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Exploring the relationship between leaf water potential, defoliation, and grape berry physical properties of Merlot (*Vitis vinifera* L.) grapevine

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Abstract
The aim of this study was to examine the impact of leaf water potential and defoliation treatments on the physical properties of grape berries. The research was conducted over two consecutive years (2019-2020) using 'Merlot'/41B graft combination grapevines grown in the Chateau Kalpak vineyards located in Tekirdağ, Şarköy. The experiment involved four distinct water stress levels (S0, S1, S2, and S3), which were determined based on leaf water potential measurements. These stress levels were subjected to different irrigation levels. Additionally, defoliation treatments were applied, including Control (C), Full Window (FW), Right Window (RW), and Left Window (LW). The results showed that the effects of water stress and defoliation treatments on berry physical properties were statistically insignificant. However, in the second year of the study, the FW treatment was observed to have led to changes in the desired direction for grapevines. This was likely due to the cumulative decrease in water reserves caused by reduced precipitation over multiple years, making the effects of FW treatment more prominent. Moreover, the study found that both current and past vegetation period conditions influence vine production year, leaf water potentials (Ψleaf), and stress levels. Finally, the data revealed that berry weight and % dry weight increased with higher stress levels.
Keywords: Abiotic stress, Grapevines, Leaf removal, Drought, Berry quality, Merlot

INTRODUCTION

Climate is one of the most important factors that affect the life cycle of vines. Temperature, wind, frost, and precipitation are among the most influential climate factors that affect vine growth and development. Additionally, the water status of the vines in the vineyard varies according to topography, cultivation practices, and soil characteristics (Jasse et al., 2021). Global climate change has led to decreased water resources, which has a significant impact on the grapevine life cycle. Adequate water availability is crucial for sustainable viticulture (Medrano et al., 2015). The amount of precipitation that falls as rain is undoubtedly important for grapevine yield and quality. However, the water-holding capacity of soil also exerts a strong influence on these factors (Blaschek et al., 2019). The water-holding capacity of soil is influenced by several factors, including soil texture, topography, and the amount of precipitation. In turn, grapevine water status is affected by both the water-holding capacity of soil and the size of the canopy (Van Leeuwen et al., 2006). The water status of grapevines is known to vary depending on whether water deficiency in the soil occurs before or after veraison (Gambetta et al., 2020). According to Korkutal et al. (2019), grapevines are more sensitive to water restriction before veraison compared to after veraison. During the early stages of berry development, water deficiency can have a significant impact on cell division and expansion, ultimately affecting both the size and structure of the berry, as noted by Bondada and Shutthanandan (2012). Furthermore, Flexas and Medrano (2002) have reported that excessive water stress can lead to a reduction in the size of grape berries. Properly managed water deficit can have a positive impact on various aspects of grapevine growth and development. This includes promoting slower leaf growth and higher water use efficiency, leading to improved cluster characteristics, berry composition, and ultimately, wine quality (De Orduna, 2010; Bahar et al., 2011; Savoi et al., 2016; Korkutal et al., 2019; Blancquaert et al., 2019; Vilanova et al., 2019). The physiological and metabolic responses to water stress also promote the formation of secondary metabolites in the berries, which are responsible for imparting desirable organoleptic properties. This is primarily attributed to the smaller berry size and higher skin-to-pulp ratio, resulting in a relatively higher skin content of tannins, anthocyanins, total phenolics, and other compounds.

Vineyard management practices, such as irrigation, training systems, leaf removal, and cluster thinning, can have a significant impact on grapevine growth and development (Alem et al., 2019). Additionally, both environmental conditions and viticulture practices can affect berry weight and composition at various stages of development (Dai et al., 2011). The attainment of optimal berry maturity and wine quality, particularly in cool climates, relies on striking a balance between leaf area and yield, as highlighted by King et al. (2015). Numerous researchers have endeavored to elucidate the impact of cultivation practices on grapevine, employing different varieties and treatments to explore this topic (Smart et al., 1990; Deloire et al., 2005; Poni et al., 2009; Korkutal and Bahar, 2013; Bahar et al., 2017; Candar et al., 2019; Korkutal et al., 2019; 2020; 2021b; Candar et al., 2020a; 2020b; Alço et al., 2023).

On the other hand, Dai et al. (2011) stated that the weight and composition of berries undergo changes depending on the genetics of the vine, environmental factors, and cultivation methods.

Leaves are vital organs that carry out crucial physiological functions in grapevines. These include establishing photosynthesis, transpiration, and carbon balance, as well as regulating the microclimate within the canopy (Kliewer and Dokoozlian, 2005). Additionally, they help maintain the plant and soil water budget balance and accumulate sugar and nitrogen in the berry (Nicotra et al., 2011; Rossouw et al., 2017; Wang et al., 2019). The amount of carbon that leaves absorb during photosynthesis is directly related to the total biomass produced by grapevines. The physiological activity of leaves is influenced by several factors, such as size, age, climatic conditions, general characteristics of the terroir, and genetic differences (Peppe et al., 2011; Tozer et al., 2015). This activity, in turn, affects the total leaf area on grapevines, yield, and biochemical processes during the ripening period. However, leaf shape and size may not always be effective in achieving desired outcomes (Chitwood et al., 2016; Candar et al., 2021).

Defoliation practices can significantly impact the production-consumption balance of the vine (Bowen, 2009). Various impacts arise from these conditions, encompassing reduced transport of photosynthesis products to the cluster, restricted root growth, and diminished water efficiency (Hunter et al., 1995; Medrano et al., 2007; Poni et al., 2008; Palliotti et al., 2013; Vaillant-Gaveau et al., 2014). Removing leaves during berry ripening can eliminate a source of carbon and nitrogen, resulting in a reduction in sugar and nitrogen accumulation (Rossouw et al., 2018) and potentially impacting the quality of the berries (Bubola et al., 2022). Moreover, reducing the total leaf area of the vine with defoliation treatments may weaken grapevine growth in the following years and cause a decrease in yield (Bahar et al., 2018). In some cases, the impact of leaf removal treatments on clusters and yield was not always statistically significant. However, treatments where the main shoot leaves were left on the plant showed slightly higher values compared to other treatments (Korkutal et al., 2017).

Understanding how grapevine varieties respond and their limits of adaptability is crucial in maintaining a balanced product load and canopy architecture that aligns with the targeted yield and quality, and in implementing effective vineyard management (Candar, 2022). When it comes to leaf area management, the seasonal effects of each vegetation period play a significant role in determining the outcome. Therefore, planning for canopy management practices should be done annually, based on long and medium-term meteorological evaluations, and these practices should be adjusted according to the phenological period and short-term meteorological evaluations (Candar et al., 2022).

Grape berries are complex and versatile biochemical units that undergo successive processes of change during their development and maturation, which influence their size, composition, color, texture, taste, and aroma (Kunter et al., 2013). The histochemical structure of grapes is composed of sugars, organic acids, phenolic substances, minerals, and flavoring substances. The process of berry ripening is a physiological period that has a significant impact on the composition of the berries and, subsequently, on the quality of the wine, depending on the characteristics of the grape variety. Throughout the ripening process, grapes undergo numerous physical and biochemical changes, including alterations in weight, volume, hardness, sugar content, acidity, color, and aroma. According to Chen et al. (2018), berry size is one of the factors that affects grape quality.

Schalkwyk (2004) states that several factors influence berry weight and size, including genetic origin, berry set, number of berries per cluster, berry position within the cluster, number of seeds per berry, number of clusters per vine (bud load), climate, water conditions, fertilization, soil type, rootstock, variety, and degree of maturity. The author also notes that the weight of clusters and berries can vary from season to season and from region to region within the same variety.

Various factors such as variety, irrigation, and canopy management can affect berry size and the proportional distribution of skin, berry flesh, and seed within the berry. These differences can also alter the ratio of berry flesh/skin ratios and the amount of solutes that pass from the skin to the wine (Roby and Matthew, 2004; Matthews and Nuzzo, 2007; Barbagallo et al., 2011).

This research focused on the 'Merlot' grape cultivar and aimed to investigate the effects of four different levels of pre-dawn leaf water potential (LWP, Ψpd) and four defoliation treatments on the physical properties of grape berries.

MATERIALS AND METHODS

Location and plant material

The study was conducted at the Chateau Kalpak vineyard in the Şarköy district of Tekirdag, in coordinates 40° 39′ 12.00″ N and 27° 03′ 20.00″ E, during the 2019 and 2020 vegetation periods for two consecutive years. The grapevines used in the study were of the 'Merlot'/41B combination and were planted with a 2.1 m and 1.0 m in-row spacing, and a 70 cm stem height. The grapevines were trained using the double arm cordon training method in the Espalye system.

'Merlot' is a wine grape variety that originates from France and has been cultivated in Turkey since the early 1990s. The population of this variety in Tekirdağ shows significant morphological variation (Aktaş, 2021). 'Merlot' grape cultivar is a moderately to strongly vigorous variety that tends to produce a lot of offshoots and suckers. Its semi-erect to horizontal bearing requires sufficient trellising, and it is better to prune it short for better fertility. In certain climatic conditions, there is a risk of coulure. The cultivar is well-suited to clay-limestone terroirs. However, it is rather sensitive to winter and spring frosts (due to early budburst) and may not be well-adapted to intense drought conditions. The berries of 'Merlot' are medium in size, while the bunches are small to medium and winged. 'Merlot' grapes produce round, powerful, and richly-colored wines with relatively low acidity. These full-bodied and structured wines, with rather supple tannins, can be aged in wood barrels. The aromas of 'Merlot' wines are complex and elegant (Plantgrape, 2023a).

According to Plantgrape (2023b), the 41B rootstock is known for its ability to adapt to limestone soils and its resistance to chlorosis. It can withstand up to 60% of "total" limestone, 40% of "active" limestone, and an ICP of 60. Additionally, it has a good capacity to absorb magnesium from the soil. However, the 41B is susceptible to temporary water excess during the spring, and its resistance to drought is moderate. It may not be well-suited for overly compact soils. Grafts with 41 B MGt exhibit moderate to high vigor, and they usually have good compatibility, though some issues have been reported with 'Merlot' and 'Pinot' cultivars, which are still frequently grafted onto this rootstock. The initial growth of the plant can be slow, and the 41B promotes the compactness of grape clusters, while delaying the vegetative cycle of grafts. Compared to other rootstocks, the fruits produced by 41B grafted varieties are slightly less rich in sugar and slightly more acidic. The 41B is sensitive to both water stress and humidity excess in the soil and may be susceptible to the decline of the grapevine trunks (Plantgrape, 2023b).

Methods

To ensure homogeneity among the grapevines measured during the 2019-2020 vegetation period, plants with extreme differences in the number of clusters and shoots were excluded from the experiment. Additionally, no empty plants were included among the trial grapevines. The number of clusters and shoots were equalized again the following year when the shoots were approximately 30 cm long. The study involved 144 homogeneous vines subjected to four 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 defoliation treatments: Control (C), Full Window (FW), Right Window (RW), and Left Window (LW).

Water Availability (Stress levels)

Irrigation was carried out as needed based on the predawn leaf water potential (LWP, Ψpd) measured at five to seven-day intervals. The irrigation was adjusted according to the predetermined stress levels, and the Ψpd was checked the next day to ensure that it was within the desired range.

The S0 treatment, which served as the control, did not receive any irrigation and was left to random precipitation. For S1, the stress level was set to -0.4 to -0.6 MPa, and the Ψpd was maintained within this range through irrigation. Similarly, for S2, the stress level was set to -0.5 to -0.7 MPa, and the Ψpd was maintained within this range through irrigation. For S3, the stress level was set to ≤ -0.7 MPa, and the Ψpd was kept below this value through irrigation.

Defoliation Treatments

The defoliation treatments (DT) were carried out about two weeks after the onset of veraison. These treatments were performed by removing shoots and leaves from the eighth node and creating a window by eliminating all the leaves between the seventh and thirteenth nodes. The experiment included 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. 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 served as the control and no defoliation was performed. During the defoliation process, special attention was paid to ensure that the grapes were between 15 and 17 °Brix according to Alço (2019).

Analysis and Measurements

Phenological development stages were recorded during the experimental years, following the guidelines of Lorenz et al. (1995). Climate data were obtained from the Turkish State Meteorological Service (MGM).

For measurements, a random sample of 18 clusters was taken from three vines in each replication. From these clusters, berries were randomly selected from all parts to determine the berry characteristics, as described by Carbonneau et al. (1991). In order to determine the characteristics of the berries, 48 representative berries from each replication of each treatment were randomly selected and their width and length were measured using a digital caliper (Mitutoyo, Japan). The values obtained were given in centimeters, following the guidelines of the International Organization of Vine and Wine (OIV, 2021). The volume of 100 representative berries was determined in cm^3 per berry using the overflow method in a measuring cylinder, as described by Bahar et al. (2011). The weight of the berries was determined using an analytical balance scale with a sensitivity of 0.001 g to obtain the fresh weight of the berries. To determine the dry weight, 48 representative berries were dried in an oven (Elektro-mag, Turkey) at 65-70°C for 72 hours and then weighed again using the analytical balance scale. The dry weight of the berries was given in g per berry, as recommended by OIV (2021). The fresh weight and dry weight of berries were used to calculate the weight values per 100 berries, using proportions. The percentage of dry weight was determined using the formula (dry weight of berries x 100) / fresh weight of berries, as described by Bahar et al. (2011). The density of the berries was calculated by dividing the berry mass by the berry volume. The berry skin area was calculated using the formula $4\pi r^2$, and the values obtained were expressed in cm^2 per berry, according to Barbagallo et al. (2011). The ratio of berry skin area to berry flesh volume was determined by dividing the berry skin area by the berry flesh volume, and the resulting value was expressed as a proportion (Palma et al., 2007).

Trail Design and Statistical Analysis

The experiment utilized a Divided Plots Trial Design, in which the main plot comprised of water stress levels, and each sub-plot was made up of defoliation practices. A total of 144 vines were examined, with four different water stress levels and four defoliation treatments. Each treatment was replicated three times, with three plants in each replication. The data collected from the berries underwent ANOVA to test for statistically significant differences between the treatments. As no statistically significant differences were detected, no multiple comparison test was conducted. Apart from ANOVA, bivariate relationships between the data were also analyzed. Additionally, a principal component analysis (PCA) was conducted to examine the physical components of the grape clusters and berries. The data analysis was carried out using the R statistical environment (R Core Team, 2016).

RESULTS AND DISCUSSION

Climate and phenology

In 2019, the total precipitation recorded was 378.4 mm, while in 2020 it was 290.00 mm. The long-term average precipitation between 1939 and 2019 was 589.5 mm. Based on this data, it can be observed that the precipitation in 2019 was 211.10 mm less than the long-term average, and in 2020, it was 299.5 mm less than the long-term average. The average temperature for 2019 was 15.60°C, while the average for 2020 was 15.30°C.

During the experimental years, phenological developmental stages were recorded and analyzed. The results showed that budburst (EL 05) occurred on 11 April in 2019 and 15 April in 2020. Full bloom (EL 23) was observed on 2 June in 2019 and 8 June in 2020, while berry set (EL 27) was recorded on 9 June in 2019 and 14 June in 2020. The verasion stage (EL 35) was observed on 20 July in 2019 and 24 July in 2020. Finally, the harvest (EL 38) was conducted on 15 September in 2019 and 16 September in 2020. The data indicate that the phenological developmental stages occurred 4-6 days later in 2020 compared to 2019. The harvest was carried out in 2019 when experimental parcels reached an average °Brix of 24.39, and average of 24.80 °Brix in 2020.

Berry Width

The results of the ANOVA revealed that the main effects of different leaf water potential and defoliation treatments were not statistically significant in both years. However, in 2019 in terms of the defoliation main effect (DME), it was observed that the C treatment had the highest berry width with a value of 12.89 mm, while the LW treatment had the lowest value of 12.72 mm. When the berry width is arranged from the largest to the smallest based on LWP Main Effect (LWPME), the following measurements were observed: S1 with 13.13 mm, S0 with 12.93 mm, S2 with 12.61 mm, and S3 with 12.53 mm. The ranking of berry width, from the largest to the smallest, based on the 2019 LWPME x DME interactions reveals that the S1 x C interaction has the highest value of 13.36 mm, while the S2 x FW interaction was the lowest with a 12.33 mm value (Figure 1A).

In 2020, during the examination of the main effects of LWP and defoliation treatments, it was discovered that both main effects did not have a significant impact, but there were measurable effects on FW and LW, with values ranging from 12.81 mm to 13.21 mm. When considering the main effect of LWP, the treatment of S3 had a higher numerical value of 13.17 mm, while the treatment of S1 had a lower numerical value of 12.79 mm (Figure1B). The effect of the interaction between LWPME and DME on berry width, was observed a higher value of 13.55 mm for S3 x LW. Conversely, the S1 x C interaction had a low value of 12.50 mm. No significant variations were observed in berry width means between the two years.

The available data is consistent with Kotseridis et al. (2012) findings that the treatment of leaf removal did not result in a change in berry size in the 'Cabernet Sauvignon' grape variety. In addition, Alco et al. (2023) reported that defoliation did not result in significant variations in berry width in the 'Gamay' grape cultivar. On the other hand, Korkutal et al. (2021) reported that the treatment of defoliation and tip removal at different phenological periods resulted in an increase in berry size in the cv. 'Michele Palieri' berries. According to Candar (2018), the effect of defoliation treatments on lateral shoots in the 'Merlot' grape cultivar varies from year to year. It has been reported that reducing defoliation during extraordinarily rainy years results in an increase in berry width. However, during years with average precipitation, more defoliation tends to increase berry width more than lateral shoots, based on several years of observations.

Therefore, the conflicting results are actually consistent with the general literature. The grapevine's response to defoliation is imprecise and depends on various factors, including the cumulative effects of the year, cultivar genetics, cultural practices, and timing of the treatment. The same argument can be applied to the LWP means as well. Although increasing water stress in 2019 resulted in smaller berry width, in 2020, the smallest berry width was observed in the C treatment. It is thought that the effect of precipitation, which was 299.50 mm less than the long-term average in 2020, outweighed the impact of cultural practices.

Berry Length

Statistical analysis showed that the changes in LWPs, defoliation treatment, and the effects of their interactions on berry size years were insignificant in both experimental years.

The ANOVA revealed that the RW treatment resulted in the smallest berry size value of 12.65 mm, whereas the FW treatment resulted in the largest berry size value of 12.98 mm. When LWPME was considered, the S1 treatment showed the highest berry length value of 13.08 mm, whereas the S2 treatment had the lowest berry length value of 12.90 mm in year of 2019. In 2020, FW treatments resulted in a small berry size of 12.71 mm in terms of DME, whereas the RW treatment produced the largest berry size value of 13.06 mm (Figure 2).

The statistical analysis of LWPME revealed that the S1 treatment had the smallest berry length value of 12.81 mm, while the S3 treatments resulted in the highest berry length value of 13.03 mm. Regarding the berry size interactions in 2020, the S3 x LW combination had the highest berry length value of 13.32 mm, while the S0 x TP combination had the lowest berry length value of 12.45 mm. Observed variations in berry lenght means between two years were not significant.

Studies by Kotseridis et al. (2012) on cv. 'Cabernet-Sauvignon' and Kılıç (2019) on cv. 'Red Globe' with

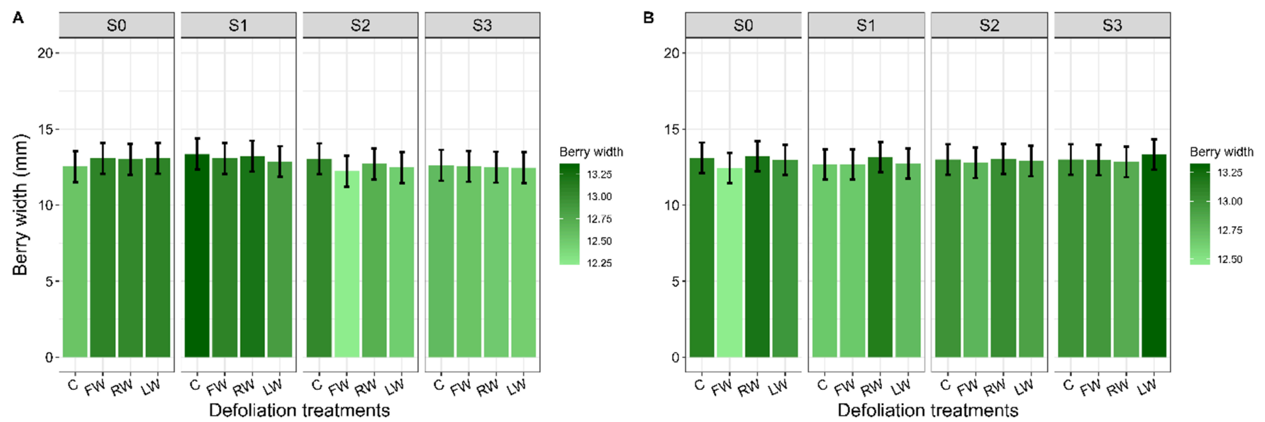

Figure 1. Effects of stress levels and defoliation treatments on berry width. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

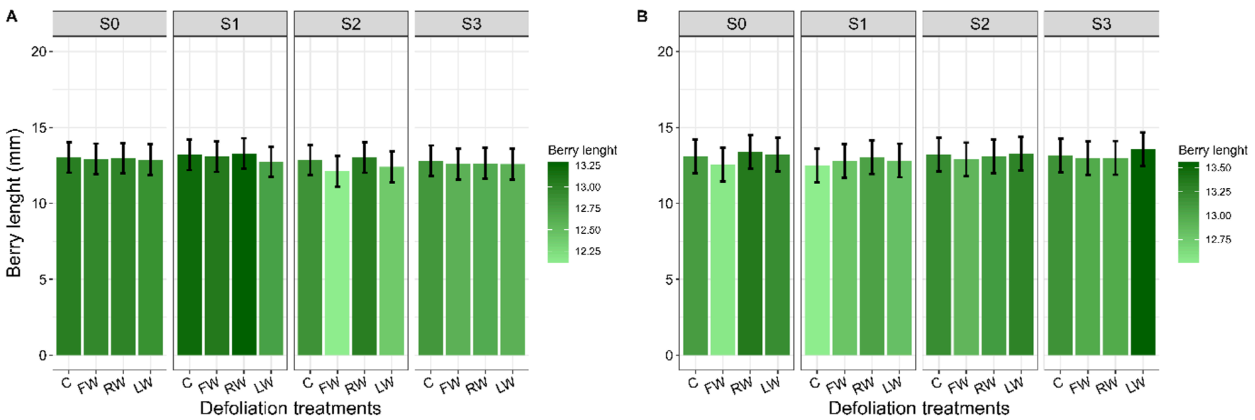

Figure 2. Effects of stress levels and defoliation treatments on berry length. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

controlled defoliation revealed no changes in berry size. In contrast, Sabır et al. (2010) reported an increase in berry size with tip removal in 'King's Ruby' grape cultivar, but found no effect on the '2B-56' grape cultivar. Candar (2018) was unable to establish the effect of mild water stress on berry size reduction that occurred with increasing main shoot length in cv. 'Merlot' grape berries, while Öner (2014) reported that mild stress influenced the development of berry width and length. Alco et al. (2023) reported that, in addition to leaf reduction interventions at different times and forms after veraison, changes in the direction of decreasing berry width and length are influenced by the amount of precipitation during the vegetation period in cv. 'Gamay'. Based on the available data, the reduction in berry width and height values was observed in relation to leaf water potential values compared to the control, although it was not statistically significant.

Berry Fresh Weight

In 2019, it was found that DME had a significant effect on the fresh weight of berries at LSD 5% level.

The RW treatment has been determined to be in the first importance group with a value of 1.37 g, while the LW and FW treatments are in the last importance group with values of 1.24 g and 1.20 g, respectively. No statistically significant effect on berry fresh weight was found with LWPME. The highest value of 1.32 g was obtained with the S0 treatment, while the lowest value of 1.22 g was obtained with the S3 treatment (Figure 3A).

The interaction between S0 and RW was found to result in a numerically high berry fresh weight of 1.46 g, while the interaction between S3 and FW was found to result

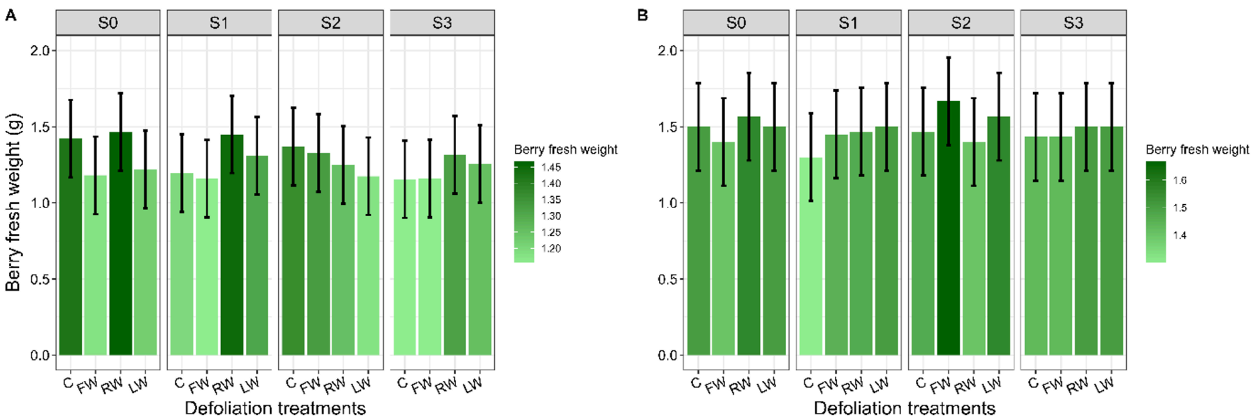

Figure 3. Effects of stress levels and defoliation treatments on berry fresh weight. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

in a numerically low fresh fruit weight of 11.15 g. In 2020, it was determined that defoliation and LWP had no statistically significant effect on berry fresh weight. Among the DME treatments, LW had the highest fresh weight with 1.51 g, while C had the lowest fresh weight with 1.42 g. The LWP means ranged from 1.42 g in S1 treatments to 1.52 g in S2 treatments, and no trend of variation proportional to stress levels was detected. Regarding the effect of treatment interactions, the highest value of 1.66 g was obtained with the S2 x FW interaction, while the lowest value of 1.30 g was obtained with the S1 x C interaction.

Although there was a statistically significant difference in fresh berry weight means between the two experimental years, this difference was not significant in terms of treatment main effects across the two years. The fresh berry means were 1.27 g in 2019 and 1.47 g in 2020 (Figure 3B).

Dimovska et al. (2000) studied the effects of defoliation treatments on the 'Beogradska Besemena' grape cultivar, while Bubola et al. (2019) investigated the same on the 'Istrian Malvasia' grape cultivar. Both studies reported a significant increase in berry weight due to defoliation. However, findings of Candar (2018) and Alco et al. (2023) suggest that defoliation did not have a significant effect on berry fresh weight in cv. 'Merlot' and cv. 'Gamay' grapes. This study predicts that berry wet weight would be lower in 2020 due to below-average rainfall compared to previous years. However, the study also reports that fresh berry weight in 2020 was higher. It is hypothesized that the higher berry set in 2019 had a more significant impact than the annual precipitation in determining the fresh berry weight in 2020. Conversely, in 2020, the weaker berry set is likely responsible for the lower berry wet weight.

Berry Dry Weight

In both experimental years, the treatment of DME and LWPME did not result in any statistically significant effects on berry dry weight. In 2019, the highest berry dry weight of 0.36 g was recorded from the C treatments, while the lowest value of 0.33 g was observed from the FW treatments. Regarding the main effect of LWP, the smallest berry dry weight of 0.34 g was numerically measured in the S0 treatments, whereas all other treatments resulted in a weight of 0.35 g. Upon ranking the LWPME x DME interactions in terms of their effect on berry dry weight from largest to smallest, the S3 x LW interaction was found to have the most substantial impact, with a value of 0.40 g. On the other hand, the S0 x FW interaction had the least effect, with a value of 0.30 g, and was ranked last (Figure 4A).

In 2020, the highest berry dry weight in terms of DME was observed in the LW treatments, with a value of 0.40 g. Conversely, the lowest dry weights were recorded in the C and FW treatments, both with a value of 0.37 g. Regarding LWPME, it was found that the lowest berry dry weight of 0.37 g was associated with the S1 treatment, while the highest berry dry weight of 0.39 g was linked to the S2. There was a statistically significant difference in berry dry weight means between 2019 and 2020, with a mean of 0.35 g in 2019 and 0.38 g in 2020 (Figure 4B).

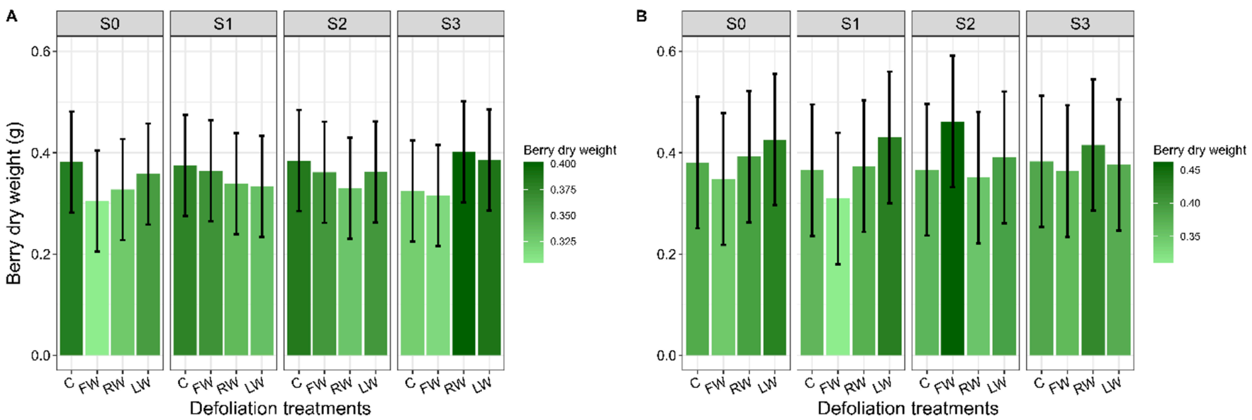

Figure 4. Effects of stress levels and defoliation treatments on berry dry weight. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

Similar to the observations made regarding fresh weights, it can be concluded that the evaluations for berry dry weights can also be repeated. The differences in the two-year averages are likely attributed to variations in berry set. While the increased stress level tended to increase the dry weight of the berry, this effect was negligible and statistically insignificant in the main effects of LWP over the two-year period. On the other hand, the defoliation treatments did not yield any significant or linear effects on berry dry weight. Candar (2018) reported that defoliation had no significant effect on berry dry weight, while Korkutal et al. (2017) and Alço et al. (2023) observed that the treatment time was more effective than the main effect of defoliation on berry dry weight. In contrast, Korkutal et al. (2021a) found that the defoliation and tipping treatments resulted in a statistically significant increase in berry dry weight.

Percentage of Berry Dry Weight

The statistical analysis showed that there was no significant difference in berry dry weight% due to the interactions between LWPME and DME in both experimental years. When it comes to DME, the highest dry weight percentage of 29.40% was observed in the LW treatment, whereas the lowest dry weight percentage of 25.73% was found in the RW treatment. Regarding the main effect of LWP on % dry weight, the values were 26.19% for S0, 28.15% for S1, 28.17% for S2, and 29.09% for S3, respectively. The highest dry weight percentage of 31.53% was observed in the S1 x FW interaction, while the lowest dry weight percentage of 22.50% was found in the S0 x RW interaction (Figure 5A).

effect percentage was 27.90% in 2019 and 25.91% in 2020 (Figure 5B). The year averages formed different statistical groups, indicating that there was a significant difference between the two years.

Similarly, according to Candar (2018), the main effect of the year on % berry dry weight was more significant than that of the defoliation treatments. However, Korkutal et al. (2017) and Alço et al. (2023) reported positive results on % berry dry weight due to the defoliation treatments.

Berry Density

Although the main effects of different stress levels and defoliation treatments on berry density in 2019 were not statistically significant, the lowest density of berries, in terms of DME, was recorded in the FW treatment at 0.95 g L^{-1}, while the highest density was observed in the C treatment at 1.01 g L^{-1}. Regarding berry density and its main effect on LWP in 2019, the S3 treatment had the highest value at 0.94 g L^{-1}, while the S1 treatment had the lowest value at 1.02 g L^{-1}.

In 2019, the highest density value in the LWPME x YUAET interaction was recorded at 1.10 g L^{-1} in the S0 x RW interaction, while the lowest density value was recorded

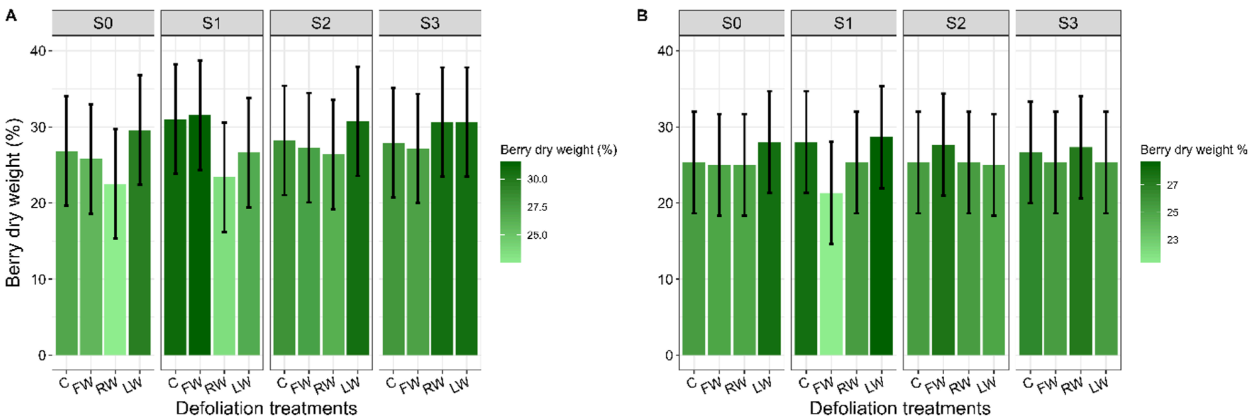

Figure 5. Effects of stress levels and defoliation treatments on berry dry weight %. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

In 2020, LW had the highest dry weight percentage of 26.75%, whereas FW had the lowest dry weight percentage of 24.83% in terms of DME. As for the main effect of LWP, the highest dry weight percentage of 26.16% was observed in the S3 treatment, while the lowest dry weight percentage of 24.83% was found in the FW treatments. In terms of interaction effects, the highest percentage of 2020 was observed in S1 x LW at 26.00%, while the lowest percentage of 21.33% was found in S1 x FW. Although the variations in the main effects of LWP and defoliation were not found to be statistically significant in the two-year average, the main

at 0.87 g L^{-1} in the S3 x TP interaction. However, in 2020, it was found that the changes in LWP and defoliation treatments had an insignificant effect on berry density compared to the LSD 5% significance level (Figure 6A).

The FW treatment was found to produce the highest berry density response at 1.13 g L^{-1} and the LW treatment produced the lowest response at 1.08 g L^{-1}. Regarding LWPME, the S3 treatment had the highest density at 1.15 g L^{-1}, while the S1 treatment had the lowest density at 1.06 g L^{-1}. In 2020, the S2 x FW combination produced the highest density value at 1.16 g L^{-1}, while the S1 x C interaction resulted in the lowest density at 0.98 g L^{-1}.

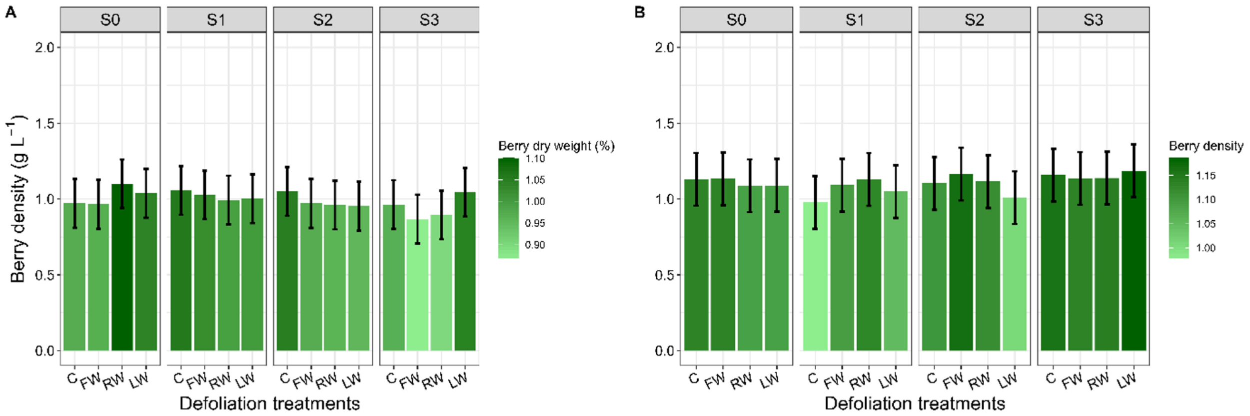

Figure 6. Effects of stress levels and defoliation treatments on berry density. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

The change in berry density between 2019 and 2020 was found to be statistically significant, with the mean density increasing from 0.99 g L^{-1} in 2019 to 1.10 g L^{-1} in 2020 (Figure 6B).

According to Candar (2018), a significant and linear relationship could not be established between defoliation and berry density in cv. 'Merlot'. However, in a recent study by Alço et al. (2023), it was highlighted that the timing of the treatment may be more crucial than the defoliation treatment itself in cv. 'Gamay'. In line with Alço et al. (2023) findings, Korkutal et al. (2021a) also supports the notion that the timing of defoliation treatments is crucial. In their research on the 'Michele Palieri' grape cultivar, defoliation performed during the berry set period resulted in reduced berry density, but the main effects of defoliation were not statistically significant. However, in a study by Bahar and Öner (2015) on the 'Cabernet-Sauvignon' grape cultivar, leaf removal treatments were found to increase berry density. It can be concluded that different cultivars may respond differently to defoliation treatments, primarily due to the cultivar genotype, treatment methods and timing, and terroir characteristics.

Berry Volume

The interactions among LWP, defoliation treatments and their mean values were not found to be statistically different for berry volume for the two experimental years, and mean of years were similar. In 2019, the treatment with the highest DME berry volume value was RW, with 1.39 cm^3, whereas the defoliation treatment with the lowest numerical value was LW, with 1.24 cm^3. The berry volume in terms of LWPME ranged from high to low as follows: S0, 1.31 cm^3; S2 and S3, 1.30 cm^3; and S1, 1.26 cm^3. The lowest value in the LWPME and DME interactions was observed in S1 x C and S1 x FW interactions, which

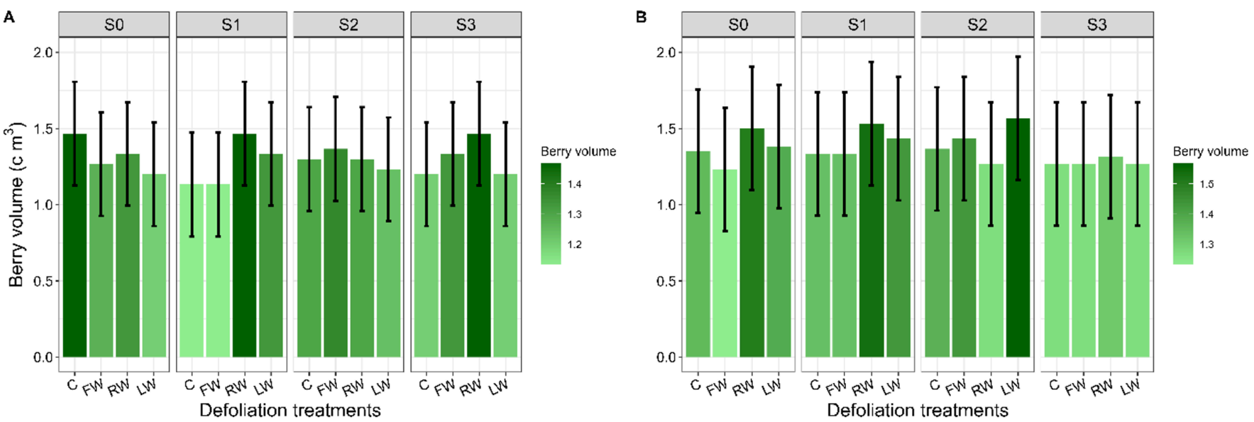

Figure 7. Effects of stress levels and defoliation treatments on berry volume. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

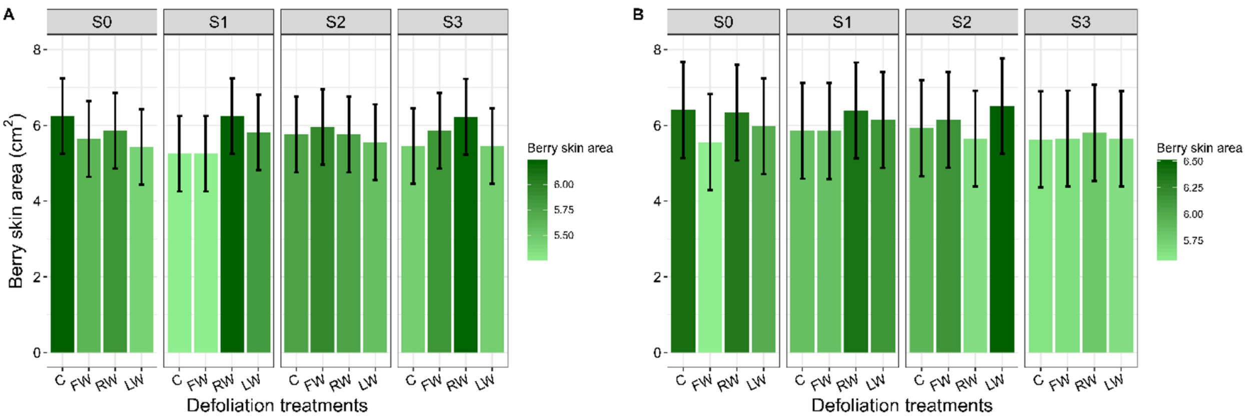

Figure 8. Effects of stress levels and defoliation treatments on berry skin area. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

reached a value of 1.13 cm^3 (Figure 7A).

In 2020, the FW treatment had the smallest DME berry volume of 1.31 cm^3, while the LW treatment had the highest with 1.41 cm^3. Among the LWPME treatments, the highest numerical value of 1.40 cm^3 was observed in S1 and S2, whereas the lowest value of 1.27 cm^3 was observed in S3. Examining the effect of interactions in 2020, the S2 x LW interaction had the highest berry volume value of 1.56 cm^3, while the S0 x FW interaction had the lowest value of 1.23 cm^3 (Figure 7B).

Korkutal et al. (2021a) found that defoliation performed during El 27 and EL31 periods increased berry volume, while treatments applied during the EL35 period decreased it. Alço et al. (2023) reported a significant increase in berry volume from veraison to maturity. However, defoliation during the 15-17° Brix period resulted in a relative reduction in berry volume, regardless of the treatment form. Candar (2018) also reported that defoliation treatments applied during the same period did not cause a significant change in berry volume similarly to Rogiers et al. (2004) which, highlighted the effect of adherence regimen in different years on volume. In 2020, despite the lower total precipitation, an increase in berry volume was detected, although it was not statistically significant. This could be due to weaker berry set in 2020, resulting in higher berry volumes.

Berry Skin Area

The interactions between LWP, defoliation treatments main effects and their interaction values did not show any significant statistical differences for berry skin area in the two experimental years, and mean of years were alike. In 2019, the FW treatment had the lowest DME berry skin area value of 5.80 cm^2, while the LW treatment had the highest value of 6.06 cm^2. Concerning LWPME, the smallest berry skin area was recorded as 5.68 cm^2 in the S3 treatment, while the highest berry skin area value of 6.06 cm^2 was observed in the S0 and S1 treatments (Figure 8A).

In 2019, when sorting LWPME x DME interactions based on berry skin area data from largest to smallest, the S0 x C and S1 x RW interactions were the lowest with 6.23 cm^2, while the S1 x C and S1 x FW interactions had the smallest numerical value with 5.25 cm^2. In 2020, the treatment with the smallest LWPME berry skin area value was FW with a value of 5.80 cm^2, and the treatment with the highest value was LW with a value of 6.06 cm^2 (Figure 8B).

In terms of LWPME, the treatment with the lowest numerical value for berry skin area was S3, with a value of 5.68cm^2. The highest numerical value for berry skin area was observed in treatments S0 and S1, with a value of 6.06 cm^2. When considering the interactions of the main effects, the combination of S2 and LW resulted in the highest berry skin area value, with 6.50 cm^2, while the combination of S0 and FW had the lowest value, with 5.56 cm^2.

Schalkwyk (2004), reported that the skin area/grape juice volume ratio is a crucial factor for wine quality. Large berries tend to produce more water and have a high grape juice ratio, while small berries offer higher color and flavor for red varieties. According to Candar (2018), defoliation treatments in various forms did not result in any statistically significant effects on the berry skin area. However, Alco et al. (2023) found that severe topping as a defoliation treatment caused a significant increase in the berry skin area from veraison to maturity but decreased it during the 15-17° Brix period across all treatment forms. In this study, the FW and S3 treatments resulted in the numerically lowest berry skin area, according to the mean of the experimental years. This finding is consistent with Alco et al.'s observation that more severe defoliation can reduce the berry skin area.

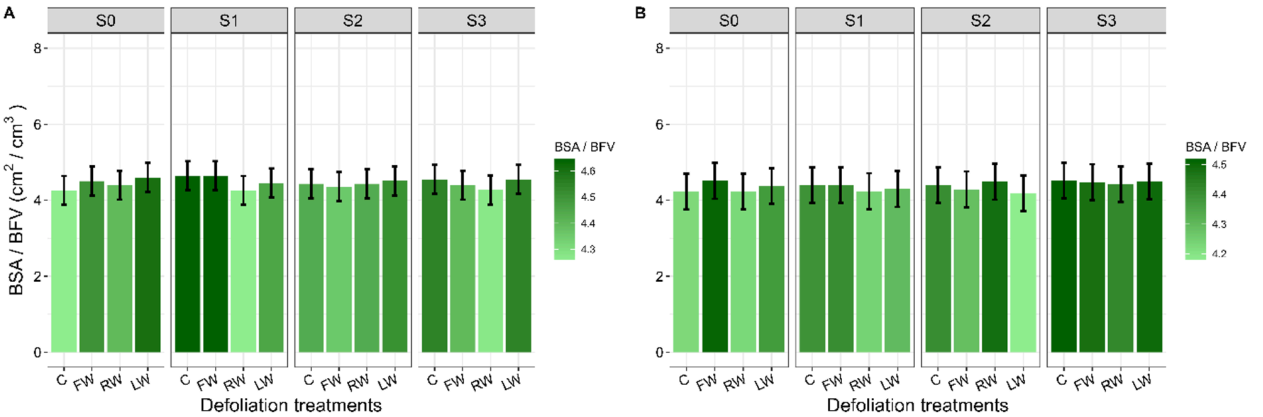

Figure 9. Effects of stress levels and defoliation treatments on berry skin area to berry flesh volume ratio. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

Ratio of Berry Skin Area to Berry Flesh Volume

There were no statistically significant differences observed in the interactions between LWP, defoliation treatments, and their main effects for berry skin area/berry flesh volume in the two experimental years. The two year means were similar, indicating that there was no significant effect of LWP or defoliation treatments on the berry skin area/berry flesh volume ratio.

When examining the berry skin area/berry flesh volume values in 2019 in terms of DME, the RW treatments had the lowest value of 4.33 cm^2/cm^3, while the LW treatments had the highest value of 4.52 cm^2/cm^3. In terms of LWPME, it was found that S1 had the highest value of 4.50 cm^2/cm^3, whereas S2 had the lowest value of 4.43 cm^2/cm^3 for berry skin area/berry flesh volume (Figure 9A).

When considering the interactions, the highest value for berry skin area/berry flesh volume was 4.64 cm^2/cm^3, observed in S1 x C and S1 x FW treatments, while the lowest value was 4 cm^2/cm^3 in S0 x C and S1 x RW treatments. The total value was calculated to be 25 cm^2/cm^3.

When ranking the values of berry skin area/berry flesh volume for 2020 from largest to smallest in terms of DME, the FW treatment had the highest value of 4.41 cm^2/cm^3, while the LW treatment had the lowest value of 4.33 cm^2/cm^3. Examining the LWP berry skin area/berry flesh volume, the S3 treatments had the highest value of 4.47 cm^2/cm^3. In terms of interactions, the S2 x LW combination had the lowest berry skin area/berry flesh volume ratio, with a value of 4.17 cm^2/cm^3 (Figure 9B).

Various factors such as variety, irrigation, canopy management can affect berry size, as reported by Sofo et al. (2012), Matthews and Kriedemann (2006), Matthews and Nuzzo (2007). Bahar et al. (2011) also stated that small grape berries have a higher berry skin area/berry flesh volume ratio than large berries, which leads to the transfer of more phenolic substances from the skin to the unit volume. Candar (2018) reported that although different responses are observed depending on the changes in physiological activity due to factors such as precipitation, humidity, and light intensity received during the vegetation period and in the total year, decreasing the total leaf area tends to decrease the berry skin area/berry flesh volume. According to Alço et al. (2023), severe topping defoliation caused a significant decrease in berry skin area/berry flesh volume towards the harvest date. However, higher berry skin area/berry flesh volume values were calculated with defoliation performed during the 15-17° Brix period. In this study, the FW and S1 treatments had the highest berry skin area/berry flesh volume ratio compared to the two-year mean.

Correlations of Berry Variables

Although no significant relationships were found between LWP levels, defoliation treatments, and berry characteristics at the p≤0.05 level according to the ANOVA results, we examined these relationships using the Pearson correlation test (Table 1).

The correlation coefficient between berry width and berry lenght is 0.852, indicating a strong positive correlation between these two variables. In general, the chart shows that there are positive correlations between berry weight and several other variables, such as berry length, berry volume, and berry skin area. However, the correlation between berry width and berry density is only weakly positive, and there is a negative correlation between berry width and bsa/bvol.

Similarly, there is a strong positive correlation between berry lenght and berry width, as well as moderate positive correlations between berry lenght and berry fresh weight, berry dry weight, berry volume, and berry skin area. There is also a weak positive correlation between berry lenght and berry density.

Other relationships in the chart include a strong positive correlation between berry fresh weight and berry dry weight, as well as moderate positive correlations between berry fresh weight and berry volume, berry dry weight %, and berry skin area. There is also a weak positive correlation between berry fresh weight and berry density.

Finally, the chart shows a strong positive correlation between berry skin area and berry volume, as well as moderate positive correlations between berry skin area and berry fresh weight, berry dry weight , and berry skin area to berry flesh volume ratio. There is also a weak negative correlation between berry skin area and berry dry weight %.

Principal Component Analysis (PCA)

To evaluate the interaction between stress levels, defoliation treatment, and the studied berry variables, we employed Principal Component Analysis (PCA). The dataset comprising eight treatments and nine berry variables was analyzed using the covariance matrix. However, two different biplots were created to investigate the effects of stress levels and defoliation treatments on berry variables separately.

According to the cumulative proportion of variance for the LWP biplot, PC1 explains 55.15% of the total variance, while PC1 and PC2 together explained 86.41% of the total variance. PC1, PC2, and PC3 combined explain 100% of the total variance. Therefore, PC1 and PC2 are the most important components in explaining the variability in the LWP data. Similarly, for the defoliation treatments, PC1 explains 68.93% of the total variance, PC1 and PC2 combined explain 92.14% of the total variance, and

Table 1. Correlations of selected berry variables

	bw	bl	bfw	bdw	bdwper	bvol	bden	bsa	bsa/ bvol
bw	1.000								
bl	0.852***	1.000							
bfw	0.191	0.326**	1.000						
bdw	0.106	0.160	0.537***	1.000					
bdwper	-0.036	-0.109	-0.309**	0.630***	1.000				
bvol	0.171	0.316**	0.696***	0.356***	-0.235*	1.000			
bden	0.055	0.026	0.303**	0.192	-0.064	-0.415***	1.000		
bsa	0.199	0.327**	0.687***	0.315**	-0.272**	0.967***	-0.383***	1.000	
bsa/bfv	-0.180	-0.304**	-0.694***	-0.326**	0.266**	-0.969***	0.403***	-0.994***	1.000

Coefficient statistical significance indicated by * symbol (absent > 0.05, * indicates < 0.05, **indicates < 0.01, *** indicates < 0.001). bw; berry widht, bl; berry length, bfw; berry fresh weight, bdw; berry dry weight, bdwper; berry dry weight %, bvol; berry volume, bden; berry density, bsa; berry skin area, bsa/bvol; berry skin area to berry flesh volume ratio.

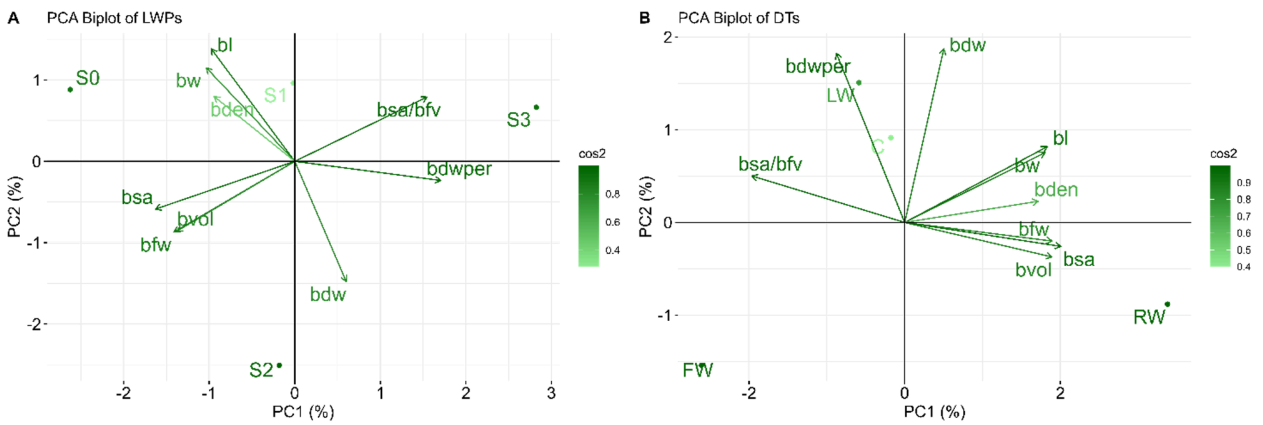

Figure 10. Principal component analysis (PCA) with the mean values of variables. A; PCA biplot of LWPs', B; PCA biplot of DTs'. All variables are displayed. The size and color of the arrows indicates the contribution strength of the variable. The color of lables reflects the magnitude of the contribution to the component. Bw; berry widht, bl; berry length, bfw; berry fresh weight, bdw; berry dry weight, bdwper; berry dry weight %, bvol; berry volume, bden; berry density, bsa; berry skin area, bsa/bvol; berry skin area to berry flesh volume ratio.

PC1, PC2, and PC3 combined explain 100% of the total variance, as indicated by the cumulative proportion of variance.

Both PCA correlation plots showed that there was a fair separation of the samples based on the treatments and variables.

Upon examination of the LWPs biplot, it is evident that variable S0 exhibits a robust negative correlation with Dim.1. This suggests that it carries a substantial weight in the first principal component and significantly contributes to the overall variability in the data. Similarly, variable S1 also negatively correlates with Dim.1, but it bears a comparatively smaller weight than S0. Conversely, variable S2 negatively correlates with Dim.2, implying that it carries a considerable weight in the second principal component and contributes significantly to the variability in that component. Lastly, variable S3 positively correlates with both Dim.1 and Dim.2, signifying that it holds a moderate weight in both principal components and contributes to the variability in both components.

Among the berry variables examined for LWP levels, berry fresh weight exhibits the highest loading (-0.809) on the first principal component, followed closely by berry volume (-0.788) and berry skin area (-0.933). These findings indicate that these three variables are highly associated with the first principal component and contribute significantly to the variability explained by this component. In contrast, the percentage of berry dry weight has the highest loading (0.979) on the second principal component, followed by the berry skin area to berry flesh volume ratio (0.886). These results suggest that these two variables are strongly associated with the second principal component and contribute the most to the variability explained by this component.

Since variables with high loadings on a specific principal component contribute the most to the variability explained by that component, it appears that the size and shape of the berry are strongly associated with the first principal component. This is evident from the high loadings of berry fresh weight, berry volume, and berry skin area (Figure 10A).

In the DTs biplot, it is observed that berry width exhibits the highest loading (0.888) on the first principal component, followed by berry length (0.902), berry volume (0.931), and berry skin area (0.987). These findings indicate that these four variables are highly associated with the first principal component and contribute the most to the variability explained by this component. On the other hand, the percentage of berry dry weight has the highest loading (-0.432) on the second principal component, followed by bsa/bfv (-0.966). These results suggest that these two variables are strongly associated with the second principal component and contribute the most to the variability explained by this component. The first principal component appears to be related to berry weight, berry length, berry volume, and berry skin area via the high loading of these criteria. Conversely, the second principal component appears to be related to bsa/bfv and the percentage of berry dry weight, as indicated by the high loading of bsa/bfv and the negative loading of the percentage of berry dry weight (Figure 10B).

CONCLUSION

In the study, it was found that the effects of water stress and defoliation treatments on berry physical properties were statistically insignificant. However, in the second year of the study, it was observed that the treatment of FW led to changes in the desired direction for grapevines. It is believed that the cumulative decrease in water reserves, resulting from reduced precipitation during the vegetation period over multiple years, caused the effects of FW treatment to become more prominent in this criterion. Additionally, the study found that vine production year, leaf water potentials (Ψleaf), and stress levels are influenced by both current vegetation period conditions as well as those from previous years. When examining the data for berry weight and % dry weight in both years, it was noted that these criteria increased with higher stress levels. Thus, it should not be assumed that cultivation practices will yield the same results for each grape cultivar, terroir, or year. Vineyard management strategies aimed at improving berry properties should be tailored to the production target, variety, and year.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors have no conflict of interest to declare.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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