

Research Article

Post-Veraison Water Stress and Pruning Level on Merlot Grapevine (*Vitis vinifera* L.): Effects on Berry Development and Composition

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The grapevine berry development and composition at harvest are major determinant of productivity and wine quality. The increase in grapevine productivity by applying proper pruning and watering may affect fruit traits of the grapevine development notably berry size, weight, and volume as well as the berry composition mainly the total soluble solids (TSSs), titratable acidity (TA), pH of the grapes, total anthocyanins, and phenolic content and concentration. In this context, this study aimed to explore the response of berry development and composition to the interactive effect of pruning level and post-veraison water stress. Four pruning levels (severe, standard, light, and minimal) in combination with four post-veraison water stresses (none, light, moderate, and intense) were tested. The interactive effect of pruning level and post-veraison water stress has significantly altered all parameters, except berry weight which was influenced by the main effect of the two factors. Generally, post-veraison water stress reduced berry volume, weight, yield, and TA, but increased the TSS, pH, total anthocyanins, and phenols. Increasing in the pruning level also reduced berry yield per vine, TSS, and pH, but increased the berry volume, weight, TA, total anthocyanins, and phenols. Specifically, the highest berry volume, weight, and TA were registered in severely pruned grapevines with adequate water supply. Conversely, the minimally pruned grapevines with intense water stress had the highest TSS and pH of grape juice. The total anthocyanins and phenols were advanced by the increment from minimal to severe pruning levels but depressed when the water stress extended from none to an intense level. In the other hand, the maximum total anthocyanins and phenols in terms of concentration and content were observed in severely pruned grapevines under intense water stress. Interestingly, higher berry yield per grapevine was scored in minimally pruned grapevines with adequate water supply, possibly due to the increased number of nodes per vine. TSS had a strong negative relationship with berry volume, weight, yield, and TA, while pH had a strong positive relationship with TSS. Total anthocyanins and phenols were also inversely correlated with berry yield per grapevine. The results showed that the combined effect of pruning levels and water stress is a powerful tool to balance berry development and composition. As Merlot is a typical red wine grapevine, it is important to increase the berry composition even though there could be a reduction in its berry development variables including berry yield per vine.

1. Introduction

The water status of grapevines is determined by a range of pedoclimatic elements, namely the rootstock's genetic features, grape variety, and production practices [1, 2]. According to Chaves et al. [3], restrictions in water supply during the season may affect plant growth, the canopy's photosynthetic capacity, yield components, and the quality

of grapes and wines. Stomatal regulation of leaf transpiration provides a short-term response of controlling water losses in vines when edaphic water stress and/or atmospheric demands rise sharply during the day [4]. The plant is able to retain its water status and stabilize the leaf water potential above the threshold required to avoid hydraulic rupture in conditions of significant water stress by controlling water flux in the plant and canopy development [5].

The grape berry quality at harvest mainly depends on the content of water, sugars, organic acids, amino acids, phenolic compounds, and aroma precursors [6–8]. Following fruit set, the increases in berry weight, volume, or diameter during berry development are typically characterized by a double sigmoid curve, resulting from two consecutive stages of rapid growth separated by a lag phase with slow or no growth [9]. During the first phase of growth, the sugar content remains low, while several organic acids are accumulated [10].

Veraison is a transition phase characterized by a change of berry skin color with a sudden increase in sugar accumulation [7]. The end of veraison coincides with the onset of ripening, which represents the second period of berry growth, mainly due to water influx and cell enlargement [10]. The most dramatic changes in grape berry composition occur during the second growth phase, or the ripening phase [6, 11]. During this phase, the berries soften, the sugar concentration increases reaching maximum levels, and the acid concentration decreases, whereas the compounds responsible for berry color, aroma, and flavor are accumulated in the berry skin [9]. At the end of the ripening period, the sugar content stabilizes, but sugar concentration may increase or decrease due to berry dehydration [10] or dilution [12].

Pruning is the most important cultural practice to balance grapevine vegetative growth, berry development, and composition [13, 14]. The yield increase is not associated with a reduction in grape quality [15]. Adaptation to pruning is achieved when the rise in production, due to the higher bud load, is compensated by canopy efficiency [16]. Dokoozlian [17] reported that when crop load is properly managed, well-pruned grapevines will produce improved berry composition and wine quality compared to poor pruned grapevines.

In grapevines, it is a common practice to keep plants under water stress at different stages of the season with the aim of reducing yield and improving the composition of the fruit [18]. However, if the tissue dehydration exceeds a critical level, a series of irreversible changes in the plant cause death [19]. Grapevines without water deficit has generally been found to cause a delay in ripening, possibly due to the accumulation of juice total soluble solids [20], the dilution effect of organic sugars in an increased berry size which is often associated with an increase in berry weight [21–23]. Generally, the effect of water stress on the ripening process may also due to a physiological response to an alteration in plant water status, which in turn impacts the regulation of pathways governing carbon metabolism in the berry [20]. Since the increase in grapevine productivity may affect berry composition, this study aimed to understand the interactive effects of pruning level and water stress on Merlot berry development and composition.

2. Materials and Method

2.1. Description of the Study Area. The experiment was carried out around Zway, located at 7° 53' 30" N latitude and 38° 43' 03" E longitude, in the Oromia region, Ethiopia. The

study area has an altitude of 1651 meters above sea level and is found 167 km south of Addis Ababa through the Ethio-Kenya highway. The general climate of the region is semi-arid, warm, with 21.6 °C and 890 mm mean annual temperature and rainfall, respectively [24]. The soil site was classified as a sandy loam with granular aggregates, well-drained, and good in porosity [25].

2.2. Experimental Design and Treatments. The study was carried out in 2019/2020 growing season on *Vitis vinifera* L. cv. Merlot grapevine. The vines were spaced at 1.5 m × 3 m and rows were oriented in an east-west direction. The pruning levels and water stress were compared in a split-plot design with three replications. Four pruning levels, i.e., severe pruning (20 nodes per grapevine), standard pruning (40 nodes per grapevine), light pruning (80 nodes per grapevine), minimal pruning (160 nodes per grapevine), and four post-veraison water stresses, i.e., none (0 MPa), light (−0.3 MPa), moderate (−0.6 MPa), and intense (−0.9 MPa) were applied. The water stress encountered was the water deficit caused by drought stress according to Rienth and Scholasch [26].

2.3. Data Collection Procedures. Berry volume, weight, yield per vine, TSS, TA, pH, total anthocyanins, and phenols per berry were assessed and randomly collected from each experimental unit. Berries were removed from the stems using a pair of scissors and cut as closely as possible between the skin and the stem thickening. The berries were dissected and the volume was measured using a densitometer balance (an accurate measuring balance to determine the volume of the water displaced) by Archimedes principle. Berry weight was measured by using a digital balance, and the grape yield per vine was determined.

At harvest, 200 berry samples had been randomly collected from each treatment to analyse TSS, TA, pH, total anthocyanins, and phenols according to OIV methods and procedures [27]. The pH of the juice sample was determined using a pH meter. TSS was measured as °Brix using a digital refractometer. Five mL of the juice sample was then diluted to 1:5 with deionised water to determine TA using an autotitrator, with the end point for the titration against 0.1 N NaOH set at a pH of 8.2.

The berry anthocyanins and phenolics were determined at harvest and homogenised using an Ultra-Turrax digital homogenizer, ensuring that both the seeds, skin, and the flesh were completely crushed and macerated for 24 hours at 20 C, according to OIV methods and procedures [27]. Extracts were placed on a rotary shaker in the dark for 1 hour. The extract samples were then centrifuged for 5 min at 10,000 g and the supernatant was retained. One mL of the supernatant was diluted in 10 mL 1N HCl and left to stand for 3 hours period, after which the absorbance of the diluted extract was determined at 520 nm and 280 nm. Spectrophotometric analysis was performed to estimate the total anthocyanins and phenols per berry.

2.4. Statistical Analysis. The data were subjected to a two-way (pruning level \times post-veraison water stress) analysis of variance (ANOVA). The significance level was set at $\alpha = 0.05$, and means were separated using Tukey's honestly significant difference (HSD) test. The statistical analysis was performed using R-programming language and statistical software (version 4.1.3, 2022). Correlation analysis was used to explore the relationships between individual components.

3. Results and Discussion

3.1. Berry Volume (ml) and Weight (g). The interaction effect of pruning level and post-veraison water stress on mean grapevine berry volume was significantly ($p < 0.001$) different. The largest berry volume was recorded in severely pruned grapevines with adequate water supply (1.10 ± 0.03) followed by in the light (1.01 ± 0.02) and moderate (0.97 ± 0.06) water stresses while the smallest berry volume was registered in minimally pruned grapevines with intense (0.49 ± 0.03) water stress followed by moderate (0.52 ± 0.01) and light (0.55 ± 0.01) water stresses (Figure 1).

Generally, berry volume in severely pruned grapevines with no water stress was statistically larger by 55.45% than in the minimally pruned grapevines with intense post-veraison water stress. Likewise, berry volume had an increment trend with increasing pruning level and declined tendency in water stressed grapevines. The consequence of berry volume decreased in minimal pruning and intense water stress is reflected in an increase in TSS concentration.

The main effect of grapevine pruning levels on berry weight has shown highly significant ($p < 0.001$) variation while the interaction effect with post-veraison water stress was not significant ($p > 0.05$). The heavier berry weight (1.13 ± 0.14) was registered in severely pruned grapevines while the lighter weight was observed in minimal pruning level (0.70 ± 0.08). Generally, berry weight had an increasing tendency during the increment of grapevine pruning from minimal to severe levels. That means, berry weight in severe pruning level was statistically heavier by 38.05% than the average berry weight in minimally pruning grapevines (Figure 2).

The main effect of grapevine post-veraison water stress on berry weight has shown highly significant variation ($p < 0.001$) while the interaction effect with the pruning levels was nonsignificant ($p > 0.05$). The heavier berry weight (1.02 ± 0.19) was registered in non-water-stressed soil while the lighter weight was observed in intense water stress (0.79 ± 0.13). That means, berry weight in the severe pruning level was statistically heavier by 38.05% than in minimally pruning grapevines (Figure 3).

The finding of this study is in line with recently published results of other authors [6, 21–23, 28, 29] who concisely reported that grapevine berry volume and weight diminished with exceeding water stress and advanced with increment of the pruning levels possibly due to berry water loss [30]. In the other hand, Eltom et al. [31] reported that temperature effect on berry volume and weight is more complex than expected. As the berry's water budget is no

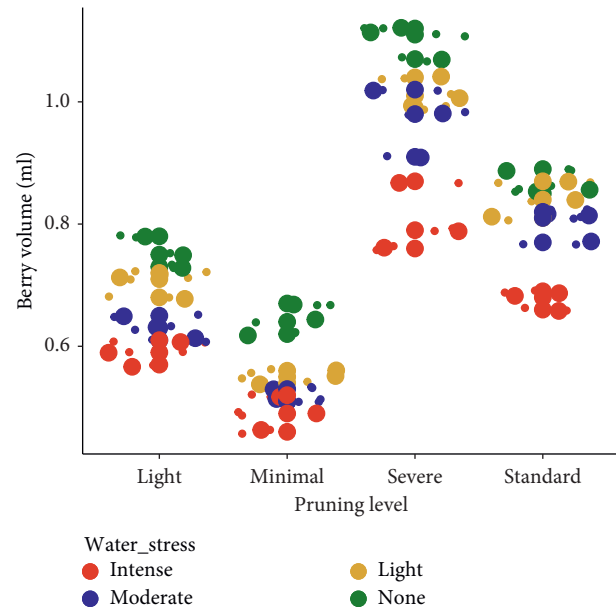


FIGURE 1: Response of grapevine berry volume to post-veraison water stress and pruning levels.

longer balanced between water loading and transpiration or water backflow to the vine, the berry may be prone to weight loss [7]. According to the studies in [30, 32], the decrease in berry weight, otherwise known as 'shriveled,' is commonly observed for Shiraz grapevines due to high temperature and water deficit. In addition, correlation analysis showed a strong relationship ($r = 0.90$) between berry volume and weight (Figure 4), while berry volume inversely correlated ($r = -0.91$) with TSS (Figure 5) as supported by studies in [33, 34].

3.2. Berry Yield (kg/Vine). The combination effect of the pruning level and postveraison water stress on mean yield per grapevine plant was significantly ($P < 0.001$) altered. In a more general context, yield per vine reached a plateau at minimal pruning level with adequate water supply (10.06 ± 0.15) followed by at light (9.76 ± 0.06) and moderate (9.44 ± 0.07) water stresses, while the lowest yield per vine was found in severely pruned grapevines with intense water stress (6.13 ± 0.04) followed by moderate (6.23 ± 0.05) and light (6.42 ± 0.04) water stresses. Merlot grapevine yield per vine in the minimal pruning level with no water stress was significantly higher by 39.07% than in severe pruning with intense water stress (Figure 6).

In this regard, several researchers reported that grape yield per vine advanced with increasing the number of nodes per vine [35–37] and applying adequate water supply [38]. Likewise, Zsófi [21], who reported that the timing of water restriction has a big impact on grape yield. The authors found that early (between anthesis and veraison) and late (between veraison and harvest) water deficit decrease yield and berry size. Similarly, Deloire et al. [7] found that water stress reduced yields by $\geq 25\%$ with consequences on berry composition and the resulting

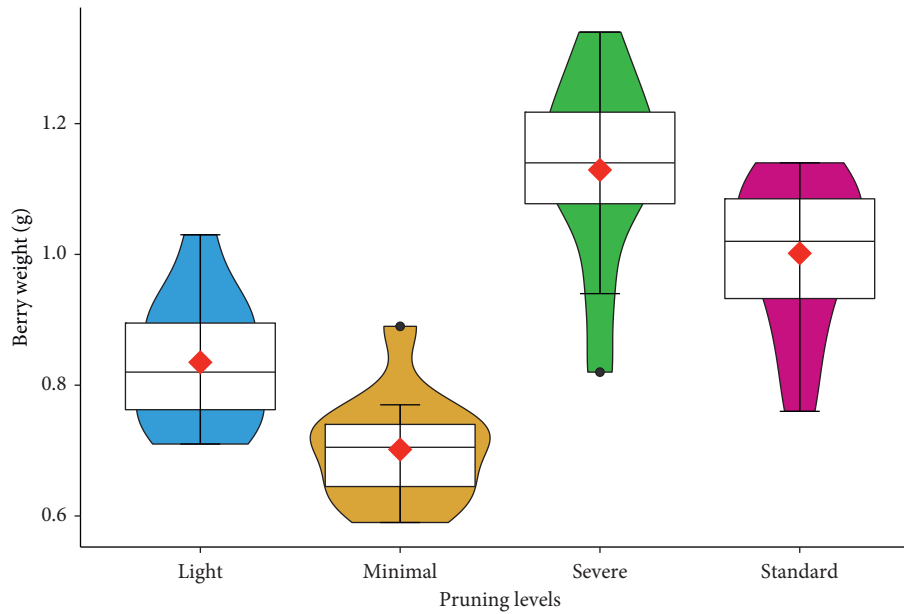


FIGURE 2: Effect of pruning level in grapevine berry weight.

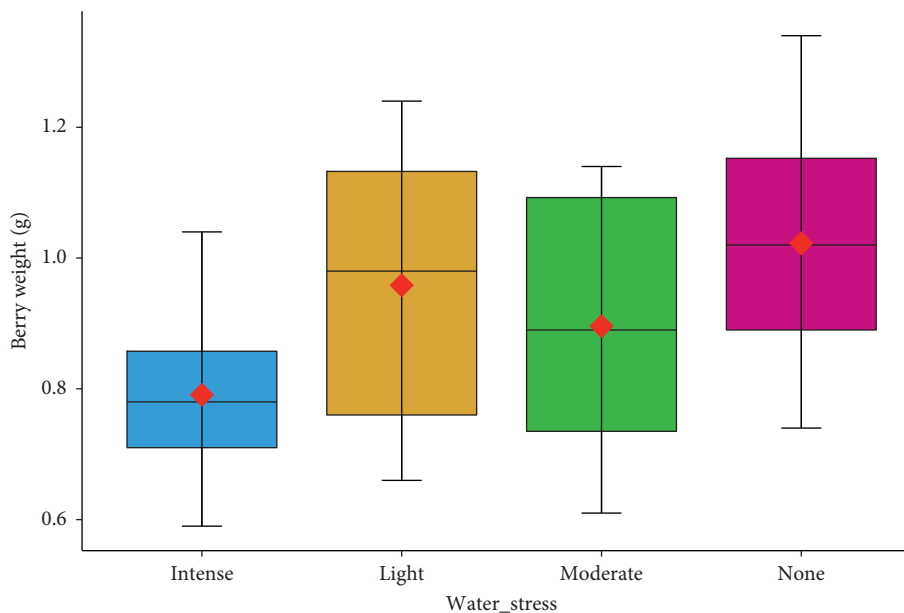


FIGURE 3: Influence of post-veraison water stress in grapevine berry weight.

wine due to predicted climate change, shifting grape development, and ripening into warmer periods. The late season dehydration can be attributed to dehydration and loss of berry cell vitality that results in losses of yield, quality, and profitability [11, 30]. It has been observed that a severe water stress can originate a sharp decrease in both yield and grape quality, while a mild water stress can help to improve quality but usually decreasing yield [22, 39]. The correlation analysis showed a strong negative relationship ($r = -0.93$) between grape yield and total anthocyanins per berry (Figure 7) as supported by Bindon et al. [36], who observed inverse correlation, with total anthocyanins decreasing as yield increased.

3.3. Total Soluble Solids ($^{\circ}$ Brix). The interaction effect of pruning level and postveraison water stress on mean grapevine TSS content showed a highly significant ($p < 0.001$) difference. The highest TSS content was registered in the minimally pruned grapevines with intense water stress (26.53 ± 0.70) followed by in moderate (25.90 ± 0.40) water stresses at the same pruning level, while the lowest TSS content recorded at severely pruned grapevines with adequate water supply (18.41 ± 0.37) followed by in the light water stress (18.92 ± 0.85) (19.42 ± 0.41). The TSS content in minimal pruning with intense postveraison water stress was statistically higher by 30.61% than TSS in severe pruning level with adequate water supply (Figure 8).

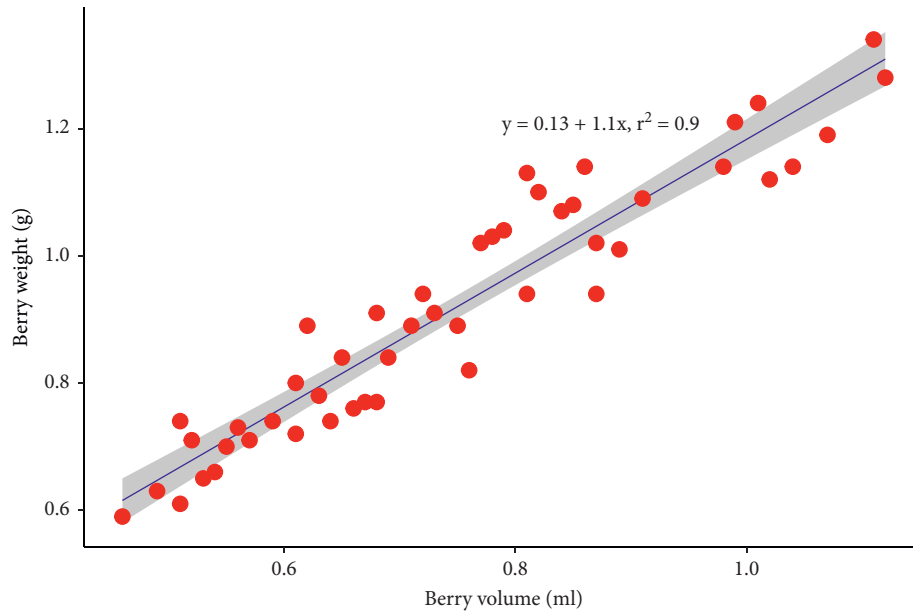


FIGURE 4: Correlation between grapevine berry volume and weight.

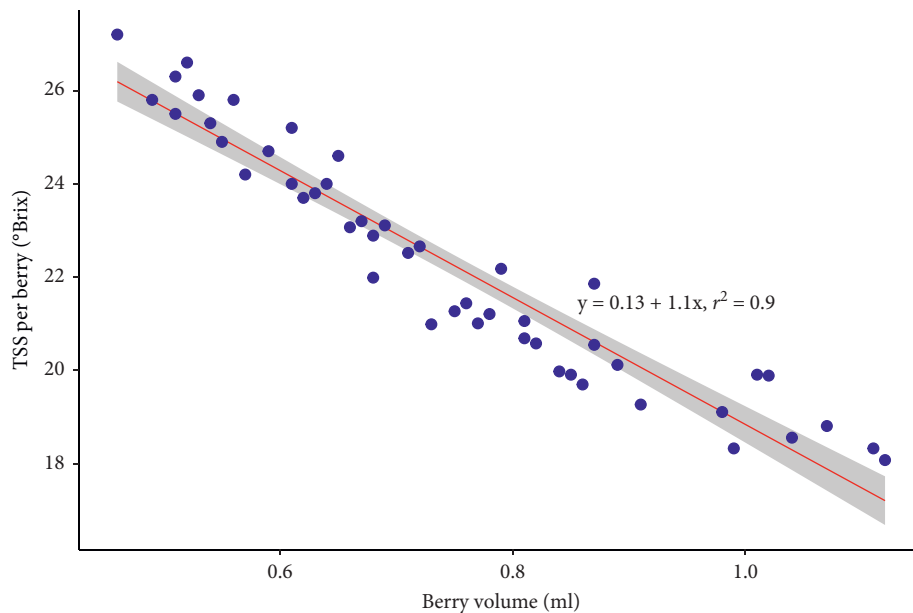


FIGURE 5: Correlation between grapevine berry volume and TSS content.

Generally, the accumulation of total soluble solids per berry decreased with increasing the pruning level from minimal to severe, while increased with postveraison water stress from none to an intense. This result is in agreement with recently published works [18, 33, 40–43], where higher crop loads per grapevine resulted in a limitation in the final sugar level attainable in the fruit at harvest. This phenomenon was also demonstrated by the studies in [44, 45], who found that water deficit combined with increased crop load led to a significant reduction in the rate of sugar accumulation. This was thought to be due to an increased yield to pruning weight ratio under water-stressed treatments where crop load was high, leading to a reduction in photosynthate

available from a limited leaf area for a large crop load, despite a consistent reduction in stomatal conductance [26, 36]. There was a tendency for a decrease in TSS with increase in berry volume which confirms that sugar loading per berry is a good indicator of the source-sink relationship. The point at which berry volume max was reached coincided with the point at which TSS per berry ceased to increase.

3.4. Titratable Acidity (TA). The interactive effect of pruning level and postveraison water stress on grapevine mean TA was shown significant ($p < 0.05$) variation. The severely pruned grapevines with adequate water supply had the

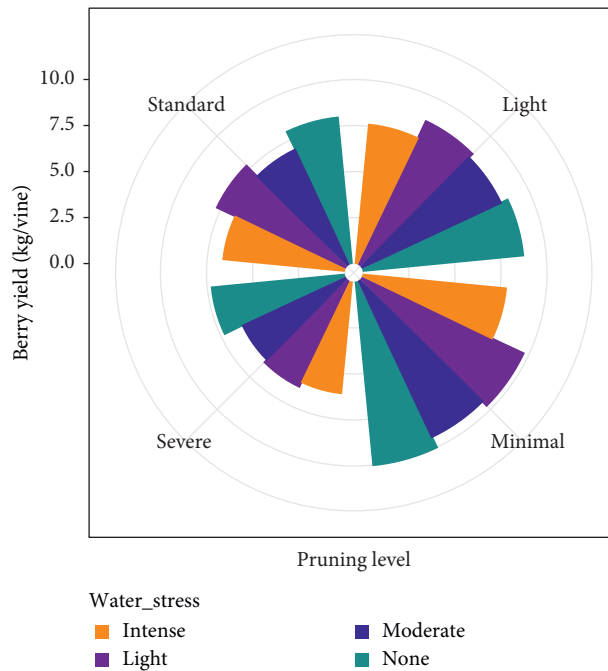


FIGURE 6: Combination effect of pruning level and water stress in grapevine yield per vine.

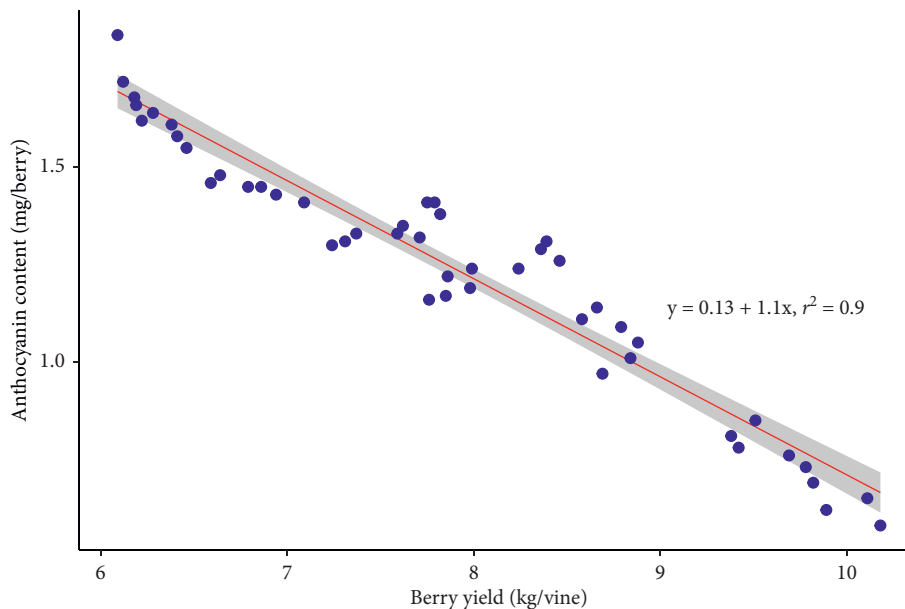


FIGURE 7: Correlation between grapevine berry yield and anthocyanin content.

highest titratable acidity (10.80 ± 0.31) followed by with light (10.30 ± 0.36) and moderate (9.82 ± 0.37) water stresses, whereas the lowest TA was found in the minimally pruned grapevines with intense (5.50 ± 0.22), moderate (5.79 ± 0.38), and light (6.10 ± 0.32) water stress levels. TA in severely pruned and adequately irrigated grapevine was higher by 49.07% compared with minimally pruned grapevines under intense water stress (Figure 9). Other authors [1, 26, 41–43, 45] have also observed higher TA content in heavy pruned grapevine with well-irrigated grapevines probably related to a delay in ripening at which the bunches

will not be able to reach target ripeness levels due to a restricted carbon allocation from the canopy and reduction of sugar accumulation.

3.5. pH Value. The average pH value of grape juices significantly ($p < 0.001$) altered by the interactive effect of pruning level and postveraison water stress on mean grapevine. The highest pH registered in the minimally pruned grapevines with intense water stress (4.33 ± 0.05) followed by in moderate (4.23 ± 0.02) and light (4.13 ± 0.03)

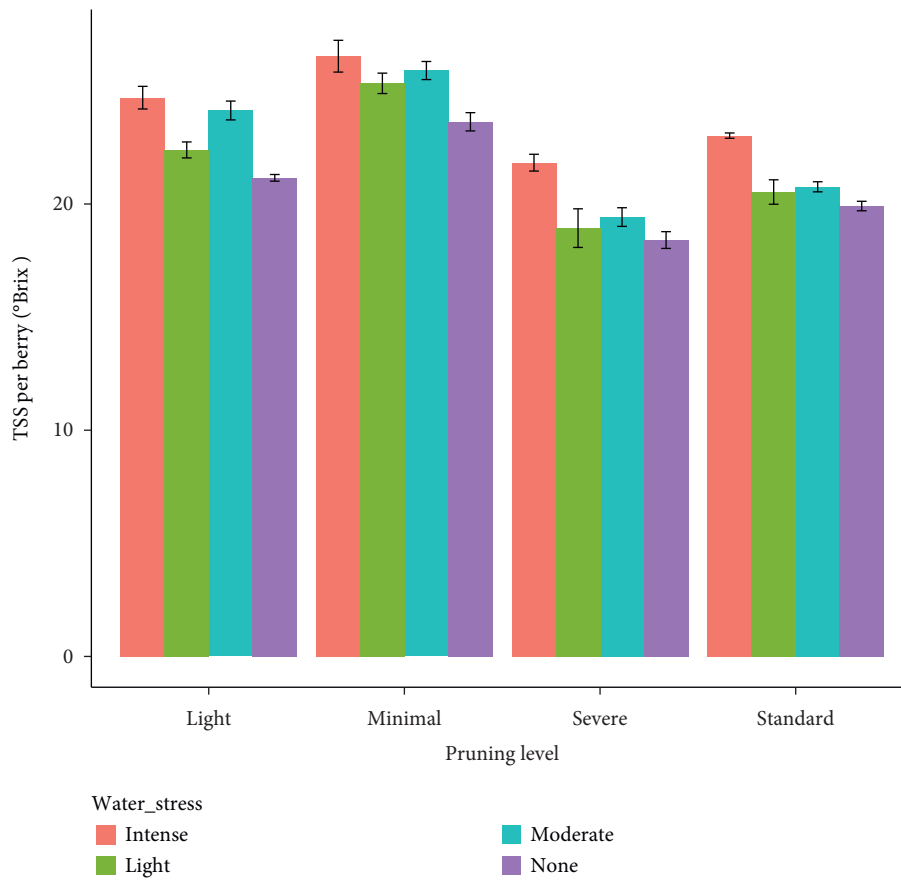


FIGURE 8: Response of TSS to combination effect of pruning level and post-veraison water stress.

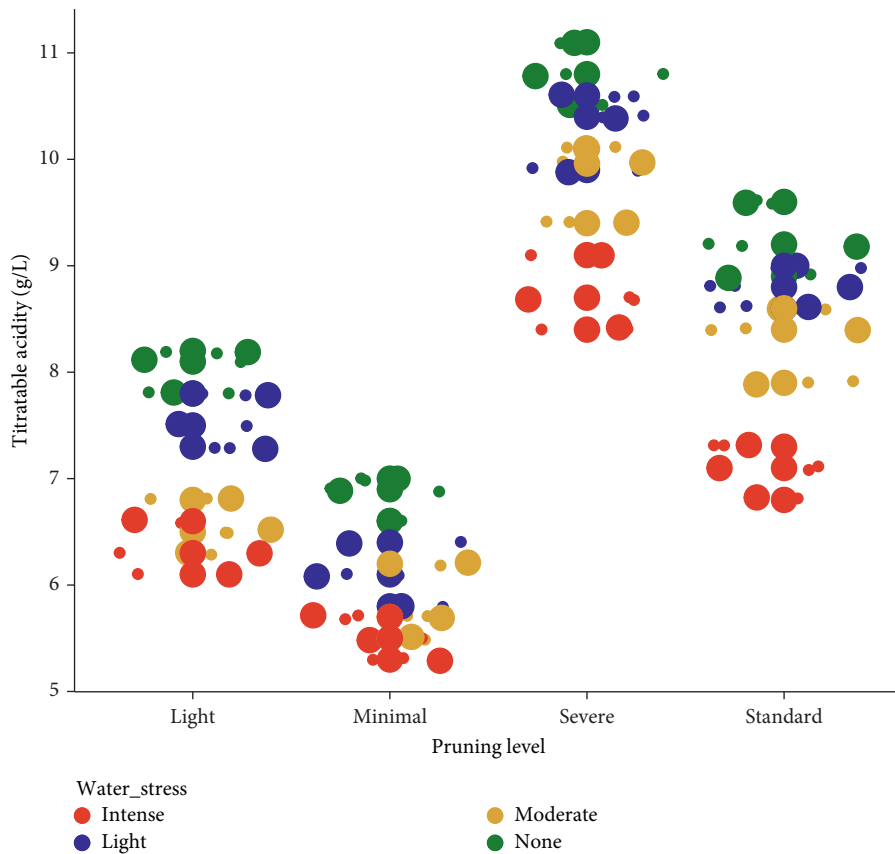


FIGURE 9: Response of berry juice titratable acidity to different pruning levels.

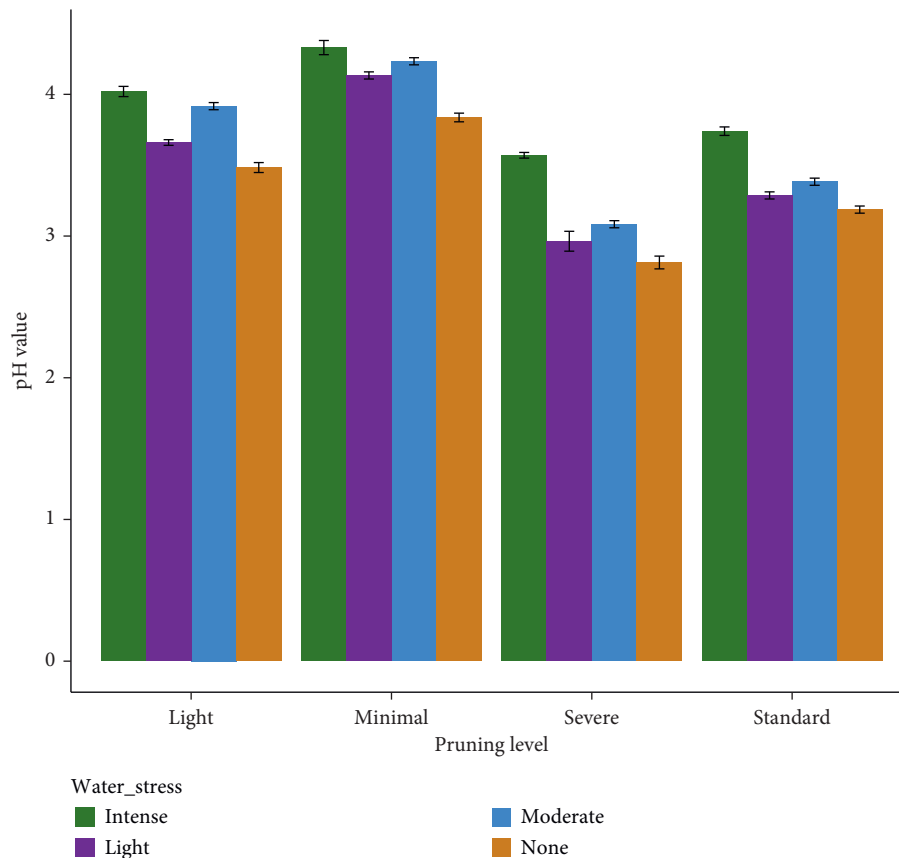


FIGURE 10: Response of berry juice pH to interactive effect of pruning level and post-veraison water stress.

water stresses at the same pruning level, while the lowest pH value recorded at severely pruned grapevines with adequate water supply (2.8 ± 0.05) followed by in the light (2.96 ± 0.07) and moderate (3.08 ± 0.02) water stresses. The pH value in minimal pruning with intense postveraison water stress was statistically higher by 35.10% than pH in severe pruning level with adequate water supply (Figure 10).

The findings of the current study is in accordance with other authors [18, 33, 40, 43] who reported that the pH of grape juice per berry decreased with increasing the pruning level, while increased with in water-stressed grapevines possibly due to an increased yield to pruning weight ratio under water-stressed treatments where crop load was high, leading to a reduction in photosynthate available from a limited leaf area for a large crop load, despite a consistent reduction in stomatal conductance [1, 26, 36]. The relationship analysis showed a strong correlation ($r=0.91$) between pH of grapes and TSS content per berry (Figure 11) while observed inverse correlation, with TA which may have led to a reduced rate of malic acid degradation as supported by Bindon et al. [36].

3.6. Total Anthocyanins and Phenols. The interactive effect of pruning level and postveraison water stress on grapevine total anthocyanins and total phenolic content per berry and concentration per gram showed highly significant ($p < 0.001$) variation. The severely pruned grapevines in an

intense water stress had the highest total anthocyanins expressed as a concentration (1.88 ± 0.03 mg/g) and content (1.75 ± 0.08 mg/berry), and total phenols in terms of concentration (1.73 ± 0.02 mg/g) and content (1.42 ± 0.03 mg/berry) while the lowest total anthocyanins concentration (1.13 ± 0.05 mg/g) and content (0.77 ± 0.04 mg/berry) and total phenolic concentration (1.03 ± 0.07 mg/g) and content (0.62 ± 0.04 mg/berry) were observed in minimally pruned Merlot grapevine with adequate water supply. At harvest (120 days after flowering), the total anthocyanins and phenols expressed as content (per berry) and concentration (per g) were found to be significantly increased with increasing in pruning level from minimal to severe and water stress from none to an intense. The severe pruning level and intense water stress increased the concentration and content by 39.89% and 56% for total anthocyanin and by 40.46% and 56.34% for total phenols compared with the minimally pruned and adequately irrigated grapevines, respectively (Table 1).

This interactive effect could relate with the decline in grape berry volume and increase in cluster number per grapevine that significantly alter the concentration and content of total anthocyanins and phenols. Likewise, the change in berry skin surface area to volume ratio may account for the increase in concentration and content of total grape berry anthocyanins and phenols as supported by the studies in [36, 46]. Besides, since berry weight was significantly affected by pruning level and water stress, it can be

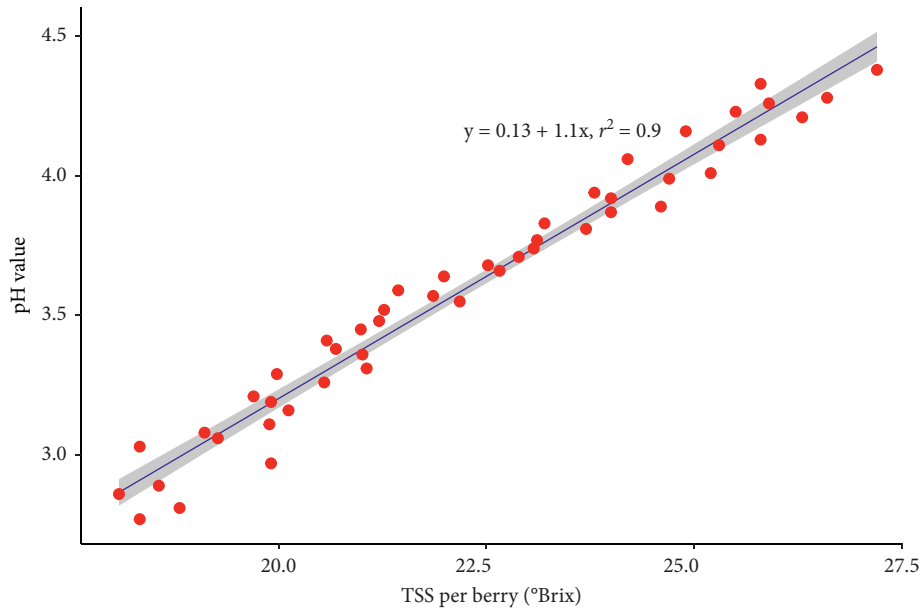


FIGURE 11: Correlation between TSS per berry and pH of grape juice.

TABLE 1: Interaction effect of pruning level and post-veraison water stress on Merlot grapevine total anthocyanins and phenols.

Pruning level	Water stress	Anthocyanin (mg/g)	Anthocyanin (mg/berry)	Phenols (mg/g)	Phenols (mg/berry)
Severe	Intense	1.88 ± 0.03 ^a	1.75 ± 0.08 ^a	1.73 ± 0.02 ^a	1.42 ± 0.03 ^a
Standard	Intense	1.72 ± 0.06 ^c	1.46 ± 0.02 ^d	1.57 ± 0.04 ^d	1.28 ± 0.03 ^{cd}
Light	Intense	1.57 ± 0.03 ^e	1.33 ± 0.02 ^f	1.47 ± 0.04 ^f	1.20 ± 0.02 ^c
Minimal	Intense	1.41 ± 0.01 ^h	1.17 ± 0.02 ^h	1.30 ± 0.03 ⁱ	1.07 ± 0.01 ^h
Severe	Moderate	1.86 ± 0.02 ^a	1.64 ± 0.02 ^b	1.67 ± 0.03 ^b	1.37 ± 0.02 ^b
Standard	Moderate	1.65 ± 0.03 ^d	1.43 ± 0.02 ^{de}	1.54 ± 0.02 ^{de}	1.27 ± 0.01 ^d
Light	Moderate	1.48 ± 0.03 ^{fg}	1.29 ± 0.03 ^{fg}	1.40 ± 0.02 ^{gh}	1.16 ± 0.01 ^{fg}
Minimal	Moderate	1.29 ± 0.02 ^j	0.89 ± 0.04 ^k	1.18 ± 0.01 ^k	0.81 ± 0.02 ^k
Severe	Light	1.78 ± 0.03 ^b	1.58 ± 0.03 ^c	1.62 ± 0.02 ^c	1.31 ± 0.02 ^c
Standard	Light	1.61 ± 0.03 ^{de}	1.40 ± 0.02 ^e	1.49 ± 0.02 ^{ef}	1.25 ± 0.01 ^d
Light	Light	1.39 ± 0.02 ^{hi}	1.11 ± 0.03 ⁱ	1.27 ± 0.03 ^{ij}	1.02 ± 0.04 ⁱ
Minimal	Light	1.21 ± 0.02 ^k	0.83 ± 0.04 ^l	1.11 ± 0.04 ^l	0.73 ± 0.01 ^l
Severe	None	1.51 ± 0.03 ^f	1.31 ± 0.02 ^f	1.42 ± 0.01 ^g	1.18 ± 0.02 ^{ef}
Standard	None	1.44 ± 0.02 ^{gh}	1.23 ± 0.01 ^g	1.36 ± 0.01 ^h	1.12 ± 0.02 ^g
Light	None	1.35 ± 0.02 ⁱ	1.01 ± 0.04 ^j	1.23 ± 0.01 ^j	0.94 ± 0.05 ^j
Minimal	None	1.13 ± 0.05 ^l	0.77 ± 0.04 ^m	1.03 ± 0.07 ^m	0.62 ± 0.04 ^m
Mean		1.52	1.26	1.40	1.11
CV		1.87	2.59	2.09	2.13
LSD		0.05	0.05	0.04	0.04
P value		***	***	***	***

Means within a column followed by same letter(s) are not significantly different at 5% LSD test.

concluded that the increase was associated with an increase in the skin:flesh ratio.

This finding is in line with other authors [40, 42, 46] whom reported that the increase in total anthocyanins and phenols under heavy pruning was due to an increase in grape berry weight and biochemical response of the anthocyanin and phenol metabolic pathway to the induced signals caused by water stress which led to increase the total anthocyanin and phenolic concentration per gram and contents per berry. In other previous studies [26, 41, 45], correlation analysis of potential factors contributing to altered

metabolism in the anthocyanin pathway showed that color was negatively correlated with vigour indices, canopy density, and stomatal conductance and positively correlated with light penetration into the canopy.

In this study, the results indicate that, where water stress does cause an increase in berry anthocyanin and phenol concentration, this is more likely to be due to a physiological response to the berry volume and weight effect. A similar observation [33, 41, 46] were made that a water stress treatment brought about an increase in anthocyanin and phenolic concentration in the berry skin.

4. Conclusion

The current study has shown that larger berry volume and heavier weight can be carried by severely pruned Merlot grapevines with adequate water supply, causing only a delay in ripening and reduced sugar per berry, and had an effect on berry composition as measured by TSS, pH, TA, anthocyanins, and phenolic concentrations. Post-veraison intense water stress and severe pruning level had a significant effect on grape composition and did cause consistent increases in TSS, pH, and concentration of anthocyanins and phenolics, associated with a decrease in TA of grape juice. The findings showed that the interactive effect of pruning levels and post-veraison water stress is a good strategy to balance berry development and composition. As Merlot is a typical red wine grapevine, it is important to increase the berry composition even though there could be a reduction in its berry development variables including berry yield per vine. Conversely, during a time when the market demands wines with lower alcohol concentration, the application of both severe pruning and adequate water supply seems to be a suitable option as the wine alcohol is directly derived from the accumulated sugar in the berry at harvest. In addition, reduction in pH of grapes is also important for wine stability especially in warm conditions, due to the global warming scenario.

Data Availability

The data that support the findings of this study are available upon request to the author.

Conflicts of Interest

The author declares that there are no conflicts of interest.

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