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Influence of Leaf Water Potential and Defoliation Techniques on Leaf Area Characteristics in 'Merlot'/41B Grapevines

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ABSTRACT
This study aimed to assess how variations in leaf water potential and different defoliation treatments influence leaf area characteristics. The research was carried out during two consecutive years (2019-2020) on 'Merlot'/41B combination grapevines cultivated in the Tekirdağ, Şarköy vineyards of Chateau Kalpak. Four distinct water stress levels (S0, S1, S2, and S3) were implemented based on measurements of leaf water potential. Additionally, defoliation treatments were applied, including Control (C), Full Window (FW), Right Window (RW), and Left Window (LW). Upon analyzing leaf characteristics, a clear trend emerged, wherein higher stress levels correlated with an increased area of primary, lateral, and total leaves per vine. Concerning leaf removal interventions, the application of FW led to a reduction in all criteria except for the total area of main leaves per vine. While FW causes a decrease in certain leaf parameters under controlled conditions, the stress-induced increase in total leaf area points to the mechanism of plasticity in grapevines and warrants further investigation under different environmental and production dynamics.

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'Merlot'/41B Asmalarında Yaprak Su Potansiyeli ve Yaprak Alma Uygulamalarının Yaprak Alanı Özelliklerine Etkisi

ÖZET
Bu çalışma, yaprak su potansiyelindeki değişikliklerin ve farklı yaprak alma işlemlerinin yaprak alanı özelliklerini nasıl etkilediğini değerlendirmeyi amaçlamıştır. Araştırma, Kalpak Şatosu'nun Tekirdağ, Şarköy bağlarında yetiştirilen 'Merlot'/41B kombinasyonlu asmalarda iki yıl süresince (2019-2020) yürütülmüştür. Yaprak su potansiyeli ölçümlerine dayalı olarak dört farklı su stresi seviyesi (S0, S1, S2 ve S3) uygulanmıştır. Ek olarak, Kontrol (C), Tam Pencere (FW), Sağ Pencere (RW) ve Sol Pencere (LW) olmak üzere dört farklı yaprak alma işlemi uygulanmıştır. Daha yüksek stres seviyelerinin, asma başına artan ana, koltuk ve toplam yaprak artış eğilimine neden olduğu belirlenmiştir. FW uygulaması, asma başına toplam ana yaprak alanı dışında tüm kriterlerde bir azalmaya yol açmıştır. FW, kontrollü koşullarda belirli yaprak parametrelerinde düşüşe neden olurken, toplam yaprak alanında stresin neden olduğu artış, asmalardaki plastidite mekanizmasına işaret etmekte ve farklı çevresel ve üretim dinamikleri altında daha fazla araştırma yapılmasını gerektirdiğini göstermektedir.

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INTRODUCTION

The grapevine canopy is a complex system of leaves, stems, and branches that play an important role in photosynthesis, fruit ripening, and water relations.

The regulation of grapevine canopy characteristics, and thus the growth and productivity of grapevines, are influenced by a range of factors, including biotic and abiotic factors such as water availability, temperature, soil nutrients, and pest and disease pressure. However, among these factors, water availability is crucial in determining grapevine growth and productivity as it directly affects the plant's physiological and biochemical processes. Human interaction with grapevine plants also plays a significant role in wine production, the direct manipulation of the grapevines' growth and health through cultivation practices, and the subsequent post-harvest handling and processing techniques meticulously employed by winemakers. Proper care and management of grapevines, including pruning, canopy management, irrigation, and fertilization, can improve vine growth and fruit quality, leading to higher-quality wine production (Brillante et al., 2018; Candar et al., 2020; Mirás-Avalos & Araujo, 2021).

In sustainable viticulture, the regulation of leaf water potential (LWP, Ψleaf) is essential, and it is a commonly used indicator of plant water status, defined as the difference in water potential between a leaf and its surroundings. Decreased water resources due to global climate change are effective in the grapevine life cycle, and monitoring and management of LWP in grapevine plants affect cluster characteristics, berry, and wine composition by promoting slower leaf growth and higher water use efficiency via leaf characteristics (Rienth & Scholasch, 2019; Deloire et al., 2020).

Defoliation is a common vineyard practice that can affect grapevine water status and productivity. However, defoliation practices can have significant physiological effects on the production-consumption balance of vines (Bowen, 2009). These effects include a decrease in the number of photosynthesis products delivered to the cluster (Poni et al., 2008; Palliotti et al., 2013; Vaillant-Gaveau et al., 2014), limited root growth (Hunter et al., 1995), and decreased water efficiency (Medrano et al., 2007). Removing leaves during berry ripening can eliminate a potential source of carbon (C) and nitrogen (N), which may lead to a reduction in sugar and nitrogen accumulation (Rossouw et al., 2018), ultimately affecting berry quality (Bubola et al., 2022). Moreover, defoliation can reduce the total leaf area of the vine, weakening grapevine growth in the following years, and leading to decreased yields (Bahar et al., 2018). Therefore, grapevine physiological activity, leaf individual size, and the total leaf area on the grapevine are in an interactive relationship with each other (Candar, 2021; Candar, 2022; Candar et al. 2022). Additionally, defoliation can alter the microclimate around grape clusters, affecting fruit quality and ripening (Bubola et al., 2019; Candar, 2019; Stefanovic et al., 2021).

Understanding the effects of LWP and defoliation on grapevine leaf characteristics can provide valuable insights into the mechanisms underlying grapevine responses to water stress and defoliation. Such insights can help optimize vineyard management practices to improve grapevine productivity and fruit quality.

Therefore, this study aimed to investigate the effects of LWP and defoliation treatments on grapevine canopy characteristics, including individual and total leaf areas of main and lateral leaves, total leaf areas of grapevines, leaf area exposed to direct sun and the sun-exposed leaf area per kilogram of grapes.

MATERIAL and METHOD

Location and plant material

The study was carried out in the vineyard of a private winery located in Tekirdağ, Şarköy, during two consecutive vegetation periods in 2019 and 2020. The grapevines utilized in the study were 'Merlot'/41B combination, planted with a 2.1 m and 1.0 m in-row spacing. The grapevines were trained to 70 cm stem height and the double arm cordon training method in the Espalier system.

Methods

To ensure the accuracy of the study, the vines in each row were carefully selected to have the same age, development stage, and approximate load. After disregarding edge effects, the selected grapevines were considered homogeneous. In the year 2020, when the shoots had grown approximately 25-35 cm, the number of shoots and clusters was found to be the same as the previous year. Routine cultural operations, including tillage, fertilization, weeding, and spraying, were performed in the vineyard throughout the two-year vegetation period from 2019 to 2020.

The experiment was designed using the divided plots trial design with three replications, and each plot was subjected to a specific level of stress measured by leaf water potential (LWP). The study included a total of 144 vines, with 48 vines in each replication, and four different stress levels (S0, S1, S2, and S3) and leaf removal treatments including Control (C, no defoliation), Full Window (FW), Right Window (RW), and Left Window (LW).

Irrigation was performed as required based on the predawn leaf water potential (LWP, Ψ_{pd}) measurements taken at five to seven-day intervals. The predetermined stress levels were used to adjust the irrigation, and the Ψ_{pd} was verified the next day to ensure it remained within the desired range. The control treatment, S0, received no irrigation and relied on random precipitation. S1 had a stress level between -0.4 to -0.6 MPa, and irrigation was used to maintain the Ψ_{pd} within this range. Similarly, S2 had a stress level between -0.5 to -0.7 MPa, and the Ψpd was maintained within this range through irrigation. Lastly, S3 was subjected to a stress level of ≤ -0.7 MPa and the Ψ_{pd} was kept below this value through irrigation. The defoliation treatments (DT) were carried out approximately two weeks after the start of veraison. The treatments involved removing shoots and leaves from the eighth node and creating a window by eliminating all the leaves between the seventh and thirteenth nodes. The study consisted of four different defoliation treatments: Control (C), Full Window (FW), Right Window (RW), and Left Window (LW). For the FW treatment, shoots and leaves were removed from the eighth node. For the RW treatment, all the leaves between the seventh and thirteenth nodes on the west side of the row were removed, while for the LW treatment, all the leaves between the seventh and thirteenth nodes on the east side of the row were removed. The C treatment was used as the control, with no defoliation being performed. The defoliation process was conducted with special care to ensure that the grapes were at a 15-17 °Brix level according to Alço (2019).

Leaf area analysis and measurements

The main phenological development dates of the bud burst (EL- 05), pre-bloom (EL- 19), full bloom (El- 23), berry set (EL- 27), veraison (EL- 35) and the harvest (EL- 38) stages were recorded using the method described by Lorenz et al. (1995). Climate data were obtained from the Turkish State Meteorological Service (MGM).

To determine the average leaf area of the main leaves developing from the main shoot and the lateral leaf areas growing from the lateral shoots, the fully grown and healthy leaves were scanned with a scanner after the harvest. The images obtained from the scanner were analyzed using the Fläeche program (Kraft, 1995), and the leaf area was calculated and recorded in cm^2.

To calculate the total main leaf (cm^2 $vine^{-1}$) and total lateral leaf area per vine (cm^2 $vine^{-1}$), the average number of leaves in the shoot and the total number of shoots after harvest were multiplied. The total leaf area per vine was determined by adding the main leaf area per stem and the lateral leaf area per vine, following the method described by Irimia and Tardea (2006) and Sanchez-de-Miguel et al. (2010).

The formula used to calculate the leaf area exposed to direct sun (m^2 da^{-1}) was;

$$\frac{1000}{RS} * [(H * 2) + CW] * (1 - CD)$$

where RS represents row spacing in meters, H represents height in meters, CW represents canopy width in meters, and CD represents canopy discontinuity (10%).

To obtain the sun-exposed leaf area per kilogram of grapes (m^2 kg^{-1}), the leaf area exposed to direct sun (m^2 da^{-1}) was divided by the yield per decare (kg da^{-1}), using the equation by Carbonneau (1980) and Carbonneau (1983).

Trail design and statistical analysis

The experiment was designed using a divided plots trial design, where the main plot represented water stress levels, and each subplot represented defoliation practices. A total of 144 vines were included in the study, with three plants per sub-plot and three replications per combination of four water stress levels and four defoliation treatments.

Statistical data analysis was performed using JMP 13.2.0. Analysis of variance (ANOVA) was used to determine the significance of differences between treatments, and significant differences were further grouped using the LSD test at a 5% significance level. correlations and principal component analysis of selected variables was carried out using R statistical environment (R Core Team, 2016).

RESULTS and DISCUSSION

Climate, phenology, yield, and total soluble solids.

Table 1 shows the viticultural climate indicators of Tekirdağ for the years 1939-2019, as well as for the years 2019 and 2020.

The mean annual precipitation decreased from 589.50 mm between 1939 and 2019 to 378.40 mm in 2019 and further decreased to 290.00 mm in 2020. The precipitation for vegetation, also decreased from 196.70 mm to 129.80 mm to 83.60 mm, respectively. The average temperature for 2019 was 15.60°C, while the average for 2020 was 15.30°C, while the long-term average temperature was 14.00°C. The mean temperature of the hottest month increased from 23.80 °C for 1939-2019 to 25.30 °C in 2019 and remained stable at 25.00 °C in 2020. These trends suggest that the region is becoming drier, which could have implications for grape production.

The Huglin index (HI) increased from 2128.20 °C in 1939-2019 to 2324.07 °C in 2019 but decreased slightly to 2229.21 °C in 2020. The Winkler index (WI-GDD) increased from 1872.00 degree-days to 2157.00 degree-days in 2019 and slightly decreased to 2124.00 degree-days in 2020. The Hydrothermal Index (HyI), which combines temperature and precipitation, decreased from 3595.20 °C mm for 1939-2019 to 2181.54 °C mm in 2019 and further decreased to 1328.10 °C mm in 2020. These changes indicate that the region is becoming warmer, with increasing heat accumulation during the growing season. The Night Cold Index (CI), increased from 16.00 °C to 17.60 °C to 19.20 °C, respectively, and the Growing Season Temperatures (GST). Finally, the GST, which is the average temperature during the growing season, increased from 18.91 °C to 20.27 °C to 20.11 °C, respectively (Table 1).

Table 1 Tekirdağ viticultural climate indicators in experimental years.
Çizelge 1. Deneme yıllarında Tekirdağ bağcılık iklim göstergeleri.

Climatic indices	Unit	1939-2019	2019	2020	References
Precipitation (Mean Annual)	mm	589.50	378.40	290.00	-
Precipitation (Vegetation)	mm	196.70	129.80	83.60	-
Mean temperature of hottest month	°C	23.80	25.30	25.00	-
Huglin index (HI)	°C	2128.20	2324.07	2229.21	(Huglin, 1978)
Winkler index (WI-GDD)	degree-day	1872.00	2157.00	2124.00	(Winkler et al, 1974)
Hydrothermic Index (HyI)	°C mm	3595.20	2181.54	1328.10	(Branas, 1946)
Night Cold Index (CI)	°C	16.00	17.60	19.20	(Tonietto, 1999)
Growing Season Temperatures (GST)	°C	18.91	20.27	20.11	(Jones, 2007)

The bud burst (EL 05), which corresponds to the appearance of green shoot tips, occurred on April 11, 2019, and on April 15, 2020, indicating that the 2020 bud burst was delayed by four days compared to 2019. The pre-bloom (EL 19) stage, occurred on May 26, 2019, and May 30, 2020, respectively. The full bloom (El 23), in 2019, occurred on June 2, while in 2020, it occurred on June 8, indicating a six-day delay in the latter year. Berry set (EL 27), was observed on June 9, 2019, and June 14, 2020, respectively. The veraison (EL 35) occurred on July 20, 2019, and July 24, 2020, respectively. The harvest (EL 38), in 2019, occurred on September 15, while in 2020, it occurred on September 16.

Overall, data showed that there were slight variations in the timing of the phenological stages between the two years, with some stages being delayed in 2020 compared to 2019. These differences could be attributed to variations in weather patterns and environmental conditions between the two years.

Since homogeneous grapevines were already selected in both years according to the trial design, the number of shoots and clusters was also homogenized, no statistical difference could be detected in kg yield per grapevine according to defoliation and stress treatments. Yield per grapevine varied between 2.20-2.22 kg per grapevine in defoliation treatments and 2.20-20.26 kg per grapevine in stress treatments.

Although defoliation treatments (FW and LW) had no overall impact on total soluble solids (TSS) accumulation over multiple years, leaf water potential (LWP) treatments did significantly affect TSS. Within the defoliation group, LW resulted in the highest average TSS (24.78 °Brix), while FW had the lowest (24.35 °Brix). Within the LWP group, the S0 treatment with the highest potential (25.00 °Brix) achieved the highest TSS, while S3 had the lowest. Notably, TSS was higher in 2020 (24.76 °Brix) compared to the previous year (24.39 °Brix).

Main leaf area

The previous studies determined that the size of the main leaves of the 'Merlot' grape cultivar ranged from 152.29 cm² to 237.60 cm² (Candar, 2018). However, the available data for experimental years ranged from 91.79 cm² to 142.94 cm², with an average of 125.93±22.69 cm². It is known that leaf size is influenced by environmental, developmental, and genetic factors during the formation process. Thus, there can be variations in leaf size from the average appearance to its actual size (Chitwood et al., 2016a). Therefore, the morphological and physiological characteristics of the leaves may be influenced by factors other than the variety itself, such as their position in the shoot or environmental effects.

In the year of 2019, there was a general decrease in the main leaf area as the level of water stress increased. This trend is supported by the declining mean values observed from S0 to S3. The highest main leaf area was recorded under S3 in 2019, which differed significantly from the other stress treatments. However, in 2020, there were no significant differences among the stress treatments. Regarding the defoliation treatments, the mean main leaf area values in the year 2019 did not show any significant differences. However, in 2020, significant differences were observed among the defoliation treatments. The main leaf area was significantly higher in the control group (C) compared to the defoliation treatments in both experimental years. This indicates that defoliation negatively affected the main leaf area (Table 2).

When considering the significance levels for the mean main leaf area concerning the main effect of defoliation treatment (DME), it was observed that the C treatment had the largest leaves, with an average size of 139.66 cm², while the FW treatment had the smallest leaves, averaging 114.97 cm². In terms of the main effect of leaf water potential (LWPME), the S3 treatment exhibited the highest main leaf size, with an average value of 134.01 cm², while the S0 treatment had the lowest main leaf size, averaging 121.01 cm² across the experimental years.

Table 2 Effects of stress levels and defoliation treatments on leaf area variables.
Çizelge 2. Stres seviyeleri ve yaprak alma uygulamalarının yaprak alanı değişkenleri üzerindeki etkileri. Values marked with different letters in the same column and row were statistically significant at $p<0.05$ level according to ANOVA and the LSD test. Results expressed as mean of three replications with ± SE.
S0; control of stress treatments, S1; Ψ_{pd} between -0.3/-0.5 MPa, S2; Ψ_{pd} between -0.5/-0.7 MPa, and S3; Ψ_{pd} <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window. LWPME= stress treatments main effect, DTME=defoliation treatments main effect, YME=year main effect.

It is reasonable to expect that increased water stress would lead to a decrease in the average main leaf area. However, in the case of grapevine plants, which are perennial woody plants, their leaf shape and size differentiation may exhibit adaptation and flexibility to various environmental factors, which may differ from evolutionary and developmental effects (Chitwood et al., 2016b). Therefore, the finding regarding the main effect of leaf water potential (LWPME) aligns with the results reported by Candar (2018). Regarding the main effect of defoliation treatment (DME), it was discovered that the FW treatment resulted in the smallest main leaf areas. This finding differs from the observations of Candar (2018), where it was noted that the main leaf size increased in grapevines with the lowest total leaf area, where only the main leaves remained on the plant. However, the available data indicate that lateral leaves have the potential to contribute to the development of main leaves, and the size of the main leaves may not play a critical role in maintaining total photosynthesis rates. Moreover, the physiological ages of main leaves and axillary leaves were found to be distinct, which is linked to their photosynthetic capacity. Schultz (1996) explained that young leaves exhibit a high photosynthetic capacity until harvest, but their position in the canopy and the light microclimate can influence the overall photosynthesis of the plant. In this regard, the available data are consistent with the findings of Schultz (1996). Furthermore, based on the data from this study, the relationship between the initiation time of stress-induced irrigation practices and leaf maturity could elucidate the observation that leaf size did not exhibit a linear and positive alteration with escalating stress levels.

However, the impact of decreasing soil water reserves becomes evident when comparing the experimental years. The main effect of the year (YME) shows statistical significance. In 2019, the average main leaf area was 138.56 cm², indicating its higher importance. However, in 2020, the average main leaf area significantly decreased to 113.29 cm², placing it in the lower-importance group. The reduced precipitation received during the vegetation period in 2020 contributed to the main leaves remaining consistently smaller compared to the previous year. It is believed that this decrease in the average main leaf area in 2020, compared to 2019, has a direct impact on photosynthesis, resulting in reduced yield per vine and, consequently, yield per decare.

Lateral leaf area

The average size of the lateral leaves of cv. 'Merlot' was determined to be 31.62±6.17 cm². Previous studies, on the other hand, report that the lateral leaf area for 'Merlot' grape cultivar varies between 55.16 cm² and 92.74 cm² (Candar, 2018). In the year of 2019, the lateral leaf area generally increased as the level of water stress increased. The highest lateral leaf area was observed under S3 in 2019, which differed significantly from the S0 and S1 treatments. However, in 2020, although S3 had the lowest value, it was significantly different only from S2. The significant differences observed in the main effect of water stress treatments (LWPME) in both experimental years indicate that the lateral leaf area was significantly influenced by the amount of water applied to grapevines (Table 2).

In both 2019 and 2020 years, the lateral leaf area exhibited some variation among the different defoliation treatments. In 2019, the lateral leaf area showed significant differences among the defoliation treatments in the control group (C). However, in 2020, significant differences were not observed between the defoliation treatments. The control group in 2020 and S3 in 2019 displayed higher lateral leaf area compared to the defoliation treatments.

When examining the mean lateral leaf area across the experimental years, the main effect of defoliation treatment (DME) was found to be statistically significant. The highest values were observed in LW with a mean of 32.76 cm² and RW with a mean of 32.28 cm², while the lowest lateral leaf area size was recorded in the FW treatment with a mean of 29.77 cm².

Analyzing the average lateral leaf area based on the main effect of leaf water potential (LWPME) across the years, ANOVA showed statistical significance at the 5% level. The S2 treatment had the highest mean value of 34.68 cm², placing it in the first importance group, while the lower values were observed in the S1, S3, and S0 treatments, respectively, in the last importance group.

Furthermore, when examining the average lateral leaf area concerning the main effect of the year (YME), statistical significance was observed. The year 2019 exhibited the highest mean value of 34.02 cm^2, placing it in the first importance group, while the year 2020 was found to be in the last importance group with a mean value of 29.21 cm^2.

Zinni et al. (2023) observed that complete removal of whole leaves was effective in increasing the average lateral leaf area in the application without tipping. The study indicated that both RW and LW applications were successful in enhancing the average lateral leaf area. The available data corroborate these findings, as similar results were achieved for the year 2019.

Total main leaf area per grapevine

In both experimental years, there was a general increase in the total main leaf area as the level of water stress increased. The highest total main leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress treatments. However, there were no significant differences in the total main leaf area among the control group and the different defoliation treatments in both 2019 and 2020. The mean values for the control group (C) and the defoliation treatments were relatively similar in both years (Table 2).

When evaluating the two-year data of the total main leaf area per grapevine together, it was found that the main effect of defoliation treatment (DME) did not show significant differences between applications with close values. On the other hand, the main effect of leaf water potential (LWPME) was statistically significant, with S3 reaching the highest value when importance levels were examined.

Regarding the main effect of year (YME), while the total main leaf area per grapevine was statistically significant throughout the experimental years, it was determined that 2019 had the highest value of 1.80 m^2 main leaf area per vine, placing it in the first importance group, and 2020 had the lowest value of 1.67 m^2 vine^{-1}, placing it in the last importance group.

Total lateral leaf area per grapevine

In both experimental years, there was a general increase in the total main leaf area as the level of water stress increased. The highest total main leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress treatments. However, there were no significant differences in the total main leaf area among the control group, and in both 2019 and 2020, there was a general increase in the total lateral leaf area as the level of water stress increased. This is evident from the increasing mean values observed from S0 to S3. The highest total lateral leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress treatments. The main effect of stress treatments (LWPME) did not significantly affect the total lateral leaf area, indicating that the interaction between stress treatments did not have a significant impact.

Similarly, the total lateral leaf area did not show significant differences among the different defoliation treatments in both 2019 and 2020. The mean values for the control group (C) and the defoliation treatments were relatively similar in both years (Table 2).

When examining the combination of years for the total lateral leaf area per grapevine, the main effects of leaf water potential (LWPME) and year (YME) were found to be statistically significant. However, the main effect of defoliation treatment (DME) was not found to be statistically significant. Among the defoliation treatments, RW had the highest value, while FW had the lowest value based on DME. According to LWPME, the S3 application was in the first importance group, followed by the other treatments. Considering the combined effect of LWP and defoliation treatments, the year 2020 reached the highest value of 3.38 m^2 vine^{-1}, placing it in the first importance group, while the year 2019 was found to be in the last importance group with a value of 2.11 m^2 vine^{-1}.

Total leaf area per grapevine

In both 2019 and 2020, there was a general increase in the total leaf area as the level of water stress increased. This trend is evident from the increasing mean values observed from S0 to S3. The highest total leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress treatments. The total leaf area also exhibited some variation among the different defoliation treatments in both 2019 and 2020. In 2019, there were no significant differences observed among the defoliation treatments. However, in 2020, significant differences were observed between the defoliation treatments (Table 3).

Table 3 Effects of stress levels and defoliation treatments on total main leaf area, sun-exposed leaf area, and yield-related variables.

Çizelge 3. Stres seviyeleri ve yaprak alma uygulamalarının toplam ana yaprak alanı, güneşe maruz kalan yaprak alanı ve verime bağlı değişkenler üzerindeki etkileri.

	Total leaf area per grapevine (m^2 vine^{-1})	Leaf area exposed to direct sun (m^2 da^{-1})	Sun exposed leaf area per kilogram of grapes (m^2 kg^{-1})

	2019	2020	2019	2020	2019	2020
			LWPs'			
S0	3.60±0.18b	4.86±0.21b	1051.50±45.49	1062.15±45.52	0.94±0.04b	1.04±0.06
S1	3.44±0.59b	4.92±0.22b	1056.83±45.47	1058.10±44.41	0.97±0.03b	1.01±0.07
S2	3.75±0.53b	4.94±0.14b	1055.91±46.34	1057.85±44.31	1.03±0.03a	1.02±0.05
S3	4.72±0.14a	5.90±0.14a	1058.33±47.50	1057.14±45.68	1.02±0.04a	1.01±0.07
LWPME (LSD0.05)	***0.46***	***0.50***	***ns***	***ns***	***0.03***	***ns***
			DTs'			
C	3.85±0.19	5.55±0.20a	1272.58±3.62a	1271.19±2.31a	1.17±0.20a	1.28±0.06a
FW	3.95±0.15	4.86±0.28b	841.33±3.72c	850.00±2.29c	0.80±0.12c	0.80±0.03c
RW	3.99±0.20	5.20±0.15ab	1055.24±5.52b	1054.52±2.59b	0.99±0.01b	0.99±0.01b
LW	3.72±0.29	5.01±0.18b	1053.33±5.09b	1059.52±3.73b	0.99±0.01b	1.02±0.03b
DME (LSD0.05)	***ns***	***0.50***	***14.31***	***8.54***	***0.03***	***0.13***
			YME			
	3.88±0.10B	5.16±0.10A	1055.59±22.35	1058.81±21.76	0.99±0.02	1.02±0.03
YME (LSD0.05)	***0.23***		***ns***		***ns***	

Values marked with different letters in the same column and row were statistically significant at $p<0.05$ level according to ANOVA and the LSD test. Results expressed as mean of three replications with ± SE.

S0; control of stress treatments, S1; Ψ_{pd} between -0.3/-0.5 MPa, S2; Ψ_{pd} between -0.5/-0.7 MPa, and S3; Ψ_{pd} <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window. LWPME= stress treatments main effect, DTME=defoliation treatments main effect, YME=year main effect.

Although the year combination did not show statistical significance in terms of the main effect of defoliation treatment (DME), it was found that the highest value for the total leaf area per grapevine was 4.70 m^2 vine^{-1} in the control (C) treatment, while the lowest value was observed in the FW treatment. When considering the main effect of leaf water potential (LWPME), it was found that the annual incorporation of LWPME is significant for the total leaf area per grapevine at the 5% level. The S3 treatment was placed in the first importance group with a value of 5.31 m^2 vine^{-1}, followed by the S2, S0, and S1 treatments in order.

Furthermore, the total leaf area per grapevine showed statistical significance at the 5% level in terms of the main effect of year (YME). In the first importance group according to YME, it reached 5.16 m^2 vine^{-1} in 2020 and 3.88 m^2 vine^{-1} in 2019 (Table 3).

Delice (2001) and Calo et al. (1999) found a significant positive correlation between grapevine yield and total leaf area, stating that brix was associated with the ratios of total leaf area/leaf area exposed to direct sun and vegetative growth/yield balance. In the present study, it was observed that the total leaf area per vine increased in 2020, and the brix values in 2020 were higher compared to the previous year.

Leaf area exposed to direct sun m^2 da^{-1}

There were no significant differences in the leaf area exposed to direct sun among the different stress treatments in both 2019 and 2020. The mean values for the leaf area exposed to direct sun were relatively similar across all stress treatments in both years.

However, there were significant differences in the leaf area exposed to direct sun among the different defoliation treatments in both 2019 and 2020. The control group (C) had the highest leaf area exposed to direct sun, which was significantly different from the other defoliation treatments. Significant differences were also observed among the defoliation treatments themselves, with the FW treatment having the lowest leaf area exposed to direct sun (Table 3).

When examining the combination of years for the main effect of defoliation treatment (DME), it was found to be statistically significant. The C treatment had the highest value of 1271.88 m^2 ha^{-1}, while the FW treatment had the lowest leaf area exposed to direct sun with a value of 845.66 m^2 ha^{-1}. In terms of the main effect of leaf water potential (LWPME), it was observed that the S0 treatment had a relatively lower leaf area exposed to direct sun with a value of 1056.82 m^2 ha^{-1}, while the S3 treatments had a higher leaf area exposed to direct sun with a value of 1057.73 m^2 ha^{-1}. Regarding the main effect of the year (YME), the year 2019 had a value of 1055.64 m^2 ha^{-1}, while the year 2020 had a slightly higher value of 1058.81 m^2 ha^{-1}.

Sun-exposed leaf area per kilogram of grapes m^2 kg^{-1}

There were significant differences in the sun-exposed leaf area per kilogram of grapes among the different stress treatments in 2019. However, in 2020, no significant differences were observed among the stress treatments.

Similarly, the sun-exposed leaf area per kilogram of grapes showed significant differences among the different defoliation treatments in both 2019 and 2020. The control group generally had the highest sun-exposed leaf area per kilogram of grapes, which differed significantly from the other defoliation treatments. Significant differences were also observed among the defoliation treatments themselves, with the FW treatment having the lowest sun-exposed leaf area per kilogram of grapes (Table 3).

When considering the combination of 2019 and 2020 in terms of the main effect of defoliation treatment (DME), it was determined that the values ranged between 2.19 m^2 kg^{-1} in the control (C) treatments and 2.00 m^2 kg^{-1} in the FW treatments. Examining the main effect of leaf water potential (LWPME), it was found to be statistically significant, with the S3 treatments reaching the highest value of 2.43 m^2 kg^{-1} in the first importance group, followed by the S2, S1, and S0 treatments in order. In terms of the main effect of the year (YME), although statistically significant, it reached a value of 2.38 m^2 kg^{-1} in 2020 and 1.77 m^2 kg^{-1} in 2019, with a higher value in 2020.

Correlations of leaf characteristics, maturation indices and yield

The variable lateral leaf area has a moderate positive correlation with the main leaf area with a correlation coefficient of 0.398. The total main leaf area per grapevine has a strong positive correlation with the main leaf area, a weak positive correlation with the lateral leaf area, and no significant correlation with the remaining variables. The variable total lateral leaf area per grapevine has a weak negative correlation with the main leaf area and no significant correlation with the other variables. The total leaf area per grapevine has no significant correlation with the main leaf area and lateral leaf area, but it has a moderate positive correlation with the total main leaf area per grapevine, a strong positive correlation with the total lateral leaf area per grapevine with a correlation coefficient of 0.944, and no significant correlation with the remaining variables. The leaf area exposed to the direct sun has a moderate positive correlation with the main leaf area and no significant correlation with the other variables. The correlation coefficient between sun-exposed leaf area per kilogram of grapes and leaf area exposed to direct sun is 0.796 and represents a strong positive relationship. The sun-exposed leaf area per kilogram of grapes also has weak positive correlations with total main leaf area per grapevine, total lateral leaf area per grapevine, and total leaf area per grapevine. The maturation indices of pH^2 x °Brix have a weak positive correlation with the main leaf area, weak negative correlations with total leaf area per grapevine and total lateral leaf area per grapevine, and no significant correlation with the other variables. The yield has no significant correlation with any of the variables except a strong negative correlation with sun-exposed leaf area per kilogram of grapes (Figure 1).

Principal component analysis (PCA) of leaf characteristics, maturation indices, and yield

To assess the interaction among stress levels, defoliation treatment, and the leaf characteristics under study, a PCA was conducted. The dataset consisted of a total of eight treatments and nine leaf variables, and the analysis was performed using the covariance matrix. Two distinct biplots were generated to examine the impact of stress levels and defoliation treatments on the leaf variables individually.

According to the cumulative proportion of variance in the LWP biplot, PC1 accounts for 56.80% of the total variance. When PC1 and PC2 are combined, they explain 95.10% of the total variance. Furthermore, PC1, PC2, and PC3 together explain 100% of the total variance. Hence, PC1 and PC2 are the primary components in capturing the variability in the LWP data. Similarly, for the defoliation treatments, PC1 explains 66.80% of the total variance, PC2 explains 23.50% of the total variance, and when PC1, PC2, and PC3 are combined, they account for 100% of the total variance, as shown by the cumulative proportion of variance. Both PCA correlation plots demonstrate a noticeable distinction among the samples based on the treatments and variables, indicating a reasonable separation (Figure 2).

Upon analyzing the LWPs biplot, it is evident that variable S0 has a loading value of -2.08 for Dim.1, indicating a negative correlation between S0 and the first principal component. Similarly, S1 has a loading value of -1.14 for Dim.1, also indicating a negative correlation with the first principal component. In contrast, S2 has a loading value of 0.11 for Dim.1, suggesting a weaker correlation. On the other hand, S3 displays a loading value of 3.11 for Dim.1, indicating a strong positive correlation with the first principal component. Regarding Dim.2, S0 has a loading value of 0.79, suggesting a positive correlation with the second principal component. Similarly, S1 has a loading value of 0.98 for Dim.2, indicating a positive correlation. In contrast, S2 demonstrates a significant negative correlation with the second principal component, as shown by its loading value of 2.78 for Dim.2. Finally, S3 has a loading value of 0.99 for Dim.2, suggesting a positive correlation.

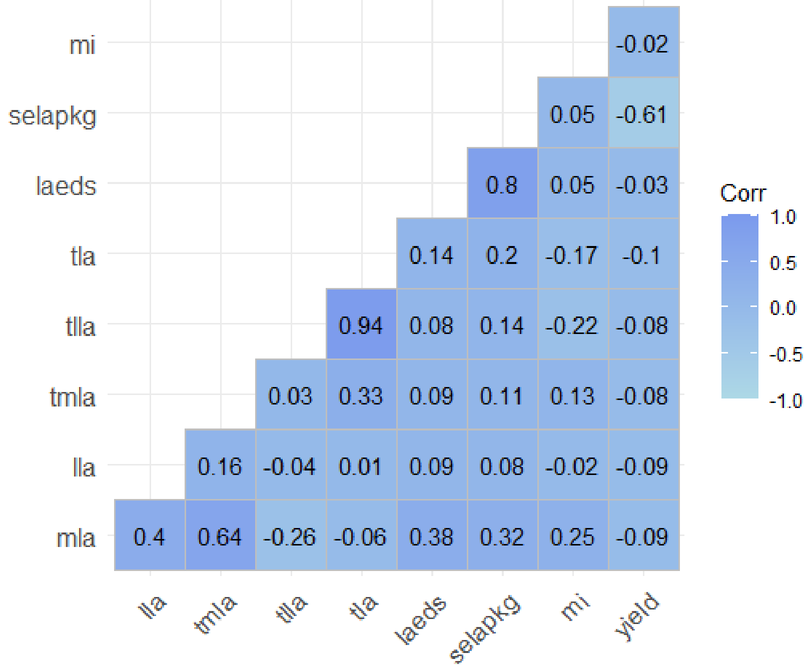

Figure 1. Correlations of selected variables. Coefficient statistical significance indicated by color of squares. mla; main leaf area (cm^2), lla; lateral leaf area (cm^2), tmla; total main leaf area per grapevine (m^2), tlla; total lateral leaf area per grapevine (m^2), tla; total leaf area per grapevine (m^2), laeds; leaf area exposed to direct sun (m^2 da^{-1}), selapkg; Sun exposed leaf area per kilogram of grapes (m^2 kg^{-1}), mi; maturation indice of pH^2 x °Brix, yield; yield per grapevine (kg)

Şekil 1. Şeçilmiş değişkenlerin korelasyonları.

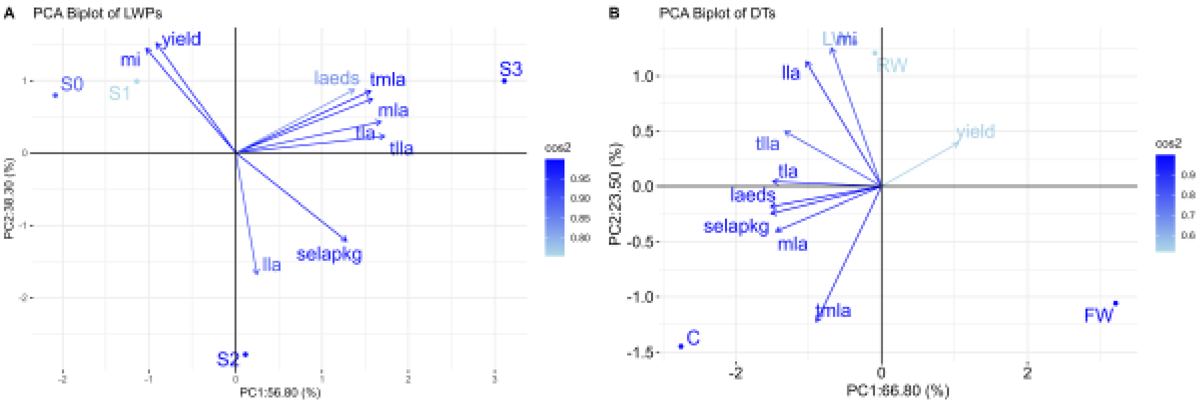

Figure 2. Principal component analysis (PCA) with the mean values of variables. A; PCA biplot of LWPs', B; PCA biplot of DTs'. mla; main leaf area (cm^2), lla; lateral leaf area (cm^2), tmla; total main leaf area per grapevine (m^2), tlla; total lateral leaf area per grapevine (m^2), tla; total leaf area per grapevine (m^2), laeds; leaf area exposed to direct sun (m^2 da^{-1}), selapkg; sun-exposed leaf area per kilogram of grapes (m^2 kg^{-1}), mi; maturation indices of pH^2 x °Brix, yield; yield per grapevine (kg)

Şekil 2. Seçilmiş değişkenlerin birincil bileşen analizi (PCA)

When examining the loading values for the leaf variables in relation to LWP levels, it is evident that the total lateral leaf area per grapevine and the $MI_{pH^2 \times °Brix}$ variable exhibit strong positive correlations with both Dim.1 and Dim.2. This implies that these variables have a significant influence on multiple aspects captured by the principal components. Their impact extends across different underlying factors represented by Dim.1 and Dim.2. In contrast, variables such as lateral leaf area and total lateral leaf area per grapevine display contrasting correlations between the two dimensions. Lateral leaf area shows a weak correlation with Dim.1 but a strong positive correlation with Dim.2. On the other hand, total lateral leaf area per grapevine demonstrates a strong positive correlation with Dim.1 but a weak correlation with Dim.2. These distinct patterns suggest that these variables contribute differently to the underlying factors represented by Dim.1 and Dim.2.

In practical terms, this information can be used to identify key variables that have a consistent and strong impact across multiple dimensions such as total lateral leaf area per grapevine and $\mathrm{MI}_{\mathrm{pH}^2 \times {}^\circ\mathrm{Brix}}$. These variables can be considered influential factors that contribute significantly to the overall structure of the data. Conversely, variables with contrasting correlations like lateral leaf area and total lateral leaf area per grapevine may require further investigation to understand their unique contributions and how they affect different dimensions of the data.

In the DTs biplot, by examining the loading values, it is observed that the variable FW has the highest loading value on Dim.1 with a value of 3.20, indicating a strong positive correlation with the first principal component. This suggests that FW is highly associated with the variability explained by Dim.1. Similarly, LW and RW exhibit positive loadings on Dim.2 by values of 1.29 and 1.20, respectively, indicating a positive correlation with the second principal component. Conversely, variable C demonstrates negative loadings on both Dim.1 and Dim.2, indicating a negative correlation with both principal components. This implies that C is inversely related to the variability explained by both dimensions. These loading values help identify the variables that contribute the most to the respective principal components. In this case, FW appears to have the strongest influence on Dim.1, while LW and RW have significant contributions to Dim.2. Variables such as total leaf area per grapevine, leaf area exposed to direct sun, and sun-exposed leaf area per kilogram of grapes exhibit relatively high loading values in both Dim.1 and Dim.2, indicating strong positive correlations with both principal components. On the other hand, variables like total main leaf area per grapevine, total lateral leaf area per grapevine, and $\mathrm{MI}_{\mathrm{pH}^2 \times {}^\circ\mathrm{Brix}}$ display moderate positive loadings in both dimensions. It's important to note that while the loading values for all variables are positive, their magnitudes differ, indicating variations in the strength of their contributions to the principal components. For instance, the leaf area exposed to direct sun and sun-exposed leaf area per kilogram of grapes have higher loading values compared to the main leaf area or yield, suggesting that they contribute more significantly to the explained variability.

CONCLUSION

Upon analyzing leaf characteristics, an observable trend emerged wherein an elevated stress level corresponded to an increased count of primary, lateral, and overall leaves per vine. This atypical occurrence, rarely documented in existing literature, is believed to activate a stress-mitigating mechanism. This mechanism involves the mobilization of stored materials through internal metabolic processes and the plastidic effects within the grapevine. Furthermore, the experiment was conducted in a uniform and well-balanced vineyard with consistent cultivation practices and longstanding production objectives. Consequently, certain criteria did not distinctly manifest the effects of the interventions; however, the outcomes did capture the underlying trends.

Regarding leaf removal interventions, the application of FW led to a reduction in all criteria except for the total number of primary leaves per vine.

It is anticipated that disparities between the outcomes will become more conspicuous in vineyards with dissimilar crop loads and/or during years when climatic variables exert more pronounced influences.

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Author contribution

The authors declare that they have contributed equally to the article.

Conflicts of interest

The authors declare no conflict of interest.

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