey

ABSTRACT

This research was carried out to determine the effects of leaf water potential and defoliation treat-ments on the oenological properties of grape must. Merlot/41B graft combination grapevines were cultivated in Chateau Kalpak vineyards in Tekirdağ, Şarköy. The trial was carried out for two con-secutive years in the vineyard in the vegetation periods of 2019–2020 and 2020–2021. Leaf water potential treatments were S0 (Control = no irrigation), S1 (-0.3/-0.5 MPa), S2 (-0.5/-0.7 MPa) and S3 (<-0.7 MPa) and defoliation treatments were implemented as; Control(C), Full Window(FW), Right Window(RW) and Left Window (LW). As a result, the S2 (-0.5 MPa and -0.7 MPa) stress level resulted in more balanced responses, as seen in the combination of years in terms of both primary and secondary metabolites. The RW defoliation treatment may cause higher primary and secondary metabolite values in all criteria.

Key words: abiotic stress, berry maturity, berry quality, canopy management, leaf removal

INTRODUCTION

Leaves are organs that have critical physiological tasks in the establishment of photosynthesis, transpiration, and carbon balance in the grapevine, the regulation of the microclimate in the canopy, the establishment of the plant and soil water budget balance, the accumulation of sugar and nitrogen in the berry. The total biomass produced by the grapevine is directly related to the amount of carbon the leaves absorb during the photosynthesis process [Candar et al. 2021].

The physiological activity of the leaf is affected by leaf size and age [Tozer et al. 2015], climatic conditions of the year, general characteristics of the viticulture region, and a variety of genetic differences. The physiological activity also affects the total leaf area on the grapevine, yield, and biochemical processes during the ripening period. However, leaf shape and size may be ineffective in some cases [Chitwood et al. 2016, Candar et al. 2021].

Defoliation practices can create significant physiological effects on the vine's production-consumption balance. These can be listed as decreased photosynthesis products to the cluster [Palliotti et al. 2013], limited root growth, and decreased water efficiency. Removal of leaves during berry ripening eliminates a potential source of carbon (C) and N that may cause a reduction in sugar and nitrogen accumulation [Rossouw et al. 2018], affecting berry quality [Bubola et al. 2022]. Reducing the total leaf area of the vine with defoli-



ation applications may weaken the grapevine growth in the following years and cause a decrease in yield. In some cases, it was reported that the effect of leaf removal applications on clusters and yield was not statistically significant. However, it was also reported that the applications where the primary shoot leaves were left on the plant received slightly higher values than other applications [Korkutal et al. 2017]. Ferlito et al. [2019] compared grapevines with early basal leaf removal with control grapevines and found that these vines showed higher water status and lower negative midday leaf water potential (LWP) than control plants in both years. Tardaguila et al. [2010] investigated the effects of mechanical and manual defoliation on the Carignane grape variety in the early period. The yield of manual defoliation practice before flowering in vines is reduced by 30%. Researchers determined that mechanical leaf removal also reduced yield by 70%. Palliotti et al. [2013] took 75-80% of the leaves before flowering in the Ciliegiolo red grape variety and noted that these vines formed light and sparse clusters compared to vines without leaf removal. Teker and Altindisli [2021] stated that the leaf removal of the Sultani Seedless grape variety does not affect the yield significantly; however, they revealed that 50% leaf removal increased yield compared to 25% leaf removal. Knowing the grapevine variety responses and plasticity limits is essential to balance the product load and canopy architecture according to the targeted yield and quality and to carry out vineyard management [Candar 2022]. Regarding leaf area management, the seasonal effects of each vegetation period are the primary determining factor. Therefore, planning canopy management practices should be done each year separately by following the long and medium-term meteorological evaluations. Canopy management practices should be applied according to the phenological period and short-term meteorological evaluations [Candar et al. 2022].

Plants develop organic compounds called primary and secondary metabolites. Primary metabolites are critical organic compounds such as carbohydrates and amino acids, nucleotides, proteins, acyl lipids, and phytosterols, which are essential in growth and development and necessary throughout the life of the plant. In contrast, secondary metabolites are abiotic compounds that are not considered the main element of plant life. Furthermore, low molecular weight phenolic compounds act against biotic stress factors. The essential substances in the composition of grapes are sugars, organic acids, phenol compounds, especially anthocyanins and tannins, aroma substances, pectic substances, nitrogenous substances, enzymes, mineral substances, and vitamins [Blouin and Guimberteau 2000, Ribéreau-Gayon et al. 2000]. The wine value of a cultivated grapevine variety can be determined to some extent by sensory and chemical analyses of the berry and most components obtained.

The berry ripening process is a physiological period that affects the composition of the berries and, later, the quality of the wine, depending on the variety of characteristics. During the ripening process, grapes undergo many physical and biochemical changes, such as weight, volume, hardness, sugar, acidity, color, and aroma. Water-soluble solids, fruit weight, and titratable acidity can determine the optimum harvest level. In order to harvest grapes at ideal maturity, it is necessary to examine their phenolic composition and organic acid profile during the ripening period. The slow, balanced, and simultaneous realization of industrial ripeness and aromatic and phenolic ripeness in grapes at the same time is one of the features that directly determines the type and quality of the wine. Canopy management, which is one of the factors affecting oenological maturity, is carried out by using strategies such as different cultivation systems and forms, winter pruning, and green pruning related to viticulture in line with a purpose.

Decreased water resources due to global climate change are effective in the grapevine life cycle. The presence of water is significant for sustainable viticulture. Drought is a critical abiotic stress. The availability of water can be considered the primary source of climatic changes manifested in the intensity and timing of precipitation and the physical properties of the soil. Severe water deficiencies experienced during the vegetation period may adversely affect shoot growth, yield, and berry composition by limiting photosynthesis [Keller et al. 2016]. For sustainability in viticulture, water should be used more efficiently. The water status of the vine causes a wide variety of effects on the vine depending on the phenological development status of the vine [Ojeda et al. 2002, Roby et al. 2004, Keller et al. 2016]. Excess water, on the other hand, induces uncontrolled vegetative growth, causes unwanted growth, increases the risk of fungal diseases, manipulates harvest time, and complicates quality management.

Appropriate water deficit positively affects cluster characteristics, berry, and wine composition by promoting slower leaf growth and higher water use efficiency [Bahar et al. 2011]. The effects of proper water stress on physiological and metabolic pathways lead to the formation of secondary metabolites in berries. These positive effects are usually explained by the smaller berries, which have a higher skin-to-pulp ratio-relatively high skin content results in high tannins, anthocyanins, total phenolics, and other organoleptic properties.

This research was carried out on the cv. Merlot grape variety. The effects of 4 different LWP(Ψ) levels and four defoliation applications on the oenological properties of grapes must be determined.

MATERIAL AND METHODS

Location and plant material. The study was conducted in Chateau Kalpak vineyard located in the Şarköy district of Tekirdağ in the 2019/2020 and 2020/2021 vegetation periods for two consecutive years. The coordinates for the location are 40°39'12.00" N and 27°03'20.00" E. Grapevines consisting of Merlot/41B combination were used. The grapevines were planted at 2.1 m and 1.0 m in-row spacing, with a 70 cm stem height, and have a double arm cordon training in the Espalye system.

Methods. In order to ensure that the grapevines measured in the 2019–2020 vegetation period are homogeneous, the number of clusters, shoots, and growth strengths were selected similarly. No empty plants were left among the trial grapevines; Grape-

vines with extreme differences in the number of clusters and shoots were excluded from the experiment. The following year, when the shoots were about 30 cm, the number of shoots and clusters was equalized again. On 144 vines, which are considered to be homogeneous, four different stress levels [S0 (Control = no irrigation), S1 (-0.3/-0.5 MPa), S2 (-0.5/-0.7 MPa) and S3(<-0.7 MPa)] and four different defoliation Control, Full Window, Right Window and Left Window application was made.

Throughout both experimental years, pest control, fertilization, tillage, and other standard cultural practices remained consistent with regional standards. Notably, there were no external factors during the experiment that could have negatively impacted plant growth or disrupted the uniformity of the procedures.

Leaf water potential (stress levels). When needed according to the pre-dawn leaf water potential (Ψ pd) measured at five to seven-day intervals, irrigation was done taking into account the determined stress intervals, and Ψ pd control was performed the next day. This way, it was checked whether the Ψ pd value was within the desired ranges (Tab. 1).

Defoliation treatments. These applications were made approximately two weeks after the veraison. They formed by removing shoots and leaves from the eighth node and removing all the leaves between the seventh and thirteenth nodes in the form of a window (Tab. 2). In the meantime, attention was paid to ensuring that the grapes were between °Brix 15 and 17, according to Alço [2019].

Trail design and statistical analysis. In the experiment established in the Divided Plots Trial Design, the main plot formed water stress levels, and each subplot formed defoliation practices. A total of 144 vines were studied, with four different water stress levels × four

Treatment	Ψpd (MPa)	Description
S0 (control, no irrigation)		irrigation was not done; it was left to random precipitation
S1 (stress level 1)	-0.4 to -0.6	Ψpd was tried to be kept between –0.4 MPa and –0.6 MPa
S2 (stress level 2)	-0.5 to -0.7	Ψpd was tried to be kept between –0.5 MPa and –0.7 MPa
S3 (stress level 3)	≤ -0.7	Ψ pd was tried to be kept below -0.7 MPa

Table 1. The description of expected water stress levels and leaf water potential values

 $\Psi pd = pre$ -dawn leaf water potential

Treatment	Description
C (control, no defoliation)	
FW (full window)	removing of shoots and leaves from the eighth node
RW (right window = west window)	removing all the leaves between the seventh and thirteenth nodes from the west side of the row
LW (left window = east window)	removing all the leaves between the seventh and thirteenth nodes from the east side of the row

defoliations \times three replications with three plants each.

JMP 13.2.0 was used for statistical data analysis. The significance of differences between treatments was determined using one-way analysis of variance (ANOVA), and significant differences were grouped using the LSD test at a 5% significance level.

Analysis and measurements. During experimental years, phenological development stages were recorded according to Lorenz et al. [1995]. Climate data were obtained from the Turkish State Meteorological Service (MGM). Huglin index (HI), Winkler index (WI-GDD), hydrothermic index (HyI), night cold index (CI), and growing season temperatures (GST) were determined using detailed calculations as outlined in Gülbasar Kandilli et al. [2022].

Randomly selected 250 berries from all parts of clusters were taken from each application to determine oenological properties. After the berries were crushed, most samples were obtained by passing them through filter paper. The amount of total-soluble solids (TSS) (°Brix) in most samples was measured by a hand refractometer (ATC brand 0-50 model, Istanbul-Turkey). Total acidity (TA) (g L^{-1}) was measured by titration method with 0.1N NaOH, and pH was measured with a digital pH meter (Hanna Instruments, HI 2210 model pH meter Bedfordshire, England). The TSS, TA, and pH were determined according to Cemeroğlu [2007]. In order to determine the sugar concentration, the sugar concentrations corresponding to the °Brix values of the samples were determined from the table [Bahar et al. 2011]. The amount of sugar per gram of grapes (mg g berry⁻¹) was calculated by dividing the amount of sugar in the berry by the fresh weight [Ferrer et al. 2014]. Total anthocyanin content (mg kg⁻¹) was determined by UV-Mini 1240 with a Shimadzu

spectrophotometer, according to Cemeroğlu [2007]. Total phenolic substance (mg kg⁻¹) was determined by spectrophotometric method according to Waterhouse [2002]. Total tannin content (g kg⁻¹) was determined according to AOAC [1998] using the spectrophotometric method. To determine the total polyphenol index, the grape must be centrifuged at 8000 rpm at 15°C for 5 minutes after filtering with a coarse filter (Nüve A.Ş., NF 1200R, Ankara/Turkey). After filtering through the coarse filter again, 1 mL of must, taken with the help of a pipette, was added to the 50 mL flask. After completing it to 50 mL with distilled water, the solution obtained was read at 280 nm with the help of a spectrophotometer [INRA 2007]. Maturity indexs, °Brix/titratable acid (g L⁻¹), and pH² × °Brix values were calculated and evaluated according to Blouin and Guimberteau [2000].

RESULTS AND DISCUSSION

Climate and phenology. The study area, Tekirdağ, has a Mediterranean climate characterized by hot and dry summers, along with mild winters, typical features of the Csa classification in the Köppen-Geiger climate classification system [Y1lmaz and Çiçek 2018]. Precipitation primarily occurs during the winter and spring seasons. While the coastal regions of Tekirdağ experience this Mediterranean climate, the interior is dominated by a continental climate, resulting in comparatively colder winters [Candar 2023]. Table 3 presents various climatic characteristics and viticultural indexs of Tekirdağ for the years 1939–2019, as well as for 2019 and 2020.

The mean annual precipitation decreased from 589.50 mm during the period 1939–2019 to 378.40 mm

in 2019 and then further dropped to 290.00 mm in 2020. Similarly, the precipitation for the vegetation period saw a decline from 196.70 mm to 129.80 mm in 2019 and subsequently decreased to 83.60 mm in 2020. Comparing the averages, the temperature for 2019 stood at 15.60°C, slightly higher than the 15.30°C average for 2020. These values differed from the longterm average temperature of 14.00°C. The mean temperature of the hottest month experienced an increase from 23.80°C during 1939–2019 to 25.30°C in 2019, and it remained relatively constant at 25.00°C in 2020. These observed patterns imply a progressing trend towards drier conditions, which could potentially impact grape production. Regarding the indices, the Huglin index (HI) showed an increase from 2128.20°C in 1939–2019 to 2324.07°C in 2019, followed by a slight decrease to 2229.21°C in 2020. Similarly, the Winkler index (WI-GDD) exhibited an increase from 1872.00 degree-days to 2157.00 degree-days in 2019 and a marginal decrease to 2124.00 degree-days in 2020. On the other hand, the hydrothermal index (HyI), which integrates temperature and precipitation, saw a decline from 3595.20°C mm during 1939-2019 to 2181.54°C mm in 2019, and further dropped to 1328.10°C mm in 2020. These shifts point to a warming trend, with rising heat accumulation during the growth season. The night cold index (CI) exhibited an increase from 16.00°C to 17.60°C and further to 19.20°C, sequentially. Moreover, the growing season temperatures (GST), representing the average temperature during the growth season, rose from 18.91°C to 20.27°C and then slightly decreased to 20.11°C, respectively (Tab. 3).

According to the phenological developmental stages recorded during experimental years, budburst (EL

05) in 2019 was determined as 11 April, and in 2020 it was 15 April. Full bloom realized (EL 23) on 2 June 2019 and 8 June 2020; berry set (EL 27) was 9 June 2019 and 14 June 2020; veraison (EL 35) was recorded on 20 July 2019, 24 July 2020, and finally the harvest (EL 38) on 15 September in 2019, and 16 September in 2020. As can be seen, the dates of the phenological developmental stages have shifted between 4–6 days.

Total soluble solids. While defoliation treatments did not cause a significant change in TSS accumulation according to the average of years results, LWP treatments affected TSS accumulation at a statistically significant level. Regarding the main effect of defoliation treatments, LW application caused the highest TSS accumulation with a value of 24.78°Brix. The lowest TSS amount was measured in the FW application with a value of 24.35°Brix. When the LWP importance levels are examined, the S0 application with 25.00°Brix is in the first significance group; The S3 treatment resulted in the lowest TSS accumulation. When the application interactions were examined, it was determined that the S0 \times LW interaction had the highest TSS, and the S3 \times FW combination had the lowest TSS value. The year effect is also statistically significant, and it has been determined that higher TSS was reached in 2020 with a value of 24.76°Brix (Tab. 4).

As many studies demonstrate, total leaf area and grapevine water potential play a vital role in determining TSS accumulation in berries with their effects on the photosynthesis process and growth parameters [Palliotti et al. 2013, Candar et al. 2019, Candar 2022]. Based on the available data, it can be speculated that these treatments do not exert a substantial influence on the total leaf area per grapevine in both experimental

Climatic indices	Unit	1939–2019	2019	2020
Precipitation (mean annual)	mm	589.50	378.40	290.00
Precipitation (vegetation)	mm	196.70	129.80	83.60
Mean temperature of the hottest month	°C	23.80	25.30	25.00
Huglin index (HI)	°C	2128.20	2324.07	2229.21
Winkler index (WI-GDD)	degree-day	1872.00	2157.00	2124.00
Hydrothermal index (HyI)	°C mm	3595.20	2181.54	1328.10
Night cold index (CI)	°C	16.00	17.60	19.20
Growing season temperatures (GST)	°C	18.91	20.27	20.11

Table 3. Tekirdağ viticultural climate indexs in experimental years

C/ 1 1	37		Defoliation	n treatments		Main	effect	
Stress level	Years	С	FW	RW	LW	LWP	ye	ear
	2019	25.66	24.80	24.20	25.53			
S 0	2020	24.86	24.53	25.20	25.26	25.00 A		
	mean	25.26	24.66	24.70	25.40			
S1	2019	24.33	24.80	24.33	23.93			
	2020	24.66	24.20	25.66	25.26	24.65 AB		
	mean	24.50	24.50	25.00	24.60		2010	2020
S2	2019	23.20	23.80	24.33	23.46		2019	2020
	2020	25.40	24.53	25.00	25.20	24.36 B	24.39 b	24.76
	mean	24.30	24.16	24.66	24.33			
S3	2019	23.86	24.26	24.33	25.40			
	2020	24.46	23.93	23.80	24.20	24.28 B		
	mean	24.16	24.10	24.06	24.80			
DTME		24.55	24.35	24.60	24.78			

Table 4. Different defoliation and LWP treatment effects on TSS (°Brix)

LWPME LSD_{0.05}: 0.44, Year ME LSD_{0.05}: 0.31

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

years (data not shown). This deduction stems from the fact that the main effect of the defoliation treatments does not manifest in any noticeable disparity in the accumulation of TSS. On the other hand, the physiological activity of leaves removed from the grapevine may be related to leaf age and position on the plant [Tozer et al. 2015]. In some cases, leaf shape and size may be ineffective [Chitwood et al. 2016]. Therefore, the leaves removed should be evaluated in this context. In a healthy and homogeneous vineyard, the plants can use their carbohydrate reserves from previous years in the ripening process according to the current year's conditions. Thus, the deficiencies caused by the total leaf area deficit can be compensated. The effect of missing reserves can be seen in later years. Similarly, Kotseridis et al. [2012] also stated that while leaf removal intensity affects the TSS amount in Cabernet-Sauvignon and Sangiovese grape varieties, it does not affect the Merlot grape variety.

Total acidity. Regarding TA, the main effect of LWP and years were statistically significant at the LSD level of 5%. Although defoliation treatments are not statistically significant, the highest TA value was measured in the RW application with 6.58 g L⁻¹, and the

lowest acidity was measured in the LW application with 6.43 g L⁻¹. In LWP applications, the lowest TA content was determined in the S3 application. Other applications were higher and in the same statistical group. The highest TA was tested at the S2 stress level of 6.76 g L⁻¹. TA was found to be higher in 2019 (Tab. 5).

The total leaf removal area regulates TA content in grapevine berries [Korkutal et al. 2017], day/night temperature changes [Sweetman et al. 2014], and the temperature regime between veraison and harvest due to being a potential source for the carbon demand in the maturation process [Candar et al. 2020]. In this study, the determination of the lowest TA content in the S3 application, which is the highest stress level, may be because the acids meet the carbohydrate deficiency caused by water stress in the berry.

When examined using annual averages, the divergence from the long-term precipitation average was more pronounced in 2020. This reduction in cumulative precipitation over recent years resulted in a statistically significant decrease in the TA content for the year 2020 compared to the preceding year.

Likewise, the night cold index (CI), which indicates day-to-night temperature fluctuations, registered at 19.20°C in 2020. It indicates that due to elevated night temperatures during 2020, the relatively modest day-to-night temperature variations, already existing in the region transitioning towards a Mediterranean climate, further declined during the same year. Consequently, the heightened nocturnal temperatures notably expedited the breakdown of malic acid in grapes and subsequently led to a reduction in TA accumulation.

pH. As a measure of active acidity, the pH of grapes is a critical determinant of wine quality. Exchanging tartaric acid compounds to potassium cations produces water-insoluble potassium bi-tartrates in grape must and reduces free acids, thus causing an increase in pH. The pH value should not exceed 3.3 in white varieties and 3.5 in colored varieties [Cox 1999] because high pH in fruit juice causes a decrease in wine quality. Musts with higher than 3.6 pH can cause wine defects by spoilage organisms and negatively affect the aging ability of wine by causing free SO₂ to bind.

Regarding defoliation treatments, the RW application numerically had the highest pH value of 3.31. A numerically low pH value was found in C and LW applications with a value of 3.29. The effect of LWP applications is significant at the LSD 5% level. While S0, S1, and S3 applications reached higher pH values, the lowest pH value was determined at 3.26 in the S2 application (Tab. 6). The climatic characteristics of the year and the soil water potential are more critical than canopy management practices in the lead of the pH value. Similar results were obtained from other nearby studies [Alço 2019, Candar 2020]. The year's main effect was also statistically significant, with a higher pH value of 3.32 in 2019.

 $pH^2 \times {}^{\circ}Brix$ maturity indice. According to the pH² × ${}^{\circ}Brix$ index, grape berries reach ideal maturity when they reach a value of 200 in white varieties and 260 in red varieties [Cox 1999, Blouin and Guimberteau 2000].

Regarding year average, LWP and year main effects were statistically significant at the LSD level of 5%. In defoliation treatment, the highest maturity was calculated with 272.04 LW, and the lowest maturity

C4	V		Defoliation	treatments		Ma	ain effect	
Stress level	Years	С	FW	RW	LW	LWP	ye	ar
	2019	7.22	8.25	7.25	7.02			
S0	2020	5.50	6.13	6.13	6.06	6.69 A		
	mean	6.36	7.19	6.69	6.54			
S1	2019	7.62	7.57	7.25	7.52	-		
	2020	5.93	6.26	5.46	4.93	6.57 A		
	mean	6.78	6.92	6.36	6.23			
S2	2019	7.63	7.20	8.37	7.75	_	2019 7.33 a	2020 5.67 t
	2020	5.23	5.30	6.63	6.00	6.76 A		
	mean	6.43	6.25	7.50	6.87			
S3	2019	6.65	6.52	6.62	6.90	-		
	2020	6.03	4.93	4.93	5.26	5.98 B		
	mean	6.34	5.73	5.78	6.08			
DTME		6.47	6.52	6.58	6.43	-		

Table 5. Different defoliation and LWP treatment effects on TA ($g L^{-1}$)

LWPME LSD $_{0.05}$: 0.28, Year ME LSD $_{0.05}$: 0.19

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

G/ 1 1	37		Defoliation	treatments		Ma	in effect	
Stress level	Years	С	FW	RW	LW	LWP	ye	ar
	2019	3.36	3.30	3.33	3.40			
S 0	2020	3.31	3.30	3.27	3.27	3.32 A		
	mean	3.34	3.30	3.30	3.33			
	2019	3.33	3.36	3.36	3.33			
S 1	2020	3.29	3.26	3.34	3.30	3.32 A		
	mean	3.31	3.31	3.35	3.31			
	2019	3.26	3.30	3.23	3.30		2019 3.32 a	2020 3.27 t
S2	2020	3.26	3.25	3.23	3.22	3.26 B		
	mean	3.26	3.27	3.23	3.26			
	2019	3.23	3.36	3.36	3.40			
S 3	2020	3.31	3.22	3.29	3.26	3.30 A		
	mean	3.27	3.29	3.33	3.33			
DTME		3.29	3.30	3.31	3.29			

Table 6. Different defoliation and LWP treatment effects on pH

LWPME LSD_{0.05}: 0.038, Year ME LSD_{0.05}: 0.027

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

was 265.29 in the FW application. Among LWP applications, the S0 stress level reached the highest maturity with a value of 276.16. S2 application was determined at the lowest maturity with a value of 259.16. In terms of the primary year effect, values of 270.72 in 2019 and 266.37 in 2020 were calculated (Tab. 7).

Similar to the available data, Alço [2019] and Candar et al. [2020] also reported that leaf removal practices did not consistently affect this index statistically significantly.

°Brix/TA maturity index. The main effects of year and LWP for the °Brix/TA index were statistically significant at the LSD 5% level. According to defoliation treatments, the highest numerical value was calculated in the 3.97 g L⁻¹ LW application, and the lowest maturity level was calculated in the RW application with a 3.84 g L⁻¹ value. Among the LWP applications, the S3 stress level reached the highest maturity with a value of 4.14 g L⁻¹, while the other stress levels formed the lower group. The °Brix/TA index was calculated as 4.42 g L⁻¹ in 2020 and 3.35 g L⁻¹ in 2019 (Tab. 8).

The ideal value range for the °Brix/TA index was reported as 3–4 g L^{-1} by Blouin and Guimberteau

[2000]. With the decrease in the total acidity level in the second year of the study, it is seen that there are statistical differences between the years. On the other hand, high stress in the S3 application created differences by reducing the total acidity in the leaf water potential applications.

Sugar concentration. The main effects of sugar concentration LWP and year were statistically significant at the LSD 5% level. Regarding LWP, the highest sugar concentrations were determined at the S0 stress level with a value of 249.64 g L⁻¹ and the lowest sugar concentrations with a value of 241.41 g L⁻¹ at the S3 stress level. A sugar concentration value of 246.96 gL⁻¹ in 2020 and 242.52 g L⁻¹ in 2019 was determined (Tab. 9).

Amount of sugar per gram of berry. When the amount of sugar in a gram of berry was calculated, the main effects of year and LWP were statistically significant at the LSD 5% level. In the mean of two years of defoliation treatment, the values were listed numerically from high to low as LW, RW, C, and FW. In LWP applications, the highest value was determined as 192.02 mg g berry⁻¹ in the S0 application, and the lowest value was 185.69 mg g berry⁻¹ in the S3

Stress level	Years		Defoliation	n treatments		Main	effect	
Suess level	1 cars	С	FW	RW	LW	LWP	ye	ear
	2019	291.00	270.33	269.00	295.00			
S0	2020	274.00	268.00	270.33	271.66	276.16 A		
	mean	282.50	269.16	269.66	283.33			
	2019	270.66	281.33	276.00	265.66			
S 1	2020	268.00	258.33	287.33	275.33	272.83 AB		
	mean	269.33	269.83	281.66	270.50			
	2019	247.66	259.66	255.00	255.66		2019 270.72 a	2020 266.37 b
S2	2020	271.00	260.33	262.00	262.00	259.16 C	270.72 a	200.370
	mean	259.33	260.00	258.50	258.83			
	2019	249.66	275.66	275.66	293.66			
S3	2020	269.33	248.66	258.33	257.33	266.04 BC		
	mean	259.50	262.16	267.00	275.50			
DTME		267.66	265.29	269.20	272.04			

Table 7. Different defoliation and LWP treatment effects on $pH^2 \times {}^{\circ}Brix$ maturity index

LWPME LSD_{0.05}: 0.038, Year ME LSD_{0.05}: 0.027

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

G(1 1	V		Defoliation	treatments		Ma	ain effect	
Stress level	Years	С	FW	RW	LW	LWP	ye	ar
	2019	3.55	3.01	3.36	3.63			
S0	2020	4.53	4.01	4.11	4.17	3.79 B		
	mean	4.04	3.51	3.73	3.90	_		
	2019	3.26	3.28	3.36	3.18	_		
S1	2020	4.16	3.87	5.18	3.87	3.87 B		
	mean	3.71	3.58	4.04	4.18			
	2019	3.05	3.30	2.90	3.03		2019 3.35 b	2020 4.42 a
S2	2020	4.86	4.67	3.78	4.20	3.72 B		
	mean	3.95	3.98	3.34	3.61			
	2019	3.59	3.72	3.68	3.70			
S3	2020	4.05	4.92	4.83	4.66	4.14 A		
	mean	3.82	4.32	4.25	4.18			
DTME		3.88	3.85	3.84	3.97			

Table 8. Different defoliation and LWP treatment effects on °Brix/TA maturity index (g L⁻¹)

LWPME LSD_{0.05}: 0.19, YearME LSD_{0.05}: 0.14

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

74 1 1	V		Defoliation	treatments		Main	effect	
Stress level	Years	С	FW	RW	LW	LWP	y	ear
	2019	257.30	247.40	239.00	255.80			
S 0	2020	248.13	244.53	252.16	252.83	249.64 A		
	mean	252.71	245.96	245.58	254.31			
	2019	241.93	247.46	241.80	237.43	_		
S1	2020	245.73	240.36	257.53	252.60	245.60 AB		
	mean	243.83	243.91	249.66	245.01			
	2019	228.73	235.73	241.93	231.86	_		2020 249.96 a
S2	2020	254.40	244.10	249.70	251.93	242.30 B		249.90
	mean	241.56	239.91	245.81	241.90			
	2019	236.46	241.26	241.83	254.40	_		
S3	2020	243.66	237.40	235.80	240.50	241.41 B		
	mean	240.06	239.33	238.81	247.45			
DTME		244.54	242.28	244.97	247.17			

Table 9. Different defoliation and LWP treatment effects on sugar concentration (g L^{-1})

LWPME LSD_{0.05}: 5.13, Year ME LSD_{0.05}: 3.63

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

application. The amount of sugar in a gram of berry was determined as 189.96 mg g berry⁻¹ in 2020 and 185.69 mg g berry⁻¹ in 2019 (Tab. 10).

Total anthocyanin content. Anthocyanins are common secondary plant metabolites that produce red, blue, and purple-black colors found in cell vacuoles and different plant tissues. They are mainly found in the skin of ripe berries and protect them from heat and extreme light stress [Castañeda-Ovando et al. 2009]. They are also the most intense natural antioxidants in red grapes and wine, along with catechins and tannins [Candar 2023], and are formed during the ripening of grapes [O'Kennedy and Reid 2006]. The total anthocyanin concentration in the berry is expressed as different degrees of maturity [Esteban et al. 2002], and ideal maturity is associated with high anthocyanin content in colored varieties. While the anthocyanin content in wine plays a role in determining the color, it is one of the essential characteristics in expressing the overall quality of the wine. Anthocyanin synthesis and accumulation are regulated under the influence of factors such as temperature and abscisic acid accumulation [Yamane and Shibayama 2006], drought [Roby et al. 2004], and light [Tarara and Lee 2019].

In terms of total anthocyanin amount, the average of years LWP and the main effects of the year were statistically significant at the LSD level of 5%. In defoliation treatments, the highest amount of anthocyanin was determined in the RW application, and the lowest amount of anthocyanin was determined in the LW application. It is difficult to understand the direct effects of canopy management practices on anthocyanins because many factors regulate the mechanisms affecting anthocyanin synthesis, accumulation, and degradation [Candar 2018], such as the year of production and the cultural processes applied in the vineyard [O'Kennedy and Reid 2006]. However, canopy management practices can affect the amount of anthocyanin with specific applications selected according to the predicted climatic characteristics [Alço 2019]. On the other hand, sugar and acidity in berries reach maturity earlier than anthocyanins, while anthocyanins are in the highest correlation with tannins in the skin and seed during the ripening process [Candar 2018].

Regarding LWP main effects, S3 and S0 stress levels reached higher values, while S2 and S1 levels were determined as 610.76 mg kg⁻¹ and 559.41 mg kg⁻¹, respectively (Tab. 11). As Ojeda et al. [2002] and Candar [2018] stated similarly, it is seen that the total anthocyanin amount increases as the stress levels increase in LWP applications, with the S3 application taking the highest value statistically. Since the stress level in the S0 application is determined depending on the annual precipitation, it is determined that this treatment differs from others depending on the natural soil water balance. It was also detected that the total anthocyanin content in 2020, which had less precipitation, was higher than the previous year. Higher anthocyanin content was also related to the cumulative effect of low precipitation during the experimental period and before, which is less than the long-term average.

Total tannin content. Harbertson et al. [2002] stated that the amount of seed tannin is about three times higher than skin, and the amount of tannin in wines varies depending on various factors. On the other hand, the amount of seed tannin decreases in the stages after the veraison stage [Ribéreau-Gayon et al. 2000]. Tan-

nins are found in organs such as skin, leaves, stems, and unripe fruits in many plants and form compounds with polymers such as proteins and polysaccharides. In grapevine plants, they are concentrated in the skin, seeds, and clusters. As the berry matures, tannins begin to soften in taste.

In terms of total tannin content, year, LWP, and, as different from the other parameters investigated, the defoliation treatment main effects were found to be statistically significant at the LSD 5% level. For defoliation treatments, it was determined that the highest tannin content belongs to the RW application with a value of 3.12 g kg⁻¹, and the lowest content belongs to the C application with a value of 2.81 g kg⁻¹. Contrary to these data, Alço [2019] and Candar [2018] reported that total tannin content was not affected by changes in total leaf area. Regarding LWP, the S2 stress level was the highest, with 3.24 g kg⁻¹ tannin content; the S1 stress level was determined in total tannin amount with 2.38 g kg⁻¹ tannin content. In 2019, the total tannin content was higher, with a value of 3.09 g kg^{-1} . The total amount of tannin in 2020 was 2.80 g kg⁻¹ (Tab. 12).

C4	V	Defoliation treatments				Main effect			
Stress level	Years	С	FW	RW	LW	LWP	ye	ear	
	2019	197.90	190.30	183.83	196.76				
SO	2020	190.86	188.06	193.96	194.50	192.02 A			
	mean	194.38	189.18	188.90	195.63				
	2019	186.10	190.33	185.96	182.63				
S 1	2020	189.03	184.93	198.10	194.30	188.92 AB			
	mean	187.56	187.63	192.03	188.46		• • • •		
	2019	175.96	181.33	186.10	178.36		2019 186.55 b	2020 189.96 a	
S2	2020	195.66	187.73	192.10	193.76	186.37 B	180.55 0	169.90 a	
	mean	185.81	184.53	189.10	186.06				
	2019	181.90	185.56	186.03	195.70				
S3	2020	187.43	182.60	181.36	184.96	185.69 B			
	mean	184.66	184.08	183.70	190.33				
DTME		188.10	186.35	188.43	190.12				

Table 10. Different defoliation and LWP treatment effects on the amount of sugar per gram of grape (mg g berry⁻¹)

LWPME LSD_{0.05}: 3.94, YearME LSD_{0.05}: 2.79

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

C/ 1 1	V		Defoliation	treatments		Main	effect	
Stress level	Years	С	FW	RW	LW	LWP	ye	ear
	2019	752.70	565.00	484.33	475.96	660.07 A		
S0	2020	679.73	875.63	773.23	674.00			
	mean	716.21	720.31	628.78	574.98			
	2019	622.96	418.16	601.30	494.03	559.41 B		
S1	2020	581.83	617.26	612.46	527.30			
	mean	602.40	517.71	606.88	510.66			
	2019	473.53	547.60	679.23	656.83	610.76 AB	2019 571.24 b	2020 676.83
S2	2020	598.36	505.16	744.70	680.70		571.240	070.05
	mean	535.95	526.38	711.96	668.76			
	2019	576.86	573.23	738.53	479.66	665.90 A		
S 3	2020	669.40	907.46	723.90	658.13			
	mean	623.13	740.35	731.21	568.90			
DTME		619.42	626.19	669.71	580.82			

Table 11. Different defoliation and LWP treatment effects on total anthocyanin content (mg kg⁻¹)

LWPME LSD_{0.05}: 74.88, Year ME LSD_{0.05}: 52.94

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

Total phenolic substance. Only the LWP main effect was statistically significant at the LSD 5% level from the year's average. In defoliation treatments, the highest total phenolic substance was determined in the LW treatment with a value of 1785.11 mg kg⁻¹, and the lowest total phenolic substance was determined in the FW treatment with a value of 1655.86 mg kg⁻¹ (Tab. 12). Phenolic compounds are secondary compounds formed from building blocks formed by the process of photosynthesis. They can be considered the total expression of tannins and color pigments. They are responsible for critical sensory properties in berries and wine. They characterized phenolic compounds chemically with hydroxyl groups and aromatic rings. Structurally, they were divided into five main groups: flavonoids (flavonols or catechins, flavanones, isoflavonoids, flavonols, flavones, total anthocyanins), phenolic acids, stilbenes, tannins and lignans [Paredes--López et al. 2010].

Unlike anthocyanins, the levels of phenolic maturity can be determined not only by evaluating the color but also by monitoring the change in sensory characteristics before harvest. Red-colored varieties have higher phenolic compounds than whites [Roby et al. 2004, Chacón et al. 2009]. Phenolic compounds play an essential role in the color and sensory properties of red grapes and wines and are responsible for their body and content. They are synthesized in berries and concentrated in the skin and seed [Candar 2023]. They viewed secondary metabolism in plants as an adaptation mechanism to defend against unwanted environmental factors that cause stress. Berry phenolic content is managed by variables such as cultivar, soil composition, climate, growing techniques, exposure to pests, and degree of maturity.

Unlike anthocyanins, phenolic maturity levels can be determined not only by evaluating the color but also by monitoring the changes in sensory properties before harvest [Esteban et al. 2002]. The slow, balanced, and simultaneous realization of industrial ripeness and aromatic and phenolic ripeness in grapes, at the same time, is one of the features that directly determines the

Stress level	Years 2019	Defoliation treatments			Main effect				
		С	FW	RW	LW	LWP	у	ear	
		3.38	3.18	3.17	3.16	3.00 B			
	2020	2.81	2.73	2.68	2.93				
	mean	3.10	2.95	2.92	3.04				
S1	2019	2.27	2.44	2.20	2.20	2.38 C			
	2020	2.59	2.31	2.58	2.48				
	mean	2.43	2.37	2.39	2.34				
S2	2019	3.21	3.50	4.66	3.61	3.24 A	2019 3.09 a	2020 2.80	
	2020	2.47	2.82	2.84	2.79				
	mean	2.84	3.16	3.75	3.20	_			
S3	2019	2.55	3.00	3.42	3.56	3.16 AB			
	2020	3.18	3.23	3.44	2.91				
	mean	2.87	3.11	3.43	3.23	_			
DTME		2.81 B	2.90 B	3.12 A	2.95 AB	_			

Table 12. Defoliation and LWP treatment effects on total tannin content ($g kg^{-1}$)

LWPME LSD_{0.05}:0.19, YearME LSD_{0.05}:0.13, DTME LSD_{0.05}:0.19

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

type and quality of the wine. These compounds do not develop in the same way as sugars during grape ripening; their maximum concentrations would typically not be the same as the maximum sugar accumulation. Therefore, the follow-up of phenolic maturity plays a vital role in evaluating total oenological maturity and quality. Phenol components are among the most critical substances in the composition of grapes and wine. The biosynthesis and concentration of phenolic compounds are essential ripening criteria for red grape varieties and a quality element in ripe grapes or the final product during processing into must and wine.

Palliotti et al. [2013] determined that the amount of phenolic substances increased in the application where they removed 75–80% of the leaves. According to the attached data, although it is not statistically significant, it is seen that different leaf removal applications positively affect the number of phenolic substances. Alço [2019] stated that among the applications, the amount of phenolic substance was the least in the application in which the leaf area was reduced the most.

It was determined that LWP treatments make a significant difference in total phenolic substance content. Similarly, Roby et al. [2004] and Chacón et al. [2009] reported that increasing water stress increased the total phenolic content. The S3 stress level has reached 1958.49 mg kg⁻¹ total phenolic substance amount. S0, S1, and S2 stress levels were found to have a total phenolic content of 1707.90 mg kg⁻¹, 1672.70 mg kg⁻¹, and 1587.81 mg kg⁻¹, respectively (Tab. 13).

CONCLUSIONS

It is observed that the production year, leaf water potentials (Ψ leaf), and stress levels in vines are under the influence of the current vegetation period conditions as well as the previous or older years. Both stress and defoliation treatments retarded sugar accumulation and resulted in a balance between maturity indices and oenological, phenolic, and aromatic maturity. In addition, the S3 stress level increased the secondary metabolites to the highest values. Although the S3 (<-0.7 MPa)

Stress level	Years 2019	Defoliation treatments				Main effect			
		С	FW	RW	LW	LWP	ye	ear	
		1750.66	1777.33	1656.33	1660.33	1707.90 B			
	2020	1808.96	1573.00	1776.96	1659.66				
	mean	1779.81	1675.16	1716.65	1660.00				
S1	2019	1459.00	1660.00	1613.33	1941.33	1672.70 B			
	2020	1679.63	1598.36	1688.96	1741.00				
	mean	1569.31	1629.18	1651.15	1841.16				
S2	2019	1695.00	1672.00	1734.66	1548.66	1587.81 B	2019 1767.04	2020 1696.41	
	2020	1591.70	1273.10	1691.66	1495.70		1707.04	1090.4	
	mean	1643.35	1472.55	1713.16	1522.18				
S3	2019	1977.33	1920.00	2048.00	2158.66	1958.49 A			
	2020	1787.63	1773.10	1927.63	2075.56				
	mean	1882.48	1846.55	1987.81	2117.11				
DTME		1718.74	1655.86	1767.19	1785.11	_			

Table 13. Different defoliation and LWP treatment effects on total phenolic substance (mg kg⁻¹)

LWPME LSD_{0.05}:157.78

Values expressed with different letters in the same column are statistically significant at the P < 0.05 level according to the LSD multiple comparison test. C – control, FW – full window, RW – right window (west window), LW – left window (east window), LWPME – leaf water potential main effect, year – year main effect, DTME – defoliation treatments main effect

treatment had high values in terms of phytochemicals, at this level, it was determined that the grapevines were blocked and reduced their photosynthetic activities. The S3 grapevines represented the lowest TSS accumulation. This situation can be an opportunity to adjust the maturity level according to the desired production target. However, it has been observed that this stress level causes water to be visibly withdrawn from the berry, causing wrinkling and shrinkage in some clusters at harvest time. Similarly, undesirable high levels of secondary metabolites are considered a factor that disrupts the balance in wine components. For this reason, the S2 (-0.5 MPa and -0.7 MPa) stress level resulted in more balanced results, as seen in the combination of years in terms of both primary and secondary metabolites. In this case, the lowest values were observed in the maturity indices. Although the FW defoliation treatment slowed down the accumulation of TSS and provided manipulations regarding maturity indexes, it showed variable effects in terms of secondary metabolites. The RW defoliation treatment may cause higher primary and secondary metabolite values in all criteria.

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AUTHOR CONTRIBUTION

The authors declare that they have contributed equally to the article. All authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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