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ORIGINAL RESEARCH ARTICLE

# Auxins seem promising as a tuning method for balancing sugars with acidity in grape musts from cv. Tempranillo, but not defoliation or application of magnesium to leaves

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## ABSTRACT

Global warming boosted by climate change affects grape quality, with increasing total soluble solids (TSS) content and decreasing total acidity (TA). However, current wine preferences increasingly include moderate alcohol content, higher acidity and the preservation of primary aromas reminiscent of grapes. Therefore, we hypothesised that applying phytohormones or mineral nutrients to leaves or carrying out defoliation can improve grape must properties in the face of climate warming and in accordance with current oenological trends. The effects of these three viticultural strategies were assessed independently from one another during three growing seasons in a *Vitis vinifera* L. cv. Tempranillo vineyard in northern Spain. Specifically, three 1-naphtaleneacetic acid (NAA) treatments, two early defoliations (ED; moderate and severe) and two foliar fertilisations with magnesium (Mg) were applied. Treatment with NAA was the most encouraging strategy for decreasing must TSS while increasing TA: it had slight effects on TSS in general and also slight effects on TA when applied close to veraison. The effects of the Mg treatments and moderate ED had null to slightly adverse effects. Finally, severe ED was clearly counter-productive. This study contributes to understanding the effects of both auxin and early defoliation treatments on grape must TSS, acidity and even yeast assimilable nitrogen (YAN) at harvest time. The favourable effects of NAA application are shown to be consistent though slight. Therefore, according to these results, the application of auxins may be an adequate choice for balancing sugars with acidity in grape musts. However, the results also suggest that more research needs to be undertaken to better characterise the effects of auxin treatments on grape must properties at harvest. In particular, different types of auxins, rates, concentrations and number of applications should be tested in the quest for more marked effects.

**KEYWORDS:** Defoliation, magnesium, malic acid, naphthaleneacetic acid, potassium, tartaric acid, Tempranillo

## INTRODUCTION

Producing consistently high quality grapes from one season to the next is a significant challenge for viticulturists from all over the world. Berry quality parameters of paramount importance, such as sugar content and acidity, and thus wine taste and aroma, are being modified by increasing temperatures and aridity due to the current global warming in many wine-producing areas (Jones *et al.*, 2005). In particular, grape must sugar (i.e., total soluble solids (TSS)) and potassium (K) are increasing, which can result in unbalanced wines with high alcoholic concentration and excessively low acidity (Villette *et al.*, 2020). An increase in grape TSS results in higher alcohol concentrations in wines, thus raising health concerns (Meurman and Vesterinen, 2000; Pinder and Sandler, 2004). Additionally, higher alcohol concentrations can decrease the impact of aroma, partly because they can suppress the perception of a wine's overall fruity character (Escudero *et al.*, 2007), and partly because they affect yeast metabolism during fermentation (Bindon *et al.*, 2013). Specifically, an increase in ethanol concentration during fermentation in conjunction with high sugar and acid concentration and low pH, as well as the addition of sulphur dioxide to the juice, exert selective pressure on the development of yeasts and bacteria during alcoholic fermentation (Du Toit and Pretorius, 2000); as a consequence, the effect of alcohol on the sensory properties of wine is such that relatively small changes in alcohol concentration can greatly influence how wines are perceived (Olego *et al.*, 2016).

Trends in wine consumption are continuously changing, a latest consumer preference being for fresh wines with moderate alcohol concentration, high acidity and a variety of primary aromas reminiscent of grapes (Morata *et al.*, 2019). However, the global warming-related tendency for grapes to have more sugar and lower acidity (Van Leeuwen and Destrac-Irvine, 2017) is not in line with this trend. Therefore, different strategies to obtain fresh and balanced wines are required; these can involve both classical and new acidification technologies in winemaking, such as: i) the direct addition of acid (Vicente *et al.*, 2022) through bipolar membrane electrodialysis or by using cation exchangers (Dequin *et al.*, 2017), ii) the addition of organic acids able to inhibit malolactic fermentation (Morata *et al.*, 2019), iii) the use of non-conventional yeasts (Morata *et al.*, 2019; Sainz *et al.*, 2022), particularly by selecting non-*Saccharomyces* yeasts (Dequin *et al.*, 2017; Morata *et al.*, 2019), and iv) the change of other key winemaking operations (Dequin *et al.*, 2017). Some of these strategies, although functional, can lead to obtain unbalanced wines (Sainz *et al.*, 2022); others are still under investigation.

In addition to acidification technologies in winemaking, viticultural strategies can be applied to obtain lower alcohol-to-acidity ratios in wine. These take advantage of the wide range of factors that can significantly affect the accumulation of both sugar and organic acids in grapes at harvest time, such as i) cultural practices; i.e., fertilisation and irrigation rates and schedules, canopy management and ripening control, ii) soil and environmental conditions, and iii) grapevine genotype – both rootstock and cultivar (Gutiérrez-Gamboa *et al.*, 2021; Olego *et al.*, 2016). Regarding cultural practices, a good deal of studies have explored how, in the context of global warming, these can improve unbalanced wines via i) the management of grape ripening time (Olego *et al.*, 2016), ii) leaf removal procedures (Gutiérrez-Gamboa *et al.*, 2021; Olego *et al.*, 2016), iii) fertilisation strategies (Marcuzzo *et al.*, 2021), which, for example, can exploit the antagonistic interactions between nutrients (Olego *et al.*, 2016), iv) transpiration regulation approaches using sprays (Di Vaio *et al.*, 2020; Gutiérrez-Gamboa *et al.*, 2021) or shading nets (Gutiérrez-Gamboa *et al.*, 2021), v) minimal pruning schemes (Gutiérrez-Gamboa *et al.*, 2021), vi) mulching strategies (Gutiérrez-Gamboa *et al.*, 2021), and vii) shoot and severe shoot trimming plans (De Toda *et al.*, 2013; Gutiérrez-Gamboa *et al.*, 2021; Zheng *et al.*, 2017).

The aforementioned cultural strategies seem to offer a promising way to mitigate global warming effects on grape quality in semi-arid Mediterranean vineyards; therefore, their actual behaviour under such climate conditions must be further investigated. However, they do not all have the same impact on vines and the environment; the management of grape ripening time and leaf removal are overall more impactful than fertilisation strategies. Regarding the management of grape ripening time, the treatment of grapes with auxins promotes a delay in the physical and biochemical changes normally associated with the later stages of grape formation; i.e., the accumulation of sugars and anthocyanins and the decrease in acidity and chlorophyll contents (Böttcher *et al.*, 2011). Specifically, 1-naphthaleneacetic acid (NAA) is a synthetic auxin that can be used to achieve such an effect in vines if properly applied; there is some evidence that the closer to veraison the NAA treatment is applied, the more effective it is in delaying veraison and therefore fruit maturity (Davies *et al.*, 2022). Grapevine canopy defoliation, if adequately performed, has shown potential for improving canopy microclimate characteristics, such as the shadow-to-light ratio and bunch temperature, with positive consequences for certain berry quality characteristics, like pigmentation, aromatic compounds and secondary metabolites; however, it can also have negative effects on some other grape parameters, such as TSS and TA (Tardaguila *et al.*, 2010; VanderWeide *et al.*, 2021).

Finally, fertilisation is perhaps the most conservative form of vine manipulation for combatting the effects of climate change on grape sugar content and acidity. Potassium is the most abundant cation in the grape berry at all stages of its development, accumulating rapidly during ripening in concert with sugar accumulation, and strongly decreasing the acid content of must and, therefore, increasing berry juice and wine pH and also colour intensity (Kodur, 2011; Rogiers *et al.*, 2017). Moreover, under current climate change conditions, with increasing temperatures and aridity in many vine cropping areas, grape berry cells adjust their osmotic potential through potassium build-up (Villette *et al.*, 2020).

This is much more relevant for red wine than for white, because in the former the grape skin is included in fermentation to enhance the anthocyanin content of the wine, and more potassium can also be extracted from the skin during the process (Mpelasoka *et al.*, 2003); additionally, potassium stimulates sugar loading into the phloem (Dreyer *et al.*, 2017), influencing the source-to-sink transport of sugar to the berry.

Plants have developed specific potassium transporters in their root cells that cannot be blocked by other nutrients, ensuring sufficient potassium uptake when its concentration in the soil solution is critically low. By contrast, magnesium (Mg) transporters are non-specific and can let other cations through, such as potassium (Senbayram *et al.*, 2015). Therefore, high potassium concentration in the rhizosphere results in antagonism with Mg, a response that can be, to some extent, inversely exploited. For example, in winter wheat the ratio of Mg in shoots to Mg in the whole plant has been found to be negatively correlated with K in the roots (Huang *et al.*, 1990). Hence, it can be conceivably hypothesised that the delivery of sugars by the phloem to sink organs, such as grapes (Lemoine *et al.*, 2013), will be influenced by foliar treatments using Mg, which would act by promoting the reduction in potassium and then the decrease in sugar loading into the phloem.

A literature search has not revealed any studies in which several cultural strategies have been developed simultaneously in a commercial vineyard to investigate their effects on the balance of sugars with acidity in grape musts. Therefore, the objective of this study was to assess, in a commercial cv. Tempranillo vineyard under Mediterranean conditions, the usefulness of three cultural strategies, namely: 1) 1-naphthaleneacetic acid (NAA) application, 2) early defoliation (ED), and 3) post-veraison magnesium foliar fertilisation (Mg), as tuning methods for grape juice composition at harvest time, especially total soluble solids and acidity, as well as potassium concentrations, which influence them both.

## MATERIALS AND METHODS

### 1. Study site

The study site was a commercial vineyard with an area of about 4,500 m$^2$ located approximately 798 m above sea level within the protected designation of origin (PDO) "Ribera del Duero" in the municipality of Aranda de Duero in Burgos, Spain, at latitude 41° 39' N and longitude 3° 40' W (Figure 1). According to a weather station located less than 10 km away from the study site in Vadocondes (Burgos, Spain), the growing seasons (from March to September) in 2017, 2018 and 2019 respectively were as follows: average temperatures of 16.3, 15.3 and 15.5 ºC; record minimum and maximum temperatures of -5.80/37.2, -2.50/36.4 and -6.27/39.1 ºC; cumulated reference evapotranspirations (according to the FAO Penman-Monteith method) of 905, 810 and 900 mm; and cumulated rainfall of 211, 365 and 187 mm (MAPA, 2022). From a climatic point of view, the site can be classified as Csb (warm-summer Mediterranean climate) based on the Köppen-Geiger classification, with an UNEP-FAO Aridity Index of Humid (Nafría García, 2013), but semi-arid based on the Thornthwaite classification (IGME, 1995). According to the FAO's World Reference Base, the soil of the study vineyard corresponds to a Calcic Fluvisol (Estévez and De Castro, 1995), which has formed on gravels and conglomerates (IGME, 1995).

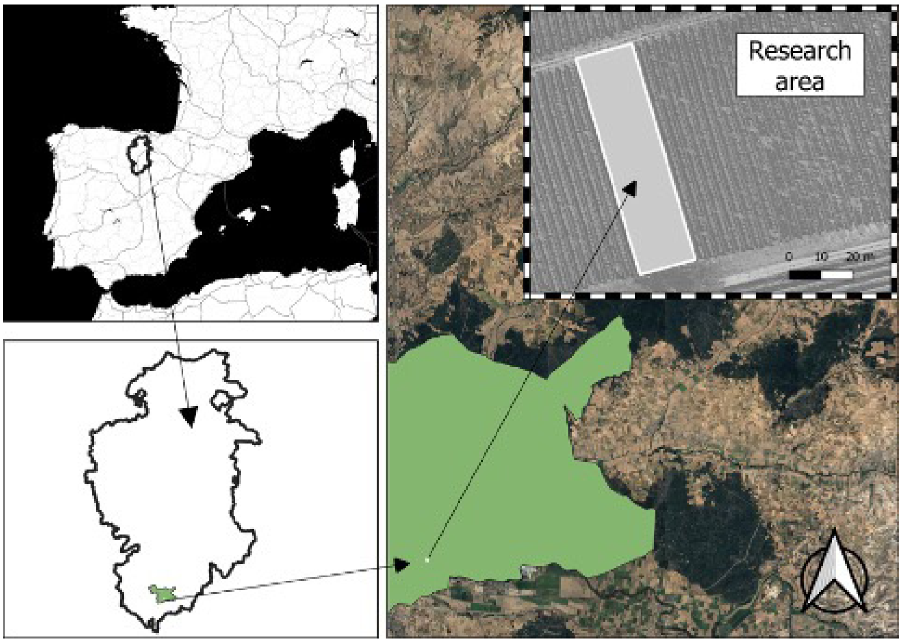

**FIGURE 1.** Location map of the research area within the municipality of Aranda de Duero in green, and this within the Burgos province surrounded by the solid line in Spain (scale and research area are shown on the upper right side, whereas north orientation is shown on the lower right side).

The research was conducted on a 20-year-old *Vitis vinifera* L. cv. 'Tempranillo' grapevine grafted onto a Richter 110 rootstock. Rows were north–south oriented, and vines were spaced 1.3 and 2.9 m within and between rows respectively, with a resulting density of 2,650 vines/ha. Plants were trellis-trained to double cordon with three two-node spurs per side retained at winter pruning. The vineyard had irrigation support provided by trickle with 0.75 m-spaced emitters and water rates programmed on the basis of dendrometer and soil moisture sensors measurements. Finally, apart from the experimental treatments, all the grapevines received the same soil and fertilisation management. In particular, a cover crop was seeded with wheat and spontaneous weed species in alternate rows and maintained until May-June (Eichhorn and Lorenz (E-L) stage 47 (Coombe, 1995)), when it was mowed and incorporated into the soil. Furthermore, neither foliar nor soil fertilisers were added during the three study seasons of 2017, 2018 and 2019.

## 2. Experimental design

Three experiments were carried out; i.e., one per cultural strategy (i.e., the treatment factor): the first comprised three levels of treatment, the second two levels, and the third also two levels, each replicated three times as explained below. The study site was split into 24 subplots with twenty vines each, and five buffer vines and one buffer row were left between the subplots. Several samples of topsoil and subsoil (n = 3), were evaluated before the experimental design was established. Because of the soil homogeneity of the area under study, the treatment replications were distributed among 8 rows with 3 subplots per row in a completely random design; three subplots constituted a common control treatment for the three experiments. Chemical treatments were applied on a subplot-by-subplot basis, and no commercial scale equipment was used.

### 2.1. 1-Naphtaleneacetic acid experiment

In the first experiment, the effects of 1-naphtaleneacetic acid at a dose of approximately 26.5 g/ha with NAA 1 % wettable powder was tested by applying three treatments differing in terms of the growth stage at which they were applied and the cumulative rate of NAA: i) a single NAA treatment at pea-size stage (E-L stage 31 (Coombe, 1995)) was applied (hereafter referred to as NAA1), ii) two NAA treatments, one at pea-size stage and another at veraison (E-L 31 and 35 stages respectively (Coombe, 1995)) were applied (NAA2), and iii) a single NAA treatment at veraison (E-L 35 stage) was applied (NAA3).

### 2.2. Early defoliation experiment

In the second experiment, the effects of manual early canopy defoliation at pea-size stage was tested by applying two treatments differing in extent of defoliation: i) all leaves and lateral shoots on each shoot between the first and second grape clusters were removed (hereafter referred to as ED1), and ii) all leaves and shoots were removed between the shoot base and the second grape cluster (ED2); i.e., in ED2 defoliation was more severe than in ED1.

### 2.3. Magnesium fertilisation experiment

In the third experiment, the effects of the foliar application of magnesium (10 % MgO chelated with EDTA dissolved in approximately 4 L of water for each subplot) fifteen days after veraison was tested by applying two treatments differing in quantity of magnesium applied per ha: 500 $g_{Mg}$/ha (hereafter referred to as Mg1), and ii) 1,000 $g_{Mg}$/ha (Mg2).

## 3. Soil sampling and analyses

An agronomic characterisation of the vineyard soil at depths of 0-30 and 30-60 cm at the beginning of the study and at the senescence phenological stage (end of leaf fall) was carried out based on the following soil properties: texture, soil pH in water (pHw), electrical conductivity (EC), soil organic matter (SOM), total carbonates, available phosphorus (P) and exchangeable potassium ($K^+$) contents (Table 1).

**TABLE 1.** Baseline soil characteristics before establishing the experimental trials for topsoil (0-30 cm) and subsoil (30-60 cm) (n = 3). Means and standard error (SE) of the mean (mean ± 1 SE) are displayed.

| Soil depth | 0-30 | 30-60 |
|---|---|---|
| Sand | 34.5 ± 0.68 | 33.8 ± 0.90 |
| Silt | 35.1 ± 0.47 | 35.8 ± 0.68 |
| Clay | 30.4 ± 0.51 | 30.4 ± 0.58 |
| Textural class | Clay loam | Clay loam |
| Carbonates | 17.5 ± 0.62 | 18.0 ± 0.63 |
| pHw | 8.33 ± 0.08 | 8.36 ± 0.10 |
| EC | 0.12 ± 0.03 | 0.12 ± 0.00 |
| SOM | 0.97 ± 0.07 | 0.86 ± 0.12 |
| P | 6.92 ± 0.50 | 4.54 ± 0.35 |
| K | 0.32 ± 0.07 | 0.23 ± 0.05 |

Soil depth in cm; Textural class from United States Department of Agriculture Textural Classification System; Sand, Silt, Clay, SOM (soil organic matter) and Carbonates in %; EC (electrical conductivity) in dS/m; P (phosphorus) in mg/kg; K (potassium) in cmol(+)/kg.

The soil samples were collected by drilling with an auger. They were then transported to the laboratory in sealed plastic bags and air-dried at room temperature. Next, they were gently disaggregated, passed through a 2-mm mesh sieve, and stored in plastic drawers at room temperature until analysis. The hydrometer method was used to determine soil texture according to the USDA (Gee & Or, 2002). Then, official methods of analysis (MAPA, 1993) were used for the determination of the soil chemical properties: i) pHw and EC were measured with a micropH 2001 pH-meter (CRISON) and a conductimeter (series 522, CRISON) respectively in the supernatants of soil-to-water 1-to-5 suspensions after 25 min of shaking and 5 min of soil settlement, ii) SOM was determined following the Walkley-Black wet oxidation procedure, iii) total carbonates were determined by the pressure calcimeter method, iv) available phosphorus was determined by ultraviolet-visible (UV-Vis) spectrometry (Libra UV/Vis spectrophotometer, Biochrom), after treatment with the Olsen-Watanabe extractant and reaction with ammonium molybdate, and v) exchangeable potassium was extracted with successive aliquots of 1 M ammonium acetate and then determined by atomic absorption spectrometry (AAS) with an atomic absorption spectrophotometer (SOLAAR 969, Unicam).

## 4. Grape sampling and analyses

The grapes were sampled at harvest each year during the first half of October, when the average TSS in the whole study area had reached at least 22 ºBx. In each subplot, 100 grape berries were randomly chosen from 15 grape clusters to determine their weight (W100), and the grape must quality parameters were obtained from the grape clusters after they had been refrigerated for approximately 12 hours. The grape must from each subplot was manually obtained from the grape clusters by gently pressing the grapes and using rubber gloves to avoid contamination.

The following grape must properties were determined based on OIV (2022): i) pH with a pH-meter (micropH 2001, CRISON), ii) total soluble solids (TSS) in ºBx using a refractometer with automatic temperature compensation (Zuzi Series 300, Auxilab), iii) titratable acidity (TA) in g/L of tartaric acid equivalent by titration with sodium hydroxide 0.1 M to an endpoint of pH 7.0, iv) potassium (Kj) in mg/L by atomic absorption spectrometry (AAS; Unicam SOLAAR 969), v) L-malic acid (MA) and tartaric acid (TcA) by enzimatic (at a wavelength of 340 nm) and colorimetric (at a wavelength of 520 nm) methods respectively, and vi) yeast assimilable nitrogen (YAN) by an enzymatic method (with Analyzer BA400, BioSystems).

In the last year of the research (2019), unlike the previous two, the grape skins from the hundred grapes were manually separated from the flesh and immediately dried at 60 ºC to a constant weight. Potassium concentration in the grape skins (Ksk) was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after microwave digestion of the skins with a mixture of chlorhydric and nitric acids at 180 ºC for 30 min (Multiwave GO Plus; Anton Paar) (Rogiers *et al.*, 2006).

## 5. Statistical analyses

Statistical analyses were performed using the R software version 4.1.3 (R Core Team., 2022). The relationships among the grape harvest parameters were assessed through Pearson's correlation coefficients. The ANOVA was applied to study the effect of each treatment on the grape weight and must quality properties. The identification of potential outliers had been previously tried, but none was found.

Mixed ANOVA was used to determine whether the differences between treatment levels (T) were statistically significant, whether the effects depended on the year of sampling (Y), and to determine the interaction between both factors. In this mixed design, the treatment factor was set as the between-group predictor and the year factor was regarded as a random factor. To reveal the overall effect of each main effect and its interactions, a hierarchical multilevel model approach was used, in which models were built up with one predictor at a time from a baseline with no predictors other than the intercept. Factors in these nested models were added in the following order: no predictors, T, Y and the interaction between T and Y. Maximum likelihood (ML) was used to compare the nested models using a variance analysis (Field *et al.*, 2012; Fox and Weisberg, 2018).

When the interaction between treatment and year was found to be significant, the main effect of T was not directly interpreted, because the higher-order interaction superseded it. In that case, the treatment effect was independently analysed and interpreted for each year. Conversely, when the interaction between factors (T × Y) was not significant, the main effect of T was directly interpreted with the advantage of gaining more statistical power to discover the effects of the fixed factors (Field *et al.*, 2012).

Even if the likelihood ratio of mixed ANOVAs was not large enough to be statistically significant at the 95 % confidence, a priori comparisons were carried out to find out if there were any significant differences in terms of harvest parameters among specific treatments groups. These a priori comparisons were performed through orthogonal contrasts in which the treatment variance was partitioned according to reasonable hypotheses. In the NAA experiment, the first hypothesis to be tested (C1) was that the effects were due to the NAA treatment itself (C vs NAA1 + NAA2 + NAA3), the second one (C2) was that the effects were due to the NAA treatment late in the growing season, specifically at veraison (NAA2 + NAA3 vs NAA1), while the third one (C3) was that the effects of the NAA treatment at veraison were modulated by another NAA treatment applied earlier in the growing season (NAA3 vs NAA2). In the ED and Mg experiments, the first hypothesis to be tested was that the effects were due to the strategy itself, while the second one was that the effects were due to the different levels at which the strategy was applied. Therefore, for the early defoliation treatment, in Contrast 1 (C1) the control group was compared with the joined treatment groups (C vs ED1 + ED2), whereas in Contrast 2 (C2) both treatment groups (ED2 vs ED1) were compared to each other. Similarly, for the post-veraison foliar Mg fertilisation treatment, in C1 the control group was compared with the joint treatment groups (C vs Mg1 + Mg2), while in C2 Mg1 to Mg2 were compared to one another.

Because the Ksk data failed to significantly meet parametric assumptions (i.e., normality and homogeneity of variances), the non-parametric Kruskal-Wallis test was applied to evaluate the differences between the treatment levels.

# RESULTS

## 1. Initial soil characterisation

Table 1 shows the baseline characteristics of the calcareous soil (at 0–30 and 30-60 cm depths) at the beginning of the study. In Table 1 it can be observed that the soil was poor in organic matter (< 1.0 % in both topsoil and subsoil), and clay-dominant throughout its depth (clay > 30 % at both depths).

The soil pH was above 8.0 and the total carbonates were moderately high. Moreover, the topsoil showed low P concentrations according to Jones (2002). Soil carbonates lead to common grape production problems, which include low P bioavailability, as can be seen at both depths, but especially at 0-30 cm. Finally, soil $K^+$ is a factor that can affect the net accumulation of K in berries (Mpelasoka *et al.*, 2003). In this regard, the topsoil $K^+$ can be considered as average (Jones, 2002).

## 2. Relationships among the grape harvest parameters

According to the Pearson's correlation coefficients calculated for the entire research period, TSS and TA were notably inversely related, thus supporting the important basis of this investigation: i.e., in general, what makes one decrease makes the other increase and vice-versa (Figure 2). In turn, TA was much more related to TcA than to MA. It is worth highlighting that TSS, TA, TcA (in particular) and Kj were related to W100. Notwithstanding, TSS was more strongly related to W100 and Kj than was TA. The strong association between YAN and TSS stood out too. In spite of these relationships lasting for the duration of the three-year time span, in 2019 most of them weakened, except for the most obvious of the different acidity parameters (Supplementary Figure 1). Notably, the basic relationship between TSS and TA almost vanished, and that between TSS and W100 was inversed; i.e., they both slightly increased in the last year.

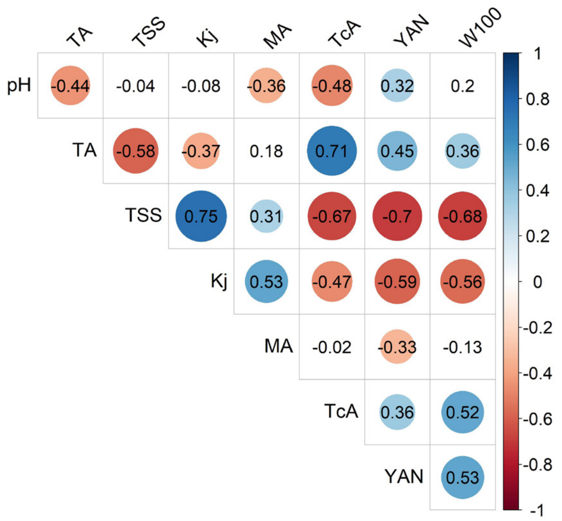

**FIGURE 2.** Pearson' product-moment correlation coefficients of grape must parameters at harvest (weight of 100 berries (W100), real acidity (pH), total acidity (TA), total soluble solids (TSS), potassium in grape juice (Kj), malic acid (MA), tartaric acid (TcA) and yeast assimilable nitrogen (YAN)) for the research period 2017-19 (n = 72). Correlation coeffcients significantly different from zero at the 95 % confidence level are within a coloured circle, whereas non-significant correlation coefficients ($p > 0.05$) are left blank (without circle).

**TABLE 2.** Analyses of variance for the three experiments performed on the harvest parameters. The variability in the harvest parameters was evaluated using the hierarchical multilevel model through maximum likelihood (ML) ratio.

| Experiment | Statistic | W100 | pH | TSS | TA | Kj | MA | TcA | YAN |
|---|---|---|---|---|---|---|---|---|---|
| NAA | ML ratio (T) | 1.21 (0.75) | 2.11 (0.55) | 5.45 (0.14) | 5.31 (0.15) | 3.67 (0.30) | 5.39 (0.15) | 6.82 (0.08) | 5.02 (0.17) |
| | ML ratio (Y) | 12.6 (**) | 9.83 (**) | 15.9 (***) | 5.04 (0.08) | 12.5 (**) | 9.91 (**) | 11.3 (**) | 12.5 (**) |
| | ML ratio (T × Y) | 12.4 (0.05) | 15.8 (*) | 8.76 (0.19) | 11.9 (0.07) | 6.24 (0.40) | 9.28 (0.16) | 22.8 (***) | 9.39 (0.15) |
| ED | ML ratio (T) | 1.02 (0.60) | 7.86 (*) | 3.77 (0.15) | 15.0 (***) | 0.36 (0.84) | 7.30 (*) | 10.6 (**) | 19.3 (***) |
| | ML ratio (Y) | 13.0 (*) | 4.29 (0.12) | 14.4 (***) | 9.12 (*) | 13.9 (**) | 11.4 (**) | 11.7 (**) | 12.8 (**) |
| | ML ratio (T × Y) | 1.60 (0.81) | 6.47 (0.17) | 10.8 (*) | 5.38 (0.25) | 2.24 (0.69) | 3.61 (0.46) | 4.35 (0.36) | 23.5 (***) |
| Mg | ML ratio (T) | 0.34 (0.84) | 12.0 (**) | 0.37 (0.83) | 4.70 (0.09) | 7.53 (*) | 7.43 (*) | 11.7 (**) | 9.33 (**) |
| | ML ratio (Y) | 13.2 (**) | 8.13 (*) | 13.9 (***) | 7.48 (*) | 13.6 (**) | 11.9 (**) | 12.9 (**) | 15.2 (***) |
| | ML ratio (T × Y) | 11.1 (*) | 15.5 (**) | 6.03 (0.20) | 23.3 (***) | 5.75 (0.22) | 6.90 (0.14) | 31.1 (***) | 18.4 (**) |

Experiments: NAA (naphthaleneacetic acid); ED (early defoliation); Mg (post-veraison magnesium foliar fertilization); Statistic: ML ratio for treatment (T) and year (Y) factors, and their interaction (T × Y); Weight of 100 grape berries (W100); total soluble solids (TSS); titratable acidity (TA); potassium content in grape juice (Kj); malic acid (MA); tartaric acid (TcA); yeast assimilable nitrogen (YAN). Results were significant at $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; non-significant values of ML ratio are shown in brackets.

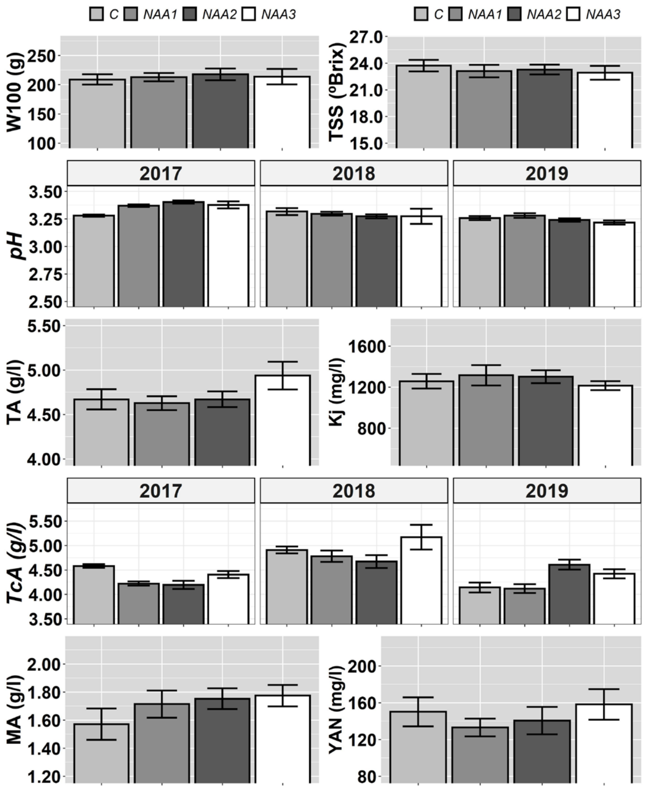

**FIGURE 3.** Mean values of the grape must parameters at harvest (weight of 100 berries (W100), total soluble solids (TSS), pH, total acidity (TA), potassium content in grape juice (Kj), tartaric acid (TcA), malic acid (MA) and yeast assimilable nitrogen (YAN)) for each naphthaleneacetic acid (NAA) treatment and control. When the interaction between treatment and year was significant ($p < 0.05$), single graphs per year are showed. Treatments: single treatment at pea-size (NAA1), double treatment at both pea-size and veraison (NAA2) and single treatment at veraison (NAA3). Error bars reflect the standard error (SE) of the mean (mean ± 1 SE).

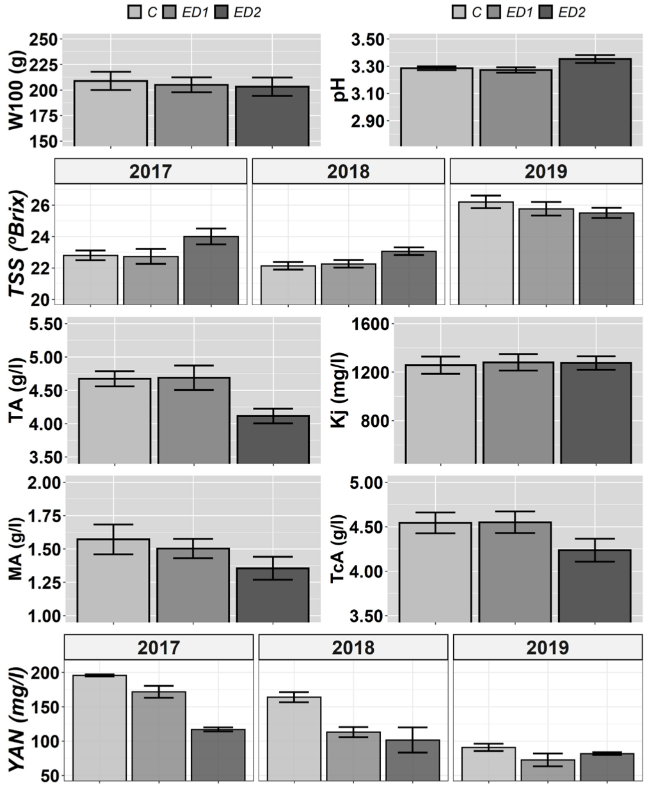

**FIGURE 4.** Mean values of the grape must parameters at harvest (weight of 100 berries (W100), pH, total soluble solids (TSS), total acidity (TA), potassium content in grape juice (Kj), malic acid (MA), tartaric acid (TcA) and yeast assimilable nitrogen (YAN)) for each early defoliation treatment and control. When the interaction between treatment and year was significant ($p < 0.05$), single graphs per year are showed. Treatments: all blades and lateral shoots on each shoot between the first and second grape clusters were removed at pea-size (ED1) and in the same way as in ED1, but between the shoot base until the second grape cluster (ED2). Error bars reflect the standard error (SE) of the mean (mean ± 1 SE).

## 3. Effects of the viticultural strategies on the grape harvest parameters

According to the mixed ANOVA results shown in Table 2, the cultural strategies differed in terms of effects on the grape properties at harvest (weight of 100 berries, pH, TSS, TA, Kj, MA, TcA and YAN). In the NAA experiment, there were differences in terms of TSS and acidity parameters among the treatment levels, although they were non-significant at the 95 % confidence interval. In the early defoliation experiment the difference in TSS among the treatment levels was also slight, but, conversely, more significant in terms of almost all the acidity parameters (pH, TA, MA and TcA), as well as of YAN. Finally, in the post-veraison foliar Mg fertilisation experiment, the difference in TSS among the treatment levels was not significant at all, but it was significant in terms of most of the acidity parameters (pH, Kj, MA, TcA), and once again of YAN. In addition to the pure strategy effects, treatment effects significantly changed in magnitude or direction with the year of sampling, as observed for YAN in the early defoliation experiment, and for pH, TcA and YAN in the post-veraison foliar Mg fertilisation experiment (Table 2).

In Figures 3, 4 and 5, the means and standard errors of the harvest quality parameters of the control and different groups of treatments during the three years of monitoring are shown for the NAA, ED and Mg experiments respectively. The numeric values of both statistical estimates are provided in Supplementary Tables 1, 2 and 3 respectively. It should be noted that in Figures 3, 4 and 5, the joint three-year means and standard errors are shown when there were no significant interactions between T and Y for a given harvest parameter (Table 2). Additionally, the previously described specific hypotheses for the treatment groups in each experiment were tested. It should be noted that for the harvest parameters for which the treatment effects significantly changed in magnitude or direction with the year of sampling, the respective hypotheses were separately tested each year.

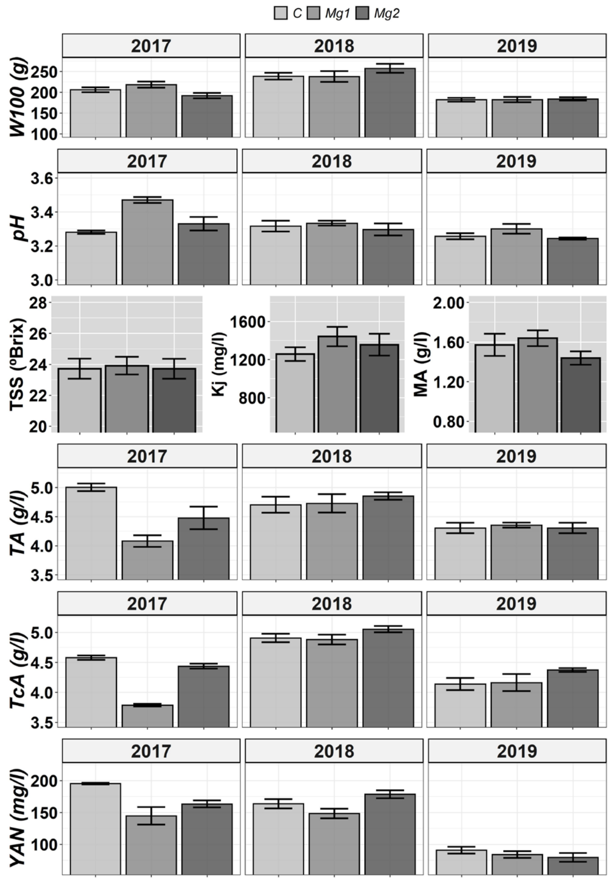

**FIGURE 5.** Mean values of the grape must parameters at harvest (weight of 100 berries (W100), pH, total soluble solids (TSS), potassium content in grape juice (Kj), malic acid (MA), total acidity (TA), tartaric acid (TcA) and yeast assimilable nitrogen (YAN)) for each Mg foliar fertilization fifteen days after veraison treatment and control. When the interaction between treatment and year was significant ($p < 0.05$), single graphs per year are showed. Treatments: 500 g/ha (Mg1) and 1,000 g/ha (Mg2). Error bars reflect the standard error (SE) of the mean (mean ± 1 SE).

**TABLE 3.** Z values of the planned contrasts (C1, C2 and C3) on the harvest parameters in the NAA experiment.

| Harvest parameter | Year | C1: NAA1+NAA2+NAA3 vs C | C2: NAA2+NAA3 vs NAA1 | C3: NAA3 vs NAA2 |
|---|---|---|---|---|
| W100 | All | 0.91 (0.84) | 0.43 (0.99) | -0.48 (0.98) |
| TSS | All | -2.20 (0.11) | -0.04 (1.00) | -1.03 (0.76) |
| TA | All | 0.63 (0.95) | 1.41 (0.55) | 1.84 (0.24) |
| Kj | All | 0.43 (0.99) | -1.16 (0.68) | -1.53 (0.42) |
| MA | All | 2.33 (0.07) | 0.61 (0.96) | 0.24 (1.00) |
| YAN | All | -0.65 (0.94) | 1.64 (0.35) | 1.52 (0.42) |
| pH | 2017 | 5.81 (***) | 1.06 (0.74) | -1.23 (0.63) |
|  | 2018 | -0.94 (0.82) | -0.58 (0.96) | 0.00 (1.00) |
|  | 2019 | -0.65 (0.95) | -2.84 (*) | -1.11 (0.71) |
| TcA | 2017 | -5.29 (***) | 1.27 (0.60) | 2.95 (*) |
|  | 2018 | -0.22 (1.00) | 0.89 (0.85) | 2.74 (*) |
|  | 2019 | 2.64 (*) | 4.10 (***) | -1.67 (0.33) |

Treatments: NAA1, in which a single NAA treatment at pea-size stage was applied; NAA2, in which two NAA treatments, one at pea-size stage and another at veraison were applied and NAA3, in which a single NAA treatment at veraison was applied. Weight of 100 grape berries (W100); total soluble solids (TSS); titratable acidity (TA); potassium content in grape juice (Kj); malic acid (MA); yeast assimilable nitrogen (YAN); tartaric acid (TcA). Results were significant at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. The word 'All' denotes that interaction between treatment and year was non-significant at $p = 0.05$.

**TABLE 4.** Z values of the planned contrasts (C1 and C2) on the harvest parameters in the early defoliation and post-veraison foliar Mg fertilization experiments.

| Harvest parameter | Year | C1: ED1+ED2 vs C | C2: ED2 vs ED1 |
|---|---|---|---|
| W100 | All | -0.97 (0.70) | -0.33 (0.98) |
| TSS | All | 0.65 (0.89) | 1.91 (0.16) |
| TA | All | -2.18 (0.09) | -4.00 (***) |
| pH | All | 1.14 (0.60) | 2.83 (**) |
| Kj | All | 0.59 (0.91) | -0.14 (1.00) |
| MA | All | -2.18 (0.09) | -1.95 (0.15) |
| TcA | All | -1.76 (0.22) | -3.21 (**) |
| YAN | 2017 | -9.65 (***) | -8.96 (***) |
|  | 2018 | -4.66 (***) | -0.82 (0.80) |
|  | 2019 | -2.17 (0.09) | 1.25 (0.51) |

| Harvest parameter | Year | C1: Mg1+Mg2 vs C | C2: Mg2 vs Mg1 |
|---|---|---|---|
| W100 | All | 0.53 (0.93) | -0.24 (0.99) |
| TSS | All | 0.31 (0.99) | -0.53 (0.93) |
| TA | All | -1.90 (0.16) | 1.27 (0.50) |
| Kj | All | 2.64 (*) | -1.37 (0.43) |
| MA | All | -0.56 (0.92) | -2.90 (*) |
| pH | 2017 | 4.65 (***) | -4.70 (***) |
|  | 2018 | -0.06 (1.00) | -1.11 (0.61) |
|  | 2019 | 0.75 (0.84) | -2.47 (*) |
| TcA | 2017 | -13.7 (***) | 16.5 (***) |
|  | 2018 | 0.87 (0.77) | 2.18 (0.09) |
|  | 2019 | 1.25 (0.51) | 1.78 (0.21) |
| YAN | 2017 | -4.80 (***) | 1.87 (0.17) |
|  | 2018 | -0.04 (1.00) | 3.71 (***) |
|  | 2019 | -1.53 (0.33) | -0.66 (0.88) |

Treatments: all blades and lateral shoots on each shoot between the first and second grape clusters were removed at pea-size (ED1) and in the same way as in ED1, but between the shoot base until the second grape cluster (ED2); 500 g/ha (Mg1); 1,000 g/ha (Mg2). Titratable acidity (TA); malic acid (MA); tartaric acid (TcA); yeast assimilable nitrogen (YAN); potassium content in grape juice (Kj). Results were significant at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. The word 'All' denotes that interaction between treatment and year was non-significant at $p = 0.05$.

The hypotheses tested in the NAA experiment revealed that NAA application, whether NAA1, NAA2 or NAA3 (i.e., C1), decreased TSS and slightly increased MA, but with p-values of 0.11 and 0.07 respectively; i.e., too low to attain significance at the 95 % confidence level (Table 3). The NAA effects on grape must TcA were even smaller and changed direction from 2017 to 2019. On the other hand, in 2019, TcA significantly increased when the NAA treatment included an application at veraison (C2) and in 2017 and 2018, TcA also significantly increased when the NAA treatment was applied only at veraison compared to at both the pea-size and veraison stages (C3, Table 3).

In response to the early defoliation strategy for the whole research period, no significant differences were found between the control and both defoliation treatments in terms of almost all TSS and acidity parameters, though TA slightly ($p = 0.09$) decreased (C1, Table 4). Conversely, compared to ED1, ED2 showed a significant decrease in TA and TcA, and a concomitant significant increase in pH (C2, Table 4). Concerning YAN, both defoliation treatments showed a significant decrease compared to the control in 2017 and 2018, whereas in 2019 this significant decrease was absent. Finally, ED2 showed a significant decrease in YAN compared to ED1 in 2017 only (Table 4).

Interestingly, in response to the post-veraison foliar Mg fertilisation strategy for the whole research period, both Mg treatments significantly increased Kj compared to the control (C1, Table 4). However, in terms of MA content, no significant differences were found between both Mg treatments and the control, although Mg2 significantly decreased MA compared to Mg1 (C2). Concerning the other characteristics, in 2017, both the Mg treatments significantly decreased TcA and YAN compared to the control, with a concomitant significant increase in pH. In the same year, Mg2 significantly increased TcA compared to Mg1, and in 2018 it significantly increased YAN (Table 4).

Because grape skin stores the greatest proportion of potassium within the berry (Rogiers *et al.*, 2017), Ksk was determined and evaluated in order to extend our knowledge of grape K maturity dynamics in the last year of the study (2019). Interestingly, according to the correlational analysis of all the harvest parameters in 2019 (Supplementary Figure 1), Kj and Ksk were found to not be correlated at all (r = -0.04). Therefore, Kj and Ksk convey different information and thus it is worth determining and assessing Ksk, in addition to Kj.

The means of both Kj and Ksk, as well as their 95 % confidence intervals, are shown in Table 5. Despite some apparent differences, the Kruskal-Wallis tests revealed that Ksk was not significantly affected by the NAA strategy (Kruskal-Wallis chi-squared = 0.64, p-value = 0.89), neither by early defoliation (Kruskal-Wallis chi-squared = 1.07, p-value = 0.59), nor by even foliar Mg fertilisation (Kruskal-Wallis chi-squared = 3.82, p-value = 0.15).

**TABLE 5.** Mean values of potassium levels in grape juice (Kj) and skins (Ksk) by treatment in year 2019 standard error (SE) of the mean (mean ± 1 SE).

| Treatment | Kj (mg/L) | Ksk (%) |
|---|---|---|
| C | 1500 ± 86.4 | 1.23 ± 0.08 |
| ED1 | 1530 ± 50.7 | 1.04 ± 0.13 |
| ED2 | 1450 ± 46.3 | 1.15 ± 0.16 |
| Mg1 | 1840 ± 90.8 | 1.14 ± 0.14 |
| Mg2 | 1760 ± 150 | 0.99 ± 0.10 |
| NAA1 | 1610 ± 175 | 1.13 ± 0.13 |
| NAA2 | 1490 ± 26.2 | 1.19 ± 0.15 |
| NAA3 | 1360 ± 17.4 | 1.20 ± 0.16 |

Treatments: control (C), all blades and lateral shoots on each shoot between the first and second grape clusters were removed at pea-size (ED1) and in the same way as in ED1, but between the shoot base until the second grape cluster (ED2); foliar fertilization of Mg at 500 g/ha (Mg1) and 1,000 g/ha (Mg2); single NAA treatment at pea-size (NAA1), double NAA treatment at both pea-size and veraison (NAA2) and single NAA treatment at veraison (NAA3).

## DISCUSSION

### 1. Naphtaleneacetic acid application

A number of recent studies have found significant delayed ripening in pre-veraison NAA-treated berries, which could be exploited for decreasing the grape juice TSS/acidity ratio for winemaking. Accordingly, Böttcher *et al.* (2011) found significant delayed ripening in Shiraz berries treated with NAA as revealed by must TSS, and the same authors found a significant increase in juice pH in Riesling berries (Böttcher *et al.*, 2012). Similarly, in pre-veraison NAA-treated vines, Ziliotto *et al.* (2012) and He *et al.* (2020) reported a slower accumulation of TSS in cv. Merlot and a surprising decline in TA in cv. Cabernet Sauvignon respectively.

In the present study, some evidence of NAA effects – albeit small – on must TSS and acidity parameters was found: the former decreased and the latter increased, which is in accordance with the aim of decreasing the TSS/TA ratio for winemaking. These effects were particularly discernible for TSS and MA, which slightly, but consistently, decreased and increased respectively in all the NAA treatments, followed by TA, which also slightly, but consistently, increased, but only in the closest-to-veraison NAA treatment (NAA3). These results are in agreement with Davies *et al.* 2022, who showed that the closer to veraison the NAA treatment is applied, the more effective it is in delaying fruit maturity.

The effect of this auxin strategy on acidity seemed to depend on the date, with the application at veraison increasing TA a little and mostly by increasing MA; meanwhile, the effect of the NAA strategy on TSS seemed not to depend at all on the application date and/or how the hormone was rated under the study conditions. Interestingly, Kj also concomitantly decreased a little, thus the NAA effect on TSS is likely due to potassium, taking into account that this cation controls the solubility of the grape acids. Moreover, W100 also increased slightly as a result of the NAA effect. Although this effect on W100 was non-significant, the mild effect of NAA might have been caused by the faster and perhaps somewhat greater growth of berries under the influence of the hormone (Davies *et al.*, 2022), thus diluting TSS a little, as well as the potassium, and in turn increasing the concentration of the acids, especially MA (Böttcher *et al.*, 2012). Therefore, the effects of NAA may have been small under the present study conditions, but nonetheless grape weight, juice potassium, acidity and TSS consistently changed in response to the hormone. While taking into account the complex network of transcriptional responses regulating the ripening process in grapevine berries (Parada *et al.*, 2017), the very slight effects observed in this study can be attributed to the low rate, low concentration and/or scarce number of NAA applications close to veraison. Furthermore, it can be hypothesised that other similar auxins have stronger impacts. Whatever the case, it was not possible to obtain more conclusive results because of the lack of statistical power in this study due to the low number of replications (n = 3).

### 2. Early defoliation

The primary objectives of early defoliation practices are: i) the mitigation of yield loss resulting from cluster rot diseases (VanderWeide *et al.*, 2021), and ii) the exposure of the fruit to the sun, which influences yield and fruit composition, with both being largely under the control of climate on the microscale; i.e., canopy and fruit zone (Šuklje *et al.*, 2014).

Our research found that pH, TA and TcA were significantly influenced by leaf removal, particularly by the most severe leaf removal, with decreasing grape juice acidity, which is not in accordance with our aim of decreasing the TSS/TA ratio. In contrast to our findings, no evidence of early defoliation effects at the pepper-corn size stage was detected by Šuklje *et al.* (2014) in terms of TA and pH in cv. Sauvignon Blanc, when all leaf and lateral shoots in the bunch zone on the morning side of the canopy had been removed. However, in accordance with the results of our study, VanderWeide *et al.* (2021) showed that early defoliation practices in this canopy area changed the grape juice acidity parameters pH, TA and TcA. In their meta-analysis of the effects of early defoliation, especially at pre-bloom stage, they reported 32 % of the observed overall decrease in TA at harvest time due to early defoliation as being significant (n = 105), and 25 % of the general pH increase as significant (n = 102). Therefore, there seem to be, in general, mild effects of defoliation on must acidity that are perhaps cultivar-dependent, which is what may have led to the inconsistent results. Specifically, Tardaguila *et al.* (2010), when removing the first eight basal leaves at the prebloom and fruit set stages (i.e., fewer leaves removed earlier than in the present study), showed a significant TA increase in cv. Carignan and a significant TA decrease in cv. Graciano, when compared with the non-defoliated control vines.

In terms of TA and pH in the present study, no significant differences among early defoliation and control vines were found, but significant differences were found between the two levels of defoliation severity. These results, alongside those of the aforementioned authors (who found faint effects) indicate that a great number of leaves have to be removed in order to significantly decrease TA and both TcA and MA, and thus increase pH. Interestingly, and in contrast to the NAA strategy, the effect of the early defoliation on acidity did not seem to work through dilution and/or potassium intermediation since no differences in terms of either W100 or Kj among treatment levels were found.

As berry weight is one of the harvest parameters that is most affected by defoliation treatments – the earlier the defoliation the larger the reduction in weight (Bledsoe *et al.*, 1988) – it is surprising that no early defoliation treatment in this research significantly affected W100. A possible explanation for this might be that the effect of defoliation on this parameter is influenced by the stage at which leaves are removed and the fact that carbon and nitrogen requirements can force the vine to further rely on reserves in the roots and wood; i.e., resulting in competition for assimilates between reproductive and vegetative organs (Cataldo *et al.*, 2021). In the light of this, our results can be explained by the fact that the leaves were removed after anthesis, when grape yield is at some risk due to most of the photosynthetically active foliage having been removed (Verdenal *et al.*, 2017). Similarly to W100, our results for the early defoliation treatments did not show any relevant differences in the potassium concentration of the juice between the early defoliation treatments and the control. These findings are somehow surprising given the fact that previous research has shown that Kj increases when produced from berries from very dense canopies (Bledsoe *et al.*, 1988).

In terms of severe early defoliation effects, since dilution and/or potassium do not seem to have been involved in decreasing TA, and both TcA and MA, they may be a result of the increase in ventilation and exposition in the fruiting zone. Such findings have also been reported by Bledsoe *et al.* (1988). In particular, the decreasing effect on MA observed in our research could be explained by temperature-driven enhanced degradation (Tardaguila *et al.*, 2010). However, because Tardaguila *et al.* (2010) observed opposite changes in TcA concentrations for cvs. Graciano and Carignan defoliated vines, it is likely that both genotype and variations in canopy openness play an important role in the plant's physiological response and could explain opposing changes in organic acid dynamics.

Regarding TSS, no significant differences between the treatment levels were found within the whole time span of the early defoliation experiment. Nevertheless, in the first two years, the effect of the most severe defoliation on TSS was significant (results not shown). It should be noted that 2019 was different from the preceding two years, as reflected in the very different relationships among the harvest parameters (Figure 2 and Supplementary Figure 1). The results suggest a mild effect of early defoliation on TSS, which was sometimes greater than the experimental error and sometimes it not. Similarly, in studies on the basal defoliation of cv. Pinot Noir at full bloom (Frioni *et al.*, 2018), and the early defoliation of cvs. Graciano and Carignan (Tardaguila *et al.*, 2010), no significant effects on TSS were found, whereas Šuklje *et al.* (2014) and VanderWeide *et al.* (2021) found that early defoliation promoted a significant increase in fruit TSS.

Interestingly, early defoliation had the effect of decreasing YAN, which was significant in two out of three of the study years. This effect of early defoliation on YAN is in accordance with findings from Verdenal *et al.* (2017): YAN was reduced when defoliation was conducted at both pre-flowering and flowering stages, with a significant decrease in the latter case. Because berry temperature is strongly dependent on direct sunlight pattern and duration, with thermal increases above ambient temperature on exposed berries (Cataldo *et al.*, 2021; Pieri and Fermaud, 2005), the decrease observed in this investigation could be linked to higher bunch temperatures. However, another simpler alternative explanation for our findings is that defoliation leads to the loss of nitrogen in two ways: i) defoliation promotes nitrogen loss in the vine by preventing nitrogen remobilisation from leaves to berries at ripening, and ii) defoliation limits the ability of the vine to absorb nitrogen from the soil, which would otherwise be transported in the xylem sap stream under the water potential gradient from soil and roots to stems and leaves and, subsequently, to the clusters. However, on a note of caution: no consistent effects of leaf removal close to bunches have been found on grape nitrogen content across cultivars and vintages (Verdenal *et al.*, 2021).

### 3. Magnesium foliar fertilisation

When Mg foliar fertilisation was applied, the results of the study showed a significant increase in Kj, a significant decrease in acidity in the first year only, and no significant effects at all on TSS. Therefore, Mg foliar fertilisation had the effect of slightly increasing the TSS/TA ratio, which is contrary to the objective of diminishing TSS and rising TA. A possible explanation for this is that, as a result of its important role in chlorophyll synthesis, the magnesium enhanced nutrient utilisation, ameliorating the nutritional status of the grapevines (Senbayram *et al.*, 2015), including an increase in potassium. Additionally, the observed effect of Mg foliar fertilisation on Kj is likely related to the strong antagonistic effect potassium exerts on Mg transport in source organs while, conversely, Mg exerts either a synergistic or no effect on potassium transport into shoots (Xie *et al.*, 2021) and, perhaps, into other sink tissues like grapes.

Previous studies have noted the importance of correlating quality parameters decisive for horticultural crops like grapevines, such as TSS and acidity, with the $K^+/Mg^{2+}$ ratio, rather than with $Mg^{2+}$ alone (Gerendás & Führs, 2013). Although the Mg concentration in grape tissues was not measured in the present study, it could conceivably be hypothesised that the effects of foliar fertilisation of Mg on grape harvest parameters can be interpreted in relation to the $K^+/Mg^{2+}$ ratio in grape tissues, instead of only to potassium. Moreover, a decrease in Kj was observed, although non-significantly, when the Mg rate was doubled from 500 $g_{Mg}$/ha to 1000 $g_{Mg}$/ha (Mg2 vs. Mg1). Hence, the study of remobilisation of $K^+$ and $Mg^{2+}$ from source organs to sink organs as a function of the $K^+/Mg^{2+}$ ratio in the various vine tissues should be taken into account in future research.

In this study, Mg did not consistently affect the other harvest parameters; i.e., W100, TSS and TA. This agrees with earlier observations made by Zatloukalová *et al.* (2011) on cv.Riesling italico, who found that both TSS and TA were not affected by summer foliar Mg treatments, and with those by Trolove *et al.* (2008) on cv. Chardonnay, who showed that increasing the leaf Mg concentration by applying five foliar sprays, giving a total rate of 2.15 kg Mg/ha, had no effect on TSS, TA or pH at harvest. Therefore, the significant decrease in acidity, due to reduced TcA in 2017 in the Mg experiment, disagrees with the aforementioned studies, and may be a consequence of uncontrolled factors. Very few studies have actually examined the impact of magnesium foliar applications on grape quality parameters; therefore, further research is required in this regard.

Finally, it is worth highlighting that, contrary to expectations, this study did not reveal a significant correlation between Kj and Ksk. The mesocarp (berry skin) houses the greatest proportion of potassium in the berry (Rogiers *et al.*, 2017). However, the Ksk/Kj ratio depends on the grapevine variety (Etchebarne *et al.*, 2009; Iland and Coombe, 1988; Rogiers *et al.*, 2006), and these findings suggest that knowing the Ksk content in addition to Kj ‒ particularly in red grapes, such as cv. Tempranillo ‒ is important, because the skin is left in contact with the must for some time after crushing to enhance anthocyanin extractability during winemaking (Rogiers *et al.*, 2017), thus determining wine acidity. Therefore, further studies focused on both Kj and Ksk are recommended.

## CONCLUSIONS

In this investigation, in the light of current climate and œnological trends, three viticultural strategies were tested with the aim of improving grape must for winemaking. The most promising strategy seems to be the use of 1-naphthaleneacetic acid (NAA), in particular close to veraison, which showed some ability for decreasing must total soluble solids, while increasing total acidity at harvest time. This effect may be a result of NAA stimulating a more rapid growth of the treated berries, with a concomitant dilution of total soluble solids, as well as potassium, in the berry. In this regard, the increase in TA may be due to increasing malic acid during ripening which, in turn, may be driven by the decline in potassium. The most unfavourable strategy seems to be that of early defoliation, which either did not exert any effect when moderately applied or promoted a decrease in total acidity when applied severely, mainly due to the decline in malic acid. With regard to foliar magnesium treatments, the clearest result of this study is that Mg foliar fertilisation had either no or faint effects on grape TSS and acidity, which does not meet the objective of decreasing the former and increasing the latter. The effects of Mg foliar fertilisation on grape harvest parameters should be interpreted in terms of the potassium/magnesium ratio in grape tissues, instead of only in terms of potassium. Moreover, the potassium concentration in the grape juice was not found to be associated with the potassium concentration in the skins; this highlights the importance of knowing the content of potassium in the skin, since it may control wine acidity, which is relevant in this study. In the face of climate warming effects and in accordance with current oenological trends, the application of auxin treatments (like NAA), particularly at veraison, as a tuning method for balancing sugars with acidity in grape musts should be explored in further research. Specifically, different types of auxins, rates, concentrations and/or number of applications should be tested.

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