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Effect of wounding on the maturity and chemical composition of Cabernet Sauvignon (*Vitis vinifera* L.) berry

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Phenolic compounds are important indicators of grape berry and wine quality. They also have many important effects on human health. Biotic and abiotic plant stressors are the factors that affect the composition and amounts of metabolites dramatically. Although wounding is one of these stressors, little is known about its effect on the accumulation and biosynthesis of the entire phenolic profile. This study aimed to identify the effects of human-made wounding to grapevine leaves on the accumulation of grape berry metabolites in cv. 'Cabernet Sauvignon' grapevines. Ten combinations of different wounding timing and treatment methods were applied to 20 years old grapevine plants. The data suggests that wounding treatments have the potential to diversify the phenolic compound profile and can be utilized for their management compared to the control group. Specifically, the E15 treatment accelerated $MI_{TSS/TA}$ by 39.83% and increased the content of trans-resveratrol by 35.78% compared to the Control. On the other hand, the E10+W5+E3 treatment resulted in a regression in maturity parameters. The wounding treatment E10+W5 increased the total phenolics by 13.74%, total anthocyanin contents by 20.48%, and trans-resveratrol content by 93.05%. Additionally, the E5+W3 treatment demonstrated a significant increase of 19.65% in syringic acid and 21.89% in vanillic acid contents. Although the control application for antioxidant activity reached its peak value, it decreased in the E15+W5 treatment. As a result, it was determined that the responses given to the treatments were quite diverse. Nevertheless, they also found it applicable to manage berry maturity and quality indicators. Early wounding treatments accelerate maturity, and treatments performed more recently at harvest stop berry sugar accumulation. While late treatments increase the total phenolics, the responses of the phenolic compounds profile differ.
Keywords: Abiotic stress, antioxidant activity, berry quality, phenolics.

INTRODUCTION

Plants are continually subject to a wide range of stressors comprising physical, chemical, and biological agents. Physical damage to a plant, known as wounding stress, triggers a physiological response in the plant. This type of stress can stem from various sources, including herbivory, infections, and mechanical damage caused by abiotic factors such as wind or anthropogenic effects (Moore *et al.*, 2022). These abiotic stress factors cause a variety of biochemical reactions that affect the synthesis and accumulation of primary and secondary metabolites.

In grapevines, compounds are classified as primary and secondary metabolites, including total soluble solids, organic acids, pH, and berry phenolics.

When a plant sustains an injury, multiple physiological processes, such as cell division and differentiation, photosynthesis, and stress tolerance, are activated. In response to the wound, plants can initiate local and systemic reactions, mitigating the injury and promoting healing (Lukaszuk and Ciereszko, 2012).

The injury response is not restricted to the injury zone; it also emerges in other parts of the plant, including leaves and organs that are distant from the injury zone. This systemic response, also known as systemic acquired resistance, helps the plant to protect itself from future injuries and pathogens (Hasegawa *et al.,* 2011). Wound signals trigger the plant's defence mechanisms, which increase its resistance to further wounding. This response is typically regulated by changes in metabolic composition and the modulation of gene expression. It can involve activating or suppressing different pathways, which work together to protect the plant and promote healing (Lukaszuk and Ciereszko, 2012).

Secondary metabolites play a significant role in addressing both abiotic and biotic stress factors. These metabolites accumulate under stress, and when their levels increase, they have a positive impact on the quality, antioxidant capacity, and aroma profiles of must, fruit, and wine. These compounds

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can be classified into three main categories: phenolic, terpenoids, and nitrogen (Revutska *et al.,* 2021).

Phenolic compounds regulate plant growth and the elongation of vegetative organs such as shoots and roots, inhibiting seed germination, promoting wound healing, and controlling cell division. Furthermore, secondary metabolites serve as a defense mechanism against stress conditions, including infection and UV radiation. These functions are essential in protecting the grapevine from external conditions and contributing to its overall resilience (Mansour *et al.,* 2022).

The composition of phenolic compounds in grapes can vary depending on a range of factors, including the grape variety, the effects of biotic and abiotic factors during the growing period, the unique characteristics of the vineyard site (terroir) and the degree of maturity of the grapes. Exposure to sunlight and UV radiation can impact various characteristics of grapes, such as °Brix and pH values, total pigment content, total phenolic content, and tannin content (Song *et al.,* 2015).

Numerous studies have examined the effects of abiotic stress on grapevines and other higher plant species. These studies have identified various stressors, including nutrient stress (Verdugo-Vásquez *et al.,* 2021), salt stress (Gohari *et al.,* 2021), drought stress (Candar *et al.,* 2023), heavy metal stress (Krupa *et al.,* 1996), cold stress (Zhao *et al.,* 2011), and variations in temperature and light (Candar *et al.,* 2019). These stressors have been found to impact secondary metabolites in plant tissues.

Grape berries and wine offer notable nutritional value, providing a range of beneficial compounds. Grape berries are a source of essential vitamins and minerals, including vitamin C, vitamin K, potassium, and manganese. They also contain dietary fiber, which promotes digestive health. Additionally, grape berries are rich in antioxidants such as resveratrol, quercetin, and anthocyanins. These antioxidants have been associated with numerous health benefits, including reducing oxidative stress, supporting heart health, and potentially offering anti-inflammatory properties (Bogdan *et al.,* 2020).

When grapes are fermented into wine, some of these nutritional compounds are preserved and transformed. Red wines, in particular, contain higher levels of antioxidants compared to white wines due to the longer contact between grape skins and the fermenting juice (Kontou *et al.,* 2012).

Additionally, there have been numerous studies on the potential health benefits of specific phenolic compounds, such as catechin, epicatechin, and trans-resveratrol (Castilla *et al.,* 2006; Lacerda *et al.,* 2014). It is widely acknowledged that plant materials with high total phenolic compound content also exhibit high antioxidant capacity (Tahmaz *et al.,* 2022).

The direction of rows within a vineyard directly affects the cluster and soil temperatures of different canopy sides (Hunter *et al.,* 2016). Depending on the row orientation, micro changes in temperature can lead to increased flavonoid content and changes in composition. Conversely, intense light can increase flavonol content while potentially altering the levels of anthocyanins and flavan-3-ols (Reshef *et al.,* 2017; Strack and Stoll, 2021). Except malvidin, overall levels of anthocyanins and cyanidin-glucoside content are reduced under severe radiation conditions (Reshef *et al.,* 2017). Under severe light, the homogeneity of the cluster improved by a response exhibited by flavonoid-glycosides, quercetin and kaempferol (Reshef *et al.,* 2017). The daily insolation regime of berries can fluctuate the levels of sugars, organic acids, amino acids, polyamines and phenylpropanoids (Reshef *et al.,* 2019; Strack and Stoll, 2021). Wines produced from grapes grown on east-west (EW) oriented rows tend to have a higher acidity, residual sugars, alcohol and color hue. In contrast, wines produced from grapes grown on north-south (NS) oriented rows have a higher color intensity, ash, pH and total phenolics (Mota *et al.,* 2021).

When a grapevine is damaged, it may experience wounding stress. This stress can have a negative impact on the grapevine's ability to recover, grow, and produce fruit. However, grapevines that are exposed to higher levels of sunlight and wind exhibit greater resistance to wounding stress. These conditions promote the formation of protective compounds and tissues in the plant. Understanding the interaction of wounding stress levels, time, and vineyard orientation on these processes can help winemakers choose the best direction for their vineyards and produce high-quality grapes and wines.

Cabernet Sauvignon, a grape variety renowned for its exceptional quality and distinctive characteristics, has achieved global acclaim. Its origins can be traced back to the Bordeaux region of France. Published genetic studies suggest that Cabernet Sauvignon is the result of a crossbreeding between Cabernet Franc and Sauvignon (Plantgrape, 2023). Today, this variety is cultivated in vineyards across the world. It is highly regarded for producing bold and full-bodied red wines that captivate the senses with intense flavors of blackcurrant and black cherry, complemented by subtle hints of herbs and spices. One of its notable attributes is its remarkable aging potential, with Cabernet Sauvignon wines often showcasing rich tannins and complex layers of flavors that evolve and mature over time.

This manuscript aimed to understand the effects of varying wounding stress on Cabernet Sauvignon (*Vitis vinifera* L.) grapevine maturity and berry composition.

MATERIAL AND METHODS

Location and plant material**:** The study was conducted during the 2021 growing season at a commercial vineyard of cv. 'Cabernet Sauvignon' grapevines planted in 2001 on SO4 rootstock (*Vitis berlandieri* x *Vitis riparia*) in Tekirdağ (41°01′ 14″ N - 27°28′ 14″ E). Vine spacing was 2.60×0.90 m in north-south rows planted in silty clay loam soil. Grapevines were pruned to 16-18-bud canes depending on and trained with a double-armed Guyot trellising system.

***Climate and phenology*:** 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). Winkler index (WI-GDD), Hydrothermic index (HyI) and Night cold index (CI) were determined according to calculations where reported in detail in Gülbasar Kandilli *et al.*, (2022).

***Wound stress treatments*:** Wound stress response was induced by a human-made systematic mechanical injury of grapevine leaves. A hand-made device was developed to evenly distribute the stress caused by mechanical wounding among the grapevine leaves. The device consisted of a 45-50 cm long PN25 PPRC (Polypropylene Random Copolymer) composite pipe handle and two 15-20 cm long whip-like wounding tips made of PA 66 (Polyamide 6.6) material mounted on the handle. Fully developed healthy leaves were uniformly subjected to mechanical leaf wounding at the time and direction combinations described in Table 1. Wounding treatment, regardless of main and lateral shoot leaves, was applied to leaves originated from fifth to ninth buds. At the end of the wounding treatments, approximately 15-20% of the total leaf area was injured as standard in each application.

***Berry sampling and maturation indices*:** Berries were sampled as a mixture of 10 individual grapevines in each iteration of each treatment. Randomly selected 400 berry samples from the bunches' middle top, middle and bottom regions were taken to the laboratory in ice boxes. All samples were kept at -80°C until analysis. Berries were taken from the freezer to be extracted and kept in the refrigerator at +4 °C to thaw. The seeds were manually sorted out, and the remaining skin and pulp parts were shredded with the help of a homogenizer (IKA-Basic T18 Ultra Turrax). Industrial maturity was analyzed by determining the total soluble solids (TSS, °Brix), pH, and total acidity. Berries were crushed by hand to extract juice, and TSS was measured with a handheld refractometer (ATC, BeyanLab Laboratuar Ürünleri San. Tic. Ltd. Şti., Türkiye). pH in the must was measured with a digital pH meter (HI 2210, Hanna Instruments, USA). Maturity indices, °Brix / Titratable acid, and pH^2 ×°Brix values were calculated and evaluated according to Blouin and Guimberteau (2000).

***Total phenolic compounds, total monomeric anthocyanins and tannins*:** Total monomeric anthocyanin content (mg kg^{-1}) was determined by the pH-differential method as malvidin-3-glucoside equivalents in mg kg^{-1} (Giusti et al., 2001). The total amount of phenolic compound was determined in gallic acid equivalent (GAE) per kilogram of berry (mg kg^{-1}) by the Folin-Ciocalteu method reported by Waterhouse (2002). The total tannin content (mg kg^{-1}) was determined according to INRA (2007). All analyzes were performed by UV Visible U-5100 spectrophotometer (Hitachi, Japan).

***Individual phenolic compounds*:** The quantification of syringic acid, vanillic acid, t-Resveratrol, (+)-catechin, and (-)-epicatechin was conducted using a modified version of the HPLC (LC-20A Shimadzu, Japan) method, as described by Meng et al. (2011). Initially, berry samples were extracted using 80% methanol. The resulting methanol samples were then filtered through 0.45 µm membrane filters and injected into the HPLC system for analysis. The mobile phase for gradient elution consisted of acetic acid:water (2:98 v/v) as (A), and acetonitrile (100 v/v) as (B). The concentration of eluent B gradually increased from 0% to 80% over a period of 55 minutes. At 57 minutes, it was further increased to 90% B and maintained at that level until 70 minutes. Subsequently, it was increased to 95% B at 80 minutes, reaching 100% B at 100 minutes, and then maintained at 100% B until 115 minutes. Finally, at 120 minutes, it was returned to 0% B. During the elution process, a flow rate of 1.5 mL min^{-1} was maintained, while the column was kept at a thermostatically controlled temperature of 22°C.

***Trolox equivalent antioxidant capacity*:** Trolox equivalent antioxidant capacity (TEAC) was determined according to DPPH (Brand-Williams et al., 1995) and ABTS (Re et al., 1999) methods. For both methods, the analytical standard Trolox was used to construct the calibration curves, and the results were expressed as equivalents of Trolox per kilogram of berry (TEAC) (mg kg^{-1}).

Table 1. Mechanical leaf wounding treatment combinations.

Treatment	Application date and direction combination
Control	No application
E15+W10+EW5	15 days prior to harvest from E + ten days prior to harvest from W + five days before harvest from E/W
E15 + W10	15 days prior to harvest from E + ten days prior to harvest from W
E15 + W5	15 days prior to harvest from E + five days prior to harvest from W
E15	15 days prior to harvest from E
E10+W5+E3	Ten days prior to harvest from E + five days prior to harvest from W + three days before harvest from E
E10+W5	Ten days prior to harvest from E + five days prior to harvest from W
E10	Ten days prior to harvest from E
E5 + W3	Five days prior to harvest from the E + three days prior to harvest from W
E5	Five days prior to harvest from E

E: East direction, W: West direction

***Trail design and statistical analysis*:** The experiment utilized a completely randomized block design, consisting of ten different wounding combinations. Each combination was replicated three times with ten individual grapevine plants. The data collected from the berries were analyzed using one-way analysis of variance (ANOVA) with JMP 13.2.0 statistical software. The results of the ANOVA indicated that there were no statistically significant differences between the treatments, thus no multiple comparison test was conducted. Additionally, the bivariate relationships were analyzed using the R statistical environment .

RESULTS

***Climate and phenology*:** Tekirdağ shows the type of Mediterranean climate with hot and dry summers and mild winters, the characteristics of the Csa according to the Koppen-Geiger climate classification. Precipitation occurs in winter and spring. The continental climate is dominant in the interior, and the winters are cold compared to the coasts. Some climatic characteristics of long and trial years are shared in Table 2.

Table 2. Some viticultural climate indexes for the 1940-2021 period and the year 2021.

	1940-2021	2021
Total precipitation (mm)	583.50	530.90
Vegetation period precipitation (mm)	190.40	192.00
Winkler index (WI-GDD)	1884.00	1992.00
Hydrothermic index (HyI)	3540.10	3439.22
Night cold index (CI)	16.10	10.60

It has been determined that the Winkler Index (WI-GDD), which was calculated as 1884.00 day-degree for the 1940-2021 period, increased to 1992.00 day-degree in 2021. Branas Hydrothermic Index (HyI) was calculated in 2021, close to the average for many years. On the other hand, the Night Cold Index (CI) was found to be 10.60 ˚C and considerably lower than the average of long years, unlike the trend of the last ten years.

The year 2021 showed a similar trend to the long-term average in the total precipitation regime. The standard of 1940-2021 was 583.50 mm, and the total precipitation in 2021 was 530.90 mm. However, it is seen that the precipitation received in the last period of the year in the average of many years is taken in the first period of the year and at the beginning of the vegetation (Figure 1).

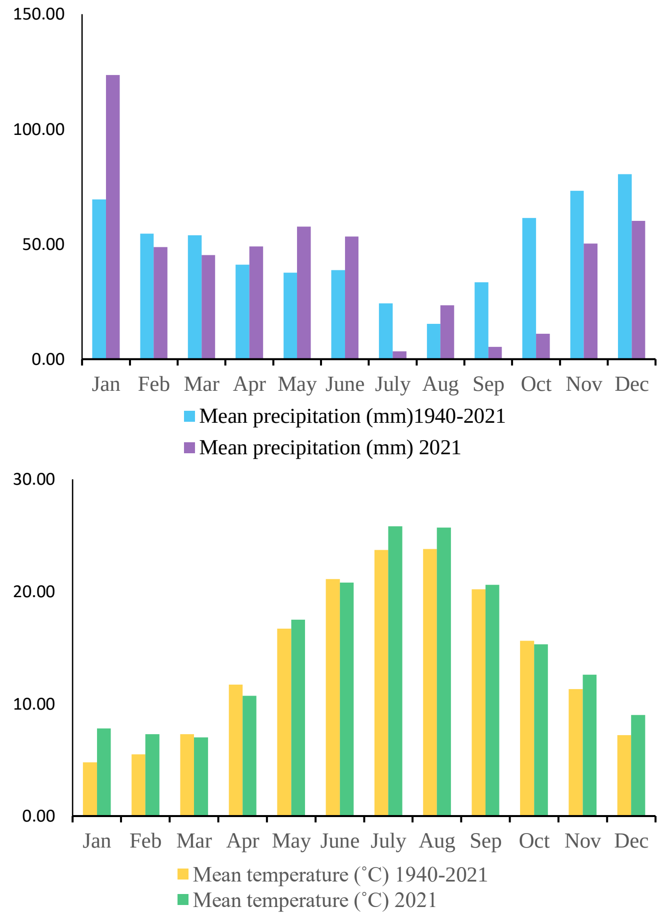

Figure 1. Mean precipitation (mm) and temperature (°C) for the 1940-2021 period and year 2021.

The year 2021 showed a similar trend to the long-term average in the total precipitation regime. The average of 1940-2021 was 583.50 mm, and the total precipitation in 2021 was 530.90. However, it is seen that the precipitation received in the last period of the year in the average of many years is taken in the first period of the year and at the beginning of the vegetation. In terms of average temperature, the continuation of the upward trend that has been going on for the last 10 years appears to be in progress. The average temperature increased by about 1 ˚C compared to the 1940-2021 period. (Figure 1). While the average temperature in 2021 measured 15.01 ˚C, the average of 23.80 ˚C in August, which was the hottest month in the 1940-2021 period, was 25.80 ˚C in 2021.

In 2021, cv. 'Cabernet Sauvignon' budburst (EL 04-07), according to Lorenz et al. (1995), was 15.04. It occurred in 2021 and flowering (EL 23-25) on 01.06.2021. Veraison (EL 35) was recorded on 08.08.2021, while the harvest (EL 38) was made on 23.09.2021 in the range of 21.00-22.00 °Brix, considering the average of all blocks and the general condition of the clusters.

***Total soluble solids, total acidity and pH*:** The differences caused by wounding treatments on total soluble solids (TSS), total acidity (TA) and pH during the study are shown in Figure 2. Variance analysis of this criteria revealed no statistically significant variations between treatments. Although no significant differences were observed in the mean values, the TSS content of the treatments were measured to vary between 20.20-22.47 °Brix. The highest TSS value of 22.47 °Brix was detected in the E15 treatment, while the lowest value was measured at 20.20 °Brix and 20.40 °Brix in E10+W5+E3 and E5 + W3 treatments. While E10+W5+E3 and E5 + W3 treatments remained below control, others accumulated TSS around or above the control. The lowest TA content was observed in the E15 treatment with a value of 5.40 g L^{-1}, and the highest content was 7.15 g L^{-1} in the control treatment. pH values varied from 3.41 to 3.53. Differences in means in TA and pH were also not statistically significant.

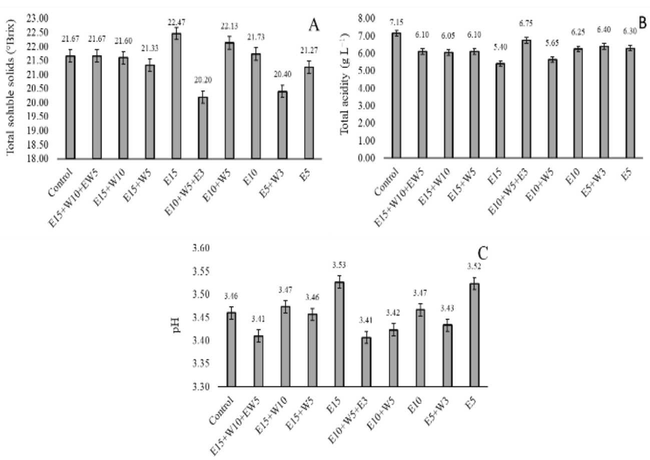

Figure 2. Effects of treatments to Total soluble solids (°Brix) (A), Total acidity (g L^{-1}) (B) and pH (C). Bars display the mean values of the treatments and ± SE, respectively. The results of variance analysis did not show a statistically significant difference between the means.

Maturity indices: The control treatment value for which the regional standards were applied slightly approached the threshold value of 259.86. E15 reached a very high MI with a weight of 281.46, followed by E5 (Figure 3A). E10+W5+E3 and E5 + W3 were determined to produce low $MI_{pH^2 \times °Brix}$ values.

Other MI, calculated as TSS/TA, considers values between 3-4. Although all applications appear to be within the expected range for MITSS/TA, the role of this indice in determining the correct harvest date is limited. There were no significant differences between the wounding treatments and the control for the studied maturity indices.

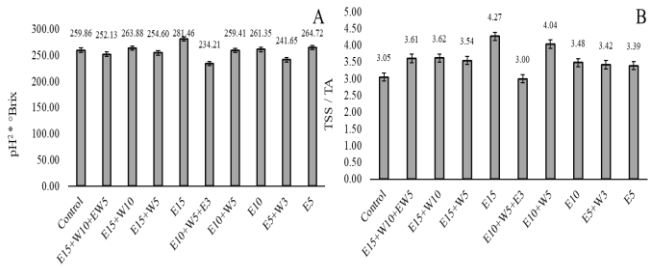

Figure 3. Effects of treatments to maturity indices. pH^2 x °Brix (A) and TSS/TA (g L^{-1}) (B). Plots display the average values of the treatments. The red central horizontal bars depict the medians, while the lower and upper limits of the box, also in red, represent the first and third quartiles, respectively. The results of variance analysis did not show a statistically significant difference between the means.

***Total phenolics, total anthocyanin and tannin*:** Regarding total phenolics, E10+W5 and E15 treatments led to higher contents than the control group. All other wounding treatments showed lower contents than the control group. As expected, E10+W5 and E15 applications achieved higher results than the control for total anthocyanin and tannin contents, in line with the total phenolics content. While the E15 treatment has a higher value in total tannin content, the E10+W5 treatment has a higher value in total anthocyanin content, and E15 has passed the control lightly. The total phenolic compounds' highest value was 3762.73 mg GAE kg^{-1} in E10+W5, and the lowest value was 2843.03 mg GAE kg^{-1} in E5 + W3. While the total tannin content was the highest in E15 with 4.93 g kg^{-1}, E5 resulted in the lowest value of 3.53 g kg^{-1}. In anthocyanin content, E10+W5 was the highest value of 2073.88 mg kg^{-1}, while E15+W10+EW5 was the lowest at 1330.10 mg kg^{-1}. E10+W5 increased both total phenolic and anthocyanin (Figure 4).

In the study, the effects of mean differences in wounding treatments on total phenolics, anthocyanins and tannins were not statistically significant.

***Profile of phenolic compounds*:** E15 was the most significant treatment for increasing (+)–Catechin with 3.30 mg kg^{-1}, followed by E10+W5+E3. (–)–Epicatechin amount reached the highest level with a value of 39.62 mg kg^{-1} in E10+W5 treatment and was followed by E10+W5+E3. It was determined that E10 treatments in (+)–Catechin content and E15+W10+EW5 in (–)–Epicatechin content caused the lowest values. Because of the strong correlation they showed in the amounts in berry must, their responses to the applications of syringic acid and vanillic acid contents were very similar. The highest amounts were detected in the E5 + W3 treatment. E10+W5+E3 treatment was the wounding combination with the lowest content for both phenolic acids. There was no significant difference between the wounding treatments and the control for any phenolic compounds studied (Fig. 5).

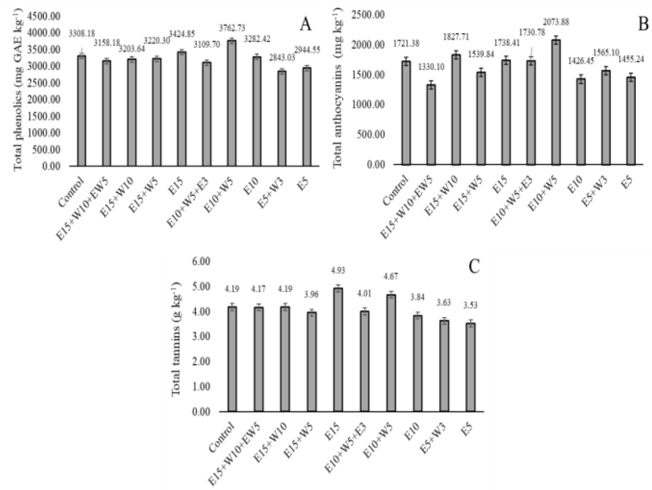

Figure 4. Effects of treatments to Total phenolics (mg GAE kg^{-1}) (A), Total anthocyanins (B) (mg kg^{-1}) and Total tannins (g kg^{-1}) (C). Bars display the mean values of the treatments and ± SE, respectively. The results of variance analysis did not show a statistically significant difference between the means.

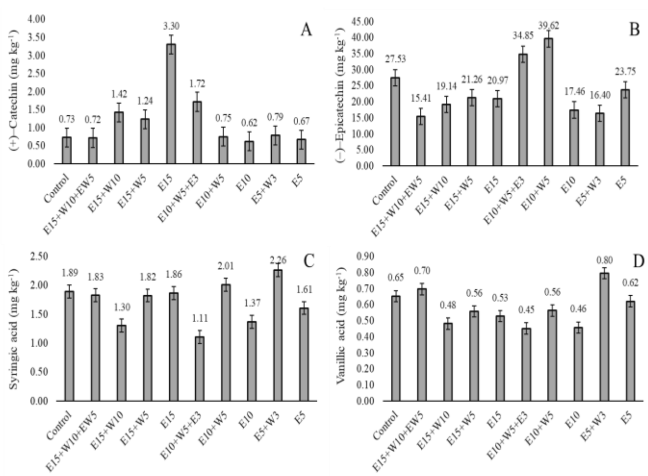

Figure 5. Effects of treatments to (+)–Catechin (mg kg^{-1}) (A), (–)–Epicatechin (mg kg^{-1}) (B), Syringic acid (mg kg^{-1}) (C) and Vanillic acid (mg kg^{-1}) (D). Bars display the mean values of the treatments and ± SE, respectively. The results of variance analysis did not show a statistically significant difference between the means.

***Trans-resveratrol and antioxidant activity*:** There were no statistically significant variations between treatments in the variance analysis of trans-resveratrol content and antioxidant activity. However, differences were observed in the mean values; the trans-resveratrol range of the treatments was measured to vary between 0.36-3.59 mg kg^{-1}. The highest trans-resveratrol value of 3.59 mg kg^{-1} was detected in the E10+W5 treatment, while the lowest value was measured in the E5 + W3 treatment (Figure 6).

Interestingly, unlike all parameters examined in the study, the highest antioxidant activity was found in the control treatment. All other treatments remained below control. The lowest antioxidant activity was observed in the E15 + W5 treatment, with 10.36 mg kg^{-1}.

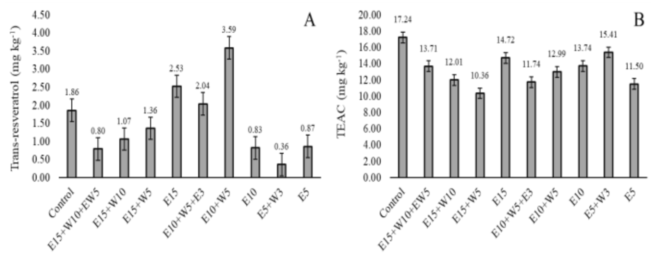

Figure 6. Effects of treatments to Trans-resveratrol (mg kg^{-1}) (A) and Antioxidant activity (TEAC) (mg kg^{-1}) (B). Bars display the mean values of the treatments and ± SE, respectively. The results of variance analysis did not show a statistically significant difference between the means.

***Bivariate analysis*:** The interrelationships of selected data are displayed as a scatterplot matrix, with bivariate scatterplots with fitted linear regression distribution and frequency histogram overlays (Figure 7).

$MI_{pH^2 \times °Brix}$ (MI) was only predictive of anthocyanins (Antho). However, total tannins (Tannin) correlated with total phenolics (Pheno) and total anthocyanins concentrations. The relationship of antioxidant activity (TEAC) with total phenolics, total tannins and anthocyanins ranged from 0.45 to 0.38. The highest correlation between these parameters was a reasonable correlation with 0.45 TEAC and total phenolics. (+)–catechin (CA) correlated negatively to MI with a -0.36 value and positively with trans-resveratrol (t-Res) with a value of 0.43. (–)–epicatechin (ECA) was found to be predictive for total anthocyanins with a value of 0.43. A more positive relationship between (–)–epicatechin and trans-resveratrol was detected with a value of 0.76. ECA had a reasonable negative correlation with vanillic acid (Vanillic) with a value of -0.54. The highest correlation between all parameters was found between vanillic acid (Vanillic) and syringic acid (Syringic), with a very significant value of 0.84 (Figure 7).

DISCUSSION

In cases where the harvest is solely based on industrial maturity monitoring, the harvest date is determined by considering the interplay of parameters such as sugar, acidity,

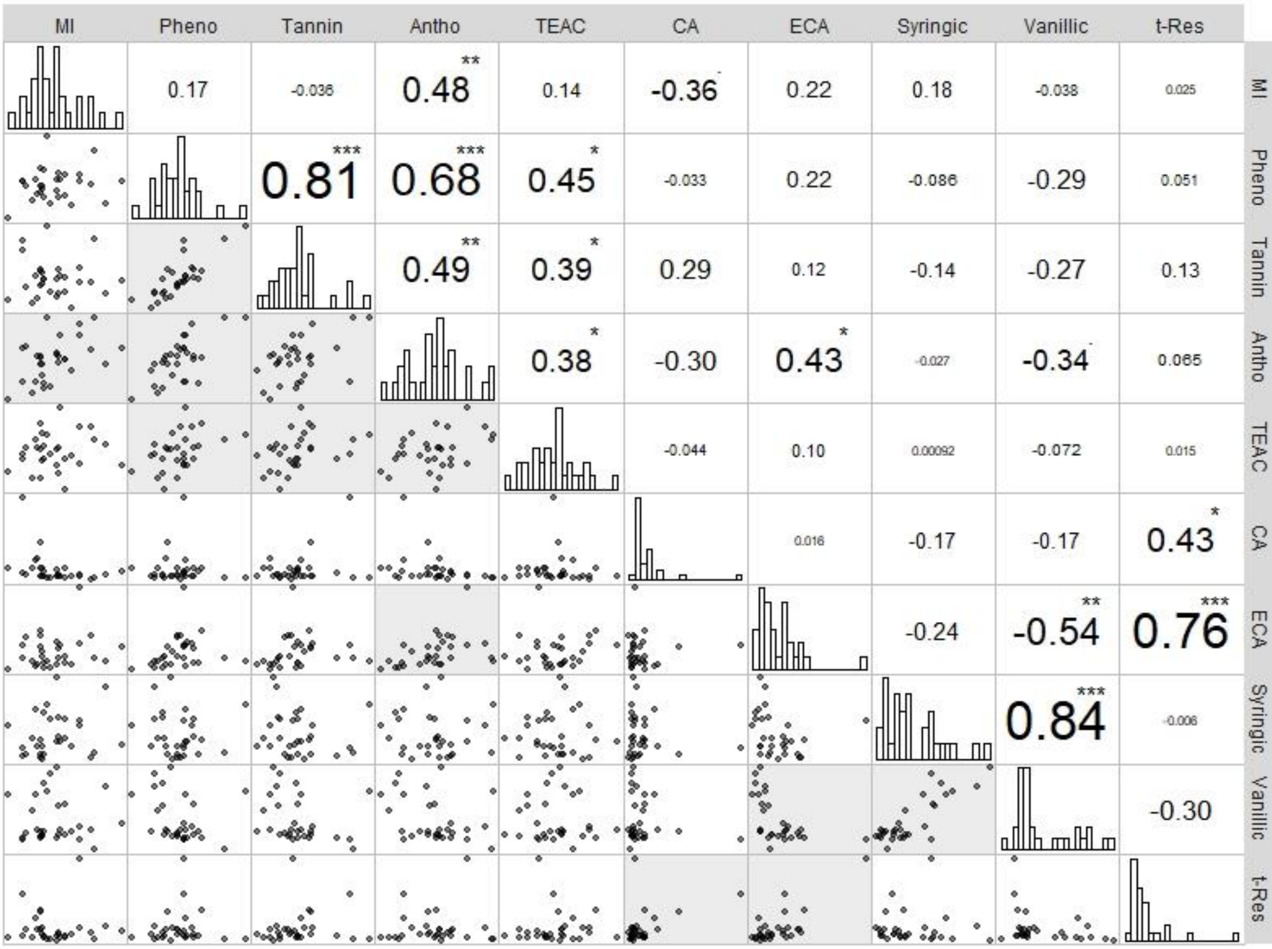

Figure 7. Scatterplot matrix of selected variables, with bivariate scatterplots with fitted linear regression distribution (below diagonal), frequency histogram overlays (on diagonal), Pearson's coefficients of determination (R^2) (above diagonal) and coefficient statistical significance indicated by text size and * symbol at above diagonal (absent > 0.05, * indicates < 0.05, **indicates < 0.01, * indicates < 0.001), and background color at below diagonal. MI; maturity indices of pH^2 x °Brix, Pheno; total phenolics (mg GAE kg^{-1}), Tannin; total tannins (g kg^{-1}), Antho; total anthocyanins (mg kg^{-1}), TEAC; antioxidant activity (mg kg^{-1}), CA; (+)–catechin (mg kg^{-1}), ECA; (–)–epicatechin (mg kg^{-1}), Syringic; syringic acid (mg kg^{-1}), Vanillic; vanillic acid (mg kg^{-1}), t-Res; trans-resveratrol (mg kg^{-1}).**

and pH. These parameters are related to maturity indexes (Blouin and Guimberteau, 2000) and are used in their calculation.

The expected phenomenon from the wounding treatments applied in the study method is that the stress response caused by the applications gives results that will improve the wine properties in the examined criteria. When TSS is considered the main criterion, the threshold value for 22 °Brix region standards can be accepted. In this case, the E15 and E10+W5 treatments, albeit with very close values, slightly precede industrial maturity. These two combinations have in common that they were applied from the past ten days or more before the designated harvest date. The E10+W5+E3 application, where the lowest TSS accumulation was seen, was made ten days before the harvest, but it was exposed to injury from the west three days before the harvest. The other low TSS accumulating E5 + W3 and E10+W5+E3 have in common is their exposure to the westerly injury three days before harvest (Figure 2).

In a similar study conducted with cv. 'Cabernet Sauvignon' in 2017 and 2019, at a location close to the experimental vineyard, Abay (2022) found that abiotic stress induced by impacts to grapevine stem and leaf wounding, treatments were not effective against TSS and TA, regard to statistical significance, however useful for manipulation of ripening. This result indicates that in early injuries, in terms of TSS accumulation, grapevine overtakes the healing process and advances maturity with response chemicals. However, in injuries close to the harvest date, the process may be reversed, and maturity may be delayed.

Accurate harvest date determination according to pH^2 x °Brix maturity index ($MI_{pH^2 \text{ x °Brix}}$) of 200 in white varieties and 260 in red varieties are another criterion used (Blouin and Guimberteau, 2000).

Although the differences in mean values calculated for both MIs are not significant, it is considered that the contrast created by E15 treatment for $MI_{pH^2 \text{ x °Brix}}$ will reflect positively on the possible fermentation process. Early wounding treatments 15-10 days before harvest are thought to accelerate ripening through carbohydrate transfer by inducing grapevine intrinsic dynamics. On the other hand, the treatments applied three-five days before the harvest ultimately limit the photosynthesis process due to the injury of leaves and stop maturation. In addition, Tok Abay (2021) also found no statistical differences in maturity indices of cv. 'Cabernet Sauvignon' and cv. 'Merlot' grape cultivars regarding to abiotic stress induced by grapevine stem impact, vibration, leaf wounding, and defoliation applications, although numerical differences have been found.

In these criteria, it is accepted that the contents depend on many different variables-however, cv. 'Cabernet Sauvignon' indicates that total phenolics vary between 1423.71 mg GAE kg^{-1} - 1963.60 mg GAE kg^{-1}, total anthocyanins vary between 297.17 mg kg^{-1} - 1250.23 mg $kg^{-1,}$ and tannins vary between 4.77 g kg^{-1} - 6.59 g kg^{-1} from limited studies conducted in the region (Bahar and Öner, 2016; Korkutal *et al.,* 2019). Girdling, a traditional cultural practice used in table grape cultivation can be considered artificial wounding. Chen et al. (2022) also reported that girdling application increased total phenolic, polymeric tannin and proanthocyanidins accumulation compared to the control. However, in this study, wounding treatments caused a fluctuation in Total anthocyanin compared to Control. Results regarding tannins are in line with the previous findings (Tok Abay, 2021; Abay, 2022). Researchers also found a relative increase in tannin content in the wound treatment compared to the control.

Although high phenolic content does not always mean high quality in terms of total wine quality, the effects of wounding treatment on phenolic substance management are manageable. The results can be improved regarding the effects of the phenolic compound on human health.

Chitarrini et al. (2017) also reported in their study that mechanical wounding, which they created with more limited applications, causes higher accumulation, especially of phenol compounds in cv. 'Bianca' grapevines. However, as reported by Chen et al. (2022), the accumulation of volatile compounds in plants can have negative effects, except for aldehyde compounds in cv. 'Hanxiangmi' grapevines.

(+)–Catechin and (–)–Epicatechin are flavonoids and types of polyphenol found in the grape berry that can impact wine quality by protecting the berry from environmental stressors such as UV radiation and high temperatures. For this reason, some wounding combinations were determined to respond to winemakers' demands of increasing (+)–Catechin and (–)–Epicatechin content. But at the same time, it has been determined that wounding treatments can also enable the establishment of the right balance, as these flavonoids can lead to a dry and bitter taste in wine (Mansour *et al.,* 2022).

Syringic acid and vanillic acid are phenolic compounds that are naturally present in grapes and wine. They have antioxidant properties that can contribute to the overall antioxidant capacity of wine and positively affect wine quality by helping preserve the wine's color, aroma, and taste. There was no relationship between the wounding stress created by the applications and the responses given to it in terms of organic acids. The reason for this may be that the organic acids synthesis period is pre-verasion, and acid contents fluctuate and fall after veraison (Geng *et al.,* 2022).

Trans-resveratrol has been extensively studied for its antioxidant properties. In grape berries, trans-resveratrol is produced in response to stress and fungal infection. In wine, protecting from oxidation, which can cause it to spoil. Additionally, it has been found to contribute to the color, aroma, and flavor of the wine (Gueguen *et al.,* 2015)

Many studies with different species have expressed the ability of plants to perceive and respond to mechanical stimuli from external sources (Benikhlef *et al.,* 2013; Chitarrini *et al.,* 2017). UV-C and leaf wounding treatments were reported to be effective in increasing trans-resveratrol in cv. 'Cabernet Sauvignon' at the time of harvest (Abay, 2022). 'Merlot' also reacted to an increase in trans-resveratrol content in artificial wounds produced by vibration, leaf scarification and severe defoliation (Tok Abay, 2021). However, in some cases, soft mechanical stimulation may not be associated with wounding responses (Benikhlef *et al.,* 2013).

***Conclusion*:** The present study aimed to analyze the influence of artificial wounding on the maturity and phenolic profile of 'Cabernet Sauvignon' grapes. The experiment was conducted in a north-south oriented vineyard, and it was observed that the direction and timing of the injury affected the responses, although not significantly, in terms of numerical value and content changes. Based on the available data, it can be concluded that wounding treatments have the potential to diversify the profile of phenolic compounds and can be used for their management compared to the control group.

The E15 treatment accelerated maturity and increased the content of trans-resveratrol. Conversely, the E10+W5+E3 treatment led to a regression in maturity parameters. The wounding treatment E10+W5 increased the total phenolics and total anthocyanin contents. Additionally, it was observed that the contents of syringic acid and vanillic acid increased in the E5+W3 treatment. The control application for antioxidant activity reached the highest value but decreased in the E15+W5 treatment.

To summarize, the findings of this study demonstrate that 'Cabernet Sauvignon' grapevines (*Vitis vinifera* L.) have the ability to perceive external mechanical stimuli, such as artificial wounding, and initiate a robust defense response.

Competing Interests**:** The author declare no conflict of interest.

Author Contributions**:** The author is responsible for all stages of the study, including the design and carrying out of the experiment, the data collection, and the article's writing**.**

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