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Application of Methyl Jasmonate and Methyl Jasmonate + Urea in Tempranillo Vines: Influence on Grape Phenolic Compounds

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Abstract

Background and goals

The aim of this work was to study, for the first time, the influence of foliar application of methyl jasmonate (MeJ) and methyl jasmonate plus urea (MeJ+Ur) on Tempranillo grape phenolic composition over two seasons.

Methods and key findings

This work examined grape phenolic compounds using high-performance liquid chromatography-diode array detection. In 2019, both treatments increased the total anthocyanins, but the MeJ+Ur treatment had a stronger effect than the MeJ treatment. MeJ foliar application decreased total flavonols, total flavanols, and total hydroxycinnamic acids in the grapes, while MeJ+Ur significantly decreased total flavonols, but did not affect total flavanols or hydroxycinnamic acids. Neither of the foliar treatments affected total stilbenes. However, in 2020, the effect of treatments was different: foliar treatments did not affect the anthocyanins, flavonols, hydroxycinnamic acids, or stilbenes, but increased the flavanol content in the grapes.

Conclusions and significance

The effect of foliar treatments was season-dependent, which can be explained by the differences in preharvest rainfall between vintages. The influence of season on grape phenolic compounds was greater than that of the treatments. The results offer information about the response of grapevine to foliar application under different climate conditions.

Key words: foliar application, methyl jasmonate, nitrogen, polyphenol, Tempranillo, urea

Introduction

Phenolic compounds, which include anthocyanins, flavonols, flavanols, stilbenes, hydroxybenzoic and hydroxycinnamic acids, are related to grape and wine quality, since these compounds affect color, mouthfeel, and wine aging potential (Santos-Buelga and Freitas 2009). The content of phenolic compounds in grapes depend on factors such as variety, climate and geography, cultural practices, and the stage of ripeness (Gil et al. 2012, Meng et al. 2012, Hornedo-Ortega et al. 2020). Due to recent increased interest in a healthy lifestyle among consumers, phenolic compounds have gained importance due to their beneficial health properties, especially stilbenes because of their anti-inflammatory, anticarcinogenic, antioxidant, and cardioprotective properties (Bertelli and Das 2009, Guerrero et al. 2009, Alesci et al. 2022). Climate change accelerates the accumulation of sugars in grapes, producing a mismatch between the technological maturity of grapes, which is achieved early, and their phenolic maturity, which is still not achieved when the grapes are ready for harvest (de Orduña 2010, Gutiérrez et al. 2021). This makes it desirable to find methods that increase grape phenolic content or accelerate biosynthesis of phenolic compounds to increase the content of phenolic compounds in grapes at harvest.

Climate-induced early ripening makes it desirable to find treatments that improve the phenolic content in grapes and wines. Foliar application of elicitors as a strategy for reducing the effect of climate change has been studied in recent years (Portu et al. 2015a, 2016, 2018a, Gil 2017, Gil-Muñoz et al. 2017, Paladines-Quezada et al. 2021). Elicitors are molecules that activate plant defense mechanisms (Delaunois et al. 2014) and can be physical or chemical. Among physical elicitors are high and low temperature and ultraviolet or gamma radiation. Chemical elicitors include compounds such as chitosan, benzothiadiazole, or methyl jasmonate (Ruiz-García and Gómez-Plaza 2013). Activating this defense mechanism stimulates accumulation of plant secondary metabolites like phenolic compounds, among others (Gutiérrez et al. 2021).

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Methyl jasmonate (MeJ) is an endogenous plant regulator that is involved in plant defense mechanisms by triggering synthesis of secondary compounds (Gil-Muñoz et al. 2018). In addition, jasmonic acid is involved in a wide range of plant developmental processes: flower and fruit development, vegetative sink, photosynthesis, senescence, and root growth (Beckers and Spoel 2006). MeJ is a plant volatile derived from jasmonic acid (Ruiz-García and Gómez-Plaza 2013) and has been studied widely as a foliar application to improve grape quality. It can affect the content of phenolic compounds; however, the outcome can be influenced by variety, season, or climate conditions (Gil 2017, Gil-Muñoz et al. 2017, 2018, Portu et al. 2018a, Gutiérrez et al. 2021). Phenolic compounds are synthesized from phenylalanine, an amino acid. The deamination of this amino acid, catalyzed by the enzyme phenylalanine ammonia-lyase (PAL), is the first step in this pathway (Ruiz-García and Gómez-Plaza 2013) and is activated by jasmonates (Vaezi et al. 2022). Therefore, MeJ application can increase content of plant polyphenols. Foliar application of MeJ significantly increased grape total anthocyanin content in Tempranillo (Portu et al. 2015a, 2016, 2018a). There was more proanthocyanidin in grapes after foliar application of MeJ, but only in one of the two seasons studied (Gil-Muñoz et al. 2018). For other phenolic compounds, the effect of MeJ application is less certain; there were more stilbenes in grapes after foliar application of MeJ in some studies (Portu et al. 2015a, 2018b), but not in others (Portu et al. 2016). Another study observed a significant effect on all phenolic compounds but the hydroxycinnamic acids (Portu et al. 2018a). Overall, the effect of foliar application of MeJ on flavanol, flavonol, and hydroxycinnamic acid content in grapes was minor (Portu et al. 2015a, 2016).

Foliar fertilization is another practice that may improve grape quality. Applications of nitrogen to vineyard foliage at veraison are an effective method to improve yeast assimilable nitrogen in must and produce changes in must amino acid profiles that do not occur when nitrogen is applied to the soil (Hannam et al. 2015). Foliar application of urea is widespread due to its small molecular size, higher water solubility, and low cost (Lasa et al. 2012, Pérez-Álvarez et al. 2021). Resveratrol and piceid synthesis was favored after urea foliar application, since the application of this nitrogen compound increased stilbene concentrations in musts and wines (Garde-Cerdán et al. 2015). There were more total anthocyanins and total flavonols in Tempranillo grapes after urea foliar application (Portu et al. 2015b). Foliar application of urea on grape phenolic composition during two consecutive vintages increased some flavanols in the second year studied, while no effect was observed in the first (Portu et al. 2017) .

We found it of interest to use MeJ and urea together as a vineyard foliar to improve grape composition. It is not known whether urea and MeJ have a synergistic effect, which could contribute to increased accumulation of secondary metabolites such as phenolic, nitrogen, or volatile compounds, therefore enhancing grape quality. To the best of our knowledge, this paper is the first to study the effect of MeJ plus urea foliar application on grape phenolic composition. As a new method to mitigate the effect of climate change on grapes and enhance grape quality, we studied the influence of foliar application of MeJ and MeJ plus urea on Tempranillo grape phenolic composition over two seasons.

Materials and Methods

Vineyard site, grapevine treatments, and samples

Tempranillo vines (*Vitis vinifera* L.) were grown in an experimental vineyard located in Finca La Grajera, Logroño, La Rioja (Spain) (42°26′; 2°30′; 456 m asl). The soil was classified as *Typic Calcixerept* (Soil Survey Staff 2010). Vines had been planted in 1997, grafted onto R-110 rootstock and trained to a vertical shoot-positioned trellis system. Vine spacing was 2.80 m × 1.25 m. During the two seasons, no nitrogen fertilizer or irrigation were used in the vineyard. Control, MeJ, and MeJ + urea (MeJ+Ur) foliar applications were studied. To carry out the treatments, aqueous solutions were prepared using Tween 80 as wetting agent (1 mL/L), with a concentration of 10 mM of MeJ that was employed for MeJ and MeJ+Ur treatments (according to previous works [Garde-Cerdán et al. 2016, 2018]), and a solution of urea with a total dose of 6 kg N/ha (according to previous work [Pérez-Álvarez et al. 2021]) for MeJ+Ur. Control plants were sprayed with water solution of Tween 80 alone. All treatments were applied to grapevines twice, at veraison and one week later. For each application, 200 mL/plant was sprayed over leaves with a Pulmic Pegasus 15 Advance sprayer (Grupo Sanz). The control and treatments were performed in triplicate and were arranged in a complete randomized block design, with 10 vines for each replication and treatment.

Grapes from all grapevines and treatments were harvested at their optimum technological maturity, when the weight of 100 berries remained constant and the potential alcoholic strength of the grapes reached 13% (v/v). A random set of 150 berries per replicate and treatment was collected and frozen at -20°C until the analyses of grape monomeric phenolic compounds were carried out. Another set of 100 berries was separated and weighed to obtain the average berry weight. Grape berries were crushed and the must was used to determine standard chemical measures.

Standard must analysis

Must enological parameters were analyzed using official methods (OIV 2009): total soluble solids (TSS; Brix), potential alcoholic strength of the grapes, pH, and total acidity. Glucose, glucose plus fructose, malic acid, and total phenols (Folin-Ciocalteu) were determined using Miura One enzymatic equipment (TDI). As the treatments were performed in triplicate, the results of these parameters are shown as the average of three analyses (n = 3).

Analysis of grape phenolic compounds by high-performance liquid chromatography-diode array detection (HPLC-DAD)

Extraction of grape phenolic compounds

Grape phenolic compounds were extracted as described (Portu et al. 2015b). Briefly, ~50 g of each frozen grape sample was weighed and immersed into 50 mL methanol/water/formic acid (50:48.5:1.5, v/v/v). The mixture was then homogenized by Ultra-Turrax T-18 (IKA) at high speed (18,000 rpm) for 1 min. Then, samples were introduced in an ultrasonic bath (ARGO LAB) for 10 min and were centrifuged at 5000 rpm at 10°C for 10 min. The supernatant was separated and the resulting pellet was extracted again using the same volume of the solvent mixture (50 mL). The supernatants were combined and the volume was recorded. Samples were transferred to vials and stored at -20°C until analysis.

Sample preparation for analysis of non-anthocyanin phenolic compounds

PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent) were used as described (Portu et al. 2015b). Cartridges were placed in the extraction system (Visiprep Vacuum Manifold, Sigma-Aldrich). First, 3 mL grape phenolic extract was diluted with 9 mL 0.1 N HCl. The PCX SPE cartridges were conditioned using 5 mL methanol and 5 mL water. Then, the diluted samples were passed through the PCX SPE cartridges, followed by washing with 5 mL 0.1 N HCl and 5 mL water. The non-anthocyanin phenolic compounds fraction was eluted with two washes of 3 mL ethanol. The non-anthocyanin phenolic compounds fraction was dried in a centrifugal evaporator (miVac, Genevac Ltd.) at 35°C and re-dissolved in 1.5 mL 20% (v/v) methanol aqueous solution. The anthocyanin-free fraction was used to analyze non-anthocyanin phenolic compounds (flavonols, hydroxycinnamic and hydroxybenzoic acids, stilbenes, and flavanols).

Analysis of phenolic compounds by HPLC-DAD

Phenolic compounds were analyzed as described (Portu et al. 2015b) using an Agilent 1260 Infinity II chromatograph equipped with DAD. Samples were filtered and injected on a Licrospher 100 RP-18 reversed-phase column (250 × 4.0 mm; 5 µm packing; Agilent) with a Licrospher 100 RP-18 precolumn (4.0 × 4.0 mm; 5 µm packing; Agilent), both at 40°C. A flow rate of 0.630 mL/min was established. For the analysis of anthocyanins, 10 µL grape extract was injected. Eluents used were (A) acetonitrile/water/formic acid (3:88.5:8.5, v/v/v), and (B) acetonitrile/water/formic acid (50:41.5:8.5, v/v/v). For the analysis of non-anthocyanin phenolic compound fractions, the injection volume was 20 µL. Eluents were (A) acetonitrile/water/formic acid (3:88.5:8.5, v/v/v), (B) acetonitrile/water/formic acid (50:41.5:8.5, v/v/v), and (C) methanol/water/formic acid (90:1.5:8.5, v/v/v).

Phenolic compounds were identified according to the retention times of available pure compounds and the UV-vis data obtained from authentic standards and/or published in previous studies (Castillo-Muñoz et al. 2009). For quantification, DAD chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols), 320 nm (hydroxycinnamic acids and stilbenes), and 280 nm (gallic acid and flavanols), and the calibration graphs of the respective standards (R^2 > 0.99) were used. When a standard was not available, quantification was made according to the calibration graph of the most similar compound. Hence, malvidin-3-O-glucoside was used for anthocyanins, quercetin-3-O-glucoside was used for flavonols, *trans*-caftaric acid was used for free hydroxycinnamic acids and the corresponding tartaric esters, catechin was used for procyanidin B1, epicatechin was used for epigallocatechin, and *trans*-piceid and *trans*-resveratrol were used for their respective *cis* isomers. Concentrations were expressed as mg/kg fresh weight. Since field treatments were performed in triplicate, the results for phenolic compounds are the average of the analyses of three samples (n = 3).

Statistical analysis

The statistical analysis of the data was performed using SPSS Version 21.0 statistical package for Windows (SPSS, Inc.). General parameters and phenolic compound data were processed using analysis of variance (ANOVA) ($p \leq 0.05$). The differences between means were compared using the Duncan test and the effects of foliar treatment, seasons, and their interaction were analyzed using a multifactor analysis and post-hoc Duncan´s multiple range test. Discriminant analyses were carried out on individual phenolic compound data to classify those variables which discriminated among samples according to the treatment or season.

Results and Discussion

Effect of MeJ and MeJ+Ur foliar applications on must chemistry

In 2019, musts from grapevines sprayed with MeJ and MeJ+Ur had less glucose and fructose content than control grapes (Table 1). In addition, MeJ musts showed a lower TSS and potential alcoholic strength than the control, which could be interesting to achieve the aim of mitigating the unbalancing effect of climate change (Gutiérrez et al. 2021). This effect of foliar MeJ treatment was observed previously, when the elicitor delayed grape maturation when rainfall was low (Paladines-Quezada et al. 2019). Grape skin cell wall is complex and dynamic, composed of polysaccharides, phenolic compounds, and proteins. Skin cell wall was reinforced after foliar MeJ treatment in grapes (Apolinar-Valiente et al. 2018), which can be related to increased phenols, proteins, and lignin observed in Monastrell grapes (Paladines-Quezada et al. 2022), and with delayed grape maturation. There were significantly more total phenols in musts from treated grapevines over the control, without differences between the treatments (Table 1). These results are in agreement with previous findings on Tempranillo, where a significant influence of MeJ on grape phenolic composition was described, but foliar application of MeJ did not produce significant differences in other grape chemistry measures (Portu et al. 2015a, 2018a). Other studies confirm little or no

effect of MeJ treatment on total acidity, pH, or malic acid content of grapes (Paladines-Quezada et al. 2021, Gutiérrez-Gamboa et al. 2019). There were no differences in general must parameters in 2020 (Table 1), in agreement with previous studies (Portu et al. 2015a, 2018a). Only the content of malic acid in MeJ musts was significantly greater than the control. Foliar application of MeJ and MeJ+Ur did not improve general grape chemistry. However, this effect was season-dependent, since MeJ foliar treatment produced an interesting reduction in TSS and increase in total phenols in 2019, but not in 2020.

Influence of foliar MeJ and MeJ+Ur treatments on grape phenolic compounds

Influence of foliar MeJ and MeJ+Ur treatments on grape anthocyanins

In 2019, both treatments produced grapes with significantly more anthocyanins than control grapes (Table 2). MeJ grapes had more delphinidin-3-glc, cyanidin-3-glc, peonidin-3-glc, peonidin-3-acglc, cyanidin-3-cmglc, petunidin-3-cmglc, peonidin-3-cmglc, and total non-acylated, total acylated, and total anthocyanins than control grapes. MeJ+Ur grapes had more of all individual non-acylated anthocyanins, peonidin-3-acglc, delphinidin-3-cmglc, cyanidin-3-cmglc, petunidin-3-cmglc, peonidin-3-cmglc, malvidin-3-*trans*-cmglc, and total non-acylated, total acylated, and total anthocyanins than control grapes (Table 2). Moreover, foliar application of MeJ+Ur produced grapes with more delphinidin-3-glc, peonidin-3-glc, total non-acylated, delphinidin-3-acglc, petunidin-3-acglc, delphinidin-3-cmglc, petunidin-3-cmglc, peonidin-3-cmglc, total acylated, and total anthocyanins than grapes from vines sprayed with MeJ. The greatest total anthocyanin concentration was found in MeJ+Ur grapes (33% more than control grapes), followed by MeJ grapes (16% more than the control) (Table 2).

Therefore, the effect of MeJ+Ur treatment on anthocyanin grape compounds was significantly higher than the effect of MeJ treatment on grape content in the 2019 season. This novel result clarifies the response of grapevine to foliar application of MeJ+Ur, since as far as we know, it is the first time that foliar applications of MeJ and urea have been studied together. There appears to be an interaction among urea and MeJ, since application of the combination is more effective in increasing some anthocyanins than application of MeJ alone, which could be explained by a synergetic effect among MeJ and urea on the synthesis of anthocyanins. The individual effect of MeJ and urea foliar applications on anthocyanins has been previously described. Foliar urea application induced anthocyanin synthesis (Portu et al. 2015b), making it a potential tool to improve grape quality. MeJ induces the activation of enzymes involved in the biosynthesis of polyphenol compounds (Ruiz-García and Gómez-Plaza 2013, Delaunois et al. 2014). One of these enzymes is PAL, of which activity is required for accumulation of phenolic compounds (Portu et al. 2016). Anthocyanin content in grapes increased after foliar MeJ application in the vineyard (Portu et al. 2015a, 2016, 2018a). It is well known that anthocyanins are responsible for the red color in grapes and wines, and increasing anthocyanin content using foliar application of MeJ and MeJ+Ur would be a good strategy to reduce the effects of climate change on grape quality and to obtain a more balanced chemical composition in the grapes at ripening.

In 2020, foliar treatments produced a different effect on anthocyanin content (Table 2). MeJ treatment increased cyanidin-3-glc and peonidin-3-cmglc, while MeJ+Ur grapes had more cyanidin-3-glc and peonidin-3-acglc than control grapes. MeJ grapes had more peonidin-3-cmglc, malvidin-3-*trans*-cmglc, and total acylated anthocyanins than MeJ+Ur grapes. MeJ+Ur grapes had fewer total acylated anthocyanins (10% less than the control), mainly due to a decrease in malvidin-3-*trans*-cmglc. These results contrast with our

Table 1 Standard chemical measures of musts from control, methyl jasmonate (MeJ), and MeJ + Urea (MeJ+Ur) treatments in 2019 and 2020. TSS, total soluble solids.

	2019			2020		
	Control	**MeJ**	**MeJ+Ur**	**Control**	**MeJ**	**MeJ+Ur**
Weight of 100 berries (g)	113.68 ± 11.07 a[a]	141.81 ± 27.18 a	131.52 ± 25.19 a	199.57 ± 7.27 a	207.67 ± 40.39 a	222.83 ± 25.25 a
TSS (Brix)	24.70 ± 0.72 b	22.23 ± 1.17 a	23.03 ± 0.60 ab	22.30 ± 0.92 a	22.17 ± 2.31 a	22.77 ± 0.74 a
Potential alcoholic strength (% v/v)	14.63 ± 0.49 b	12.92 ± 0.80 a	13.48 ± 0.42 ab	12.97 ± 0.63 a	12.89 ± 1.58 a	13.29 ± 0.51 a
pH	3.83 ± 0.05 a	3.78 ± 0.10 a	3.80 ± 0.04 a	3.76 ± 0.01 a	3.70 ± 0.07 a	3.71 ± 0.03 a
Total acidity (g/L)[b]	4.61 ± 0.11 a	5.20 ± 0.36 a	5.11 ± 0.36 a	4.12 ± 0.33 a	4.54 ± 1.08 a	3.83 ± 0.13 a
Glu+Fru (g/L)[c]	249.86 ± 9.97 b	215.50 ± 12.29 a	226.67 ± 5.67 a	216.42 ± 10.70 a	218.62 ± 26.56 a	228.85 ± 9.85 a
Glu (g/L)	120.18 ± 5.13 b	102.88 ± 6.89 a	107.43 ± 3.65 a	107.31 ± 4.54 a	106.08 ± 12.84 a	113.11 ± 6.85 a
Fru (g/L)	129.68 ± 4.84 b	112.62 ± 5.43 a	119.25 ± 2.52 a	109.11 ± 6.53 a	112.54 ± 13.76 a	115.75 ± 3.49 a
Malic acid (g/L)	2.24 ± 0.24 a	2.54 ± 0.32 a	2.45 ± 0.46 a	1.21 ± 0.08 a	1.54 ± 0.22 b	1.42 ± 0.05 ab
Total phenols (mg/L)	1185.33 ± 72.31 a	1306.57 ± 61.35 b	1351.83 ± 29.05 b	541.60 ± 64.02 a	603.07 ± 73.82 a	578.17 ± 82.64 a

[a]All parameters are listed with their standard deviation (n = 3). For each season and parameter, different letters indicate significant differences between the samples ($p \leq 0.05$).
[b]As g/L tartaric acid.
[c]Glu, glucose; Fru, fructose.

findings in 2019, when both treatments increased the content of several individual anthocyanins and the total non-acylated, acylated, and total anthocyanin content and MeJ+Ur treatment had a greater effect on anthocyanins than MeJ alone. These differences between years in the effects of foliar treatments could be explained by external factors such as plant nutrient status and climate conditions, that could affect the response of grapevines to the foliar treatment (Portu et al. 2017). However, analysis of vine status was not carried out in this study.

Our study is not the only one reporting that response to foliar application of MeJ depends on seasonal conditions (Paladines-Quezada et al. 2019, González-Lázaro et al. 2022). In August 2020, our vineyard received more rainfall than in August 2019 (11.5 L/m^2 in 2019 versus 32.9 L/m^2 in 2020) and these differences may explain the different effect of treatments among seasons. In addition, environmental conditions can affect the accumulation and composition of skin anthocyanins and dilute polyphenols through greater absorption of water by the grapevines (Paladines-Quezada et al. 2021). This effect can be observed in the weight of 100 berries: due to more preharvest rain in 2020, the weight of 100 berries was greater than in 2019 (Table 1). In addition, water deficit could promote grape quality, since reduced berry size increases skin phenolics (van Leeuwen and Destrac-Irvine 2017). Vine water status was not measured in this study, but the differences in preharvest rain between seasons could explain the observed differences in phenolic content and in the effect of foliar treatments. Overall, foliar treatments did not significantly affect total anthocyanin content in 2020.

Non-acylated forms were the major contributor to total anthocyanins in grapes (Table 2). In both seasons, malvidin-3-glc was the primary anthocyanin (representing ~33% of total anthocyanins in 2019 and ~38% of total anthocyanins in 2020).

Influence of foliar MeJ and MeJ+Ur treatments on grape flavonols

In 2019, MeJ and MeJ+Ur grapes had less quercetin-3-glcU, kaempferol-3-gal, and total flavonols than control grapes (Table 3). Total flavonol content decreased 11% due to MeJ treatment and 14% due to MeJ+Ur treatment. This decrease in total flavonols can be explained by the reduction in quercetin-3-glcU and kaempferol-3-gal in treated grapes. However, MeJ grapes had more isorhamnetin-3-glc than MeJ+Ur grapes, with intermediate values in control grapes (Table 3). An unclear effect of MeJ foliar application on flavonols was described previously (Portu et al. 2018a). This study concluded that the effect of MeJ foliar application changed with variety and season, although there was no decrease in total flavonols.

Flavonols are important compounds for wine quality since they act as copigments and, therefore, contribute to wine color stabilization (Boulton 2001). They also contribute to wine astringency (Gonzalo-Diago et al. 2014), although the predominant compounds implicated in astringency sensation are flavanols. In 2020, MeJ grapes had more laricitrin-3-glc and kaempferol-3-glcU+3-glc than control grapes, while

Table 2 Anthocyanin content (mg/kg fresh weight) in grapes from control, methyl jasmonate (MeJ), and MeJ + Urea (MeJ+Ur) treatments in 2019 and 2020.

	2019			2020		
	Control	**MeJ**	**MeJ+Ur**	**Control**	**MeJ**	**MeJ+Ur**
Delphinidin-3-glc[a]	123.77 ± 12.34 a[b]	148.36 ± 10.88 b	171.99 ± 7.88 c	50.58 ± 1.69 a	57.09 ± 7.80 a	56.08 ± 10.13 a
Cyanidin-3-glc	25.83 ± 3.66 a	44.14 ± 3.96 b	45.65 ± 2.93 b	8.44 ± 0.34 a	10.95 ± 1.16 b	10.80 ± 1.30 b
Petunidin-3-glc	85.84 ± 6.28 a	97.26 ± 16.47 ab	117.78 ± 6.68 b	47.93 ± 1.59 a	53.03 ± 5.03 a	52.61 ± 8.93 a
Peonidin-3-glc	45.73 ± 3.95 a	57.46 ± 3.93 b	76.50 ± 6.57 c	19.77 ± 0.91 a	24.72 ± 1.14 a	24.61 ± 3.94 a
Malvidin-3-glc	215.76 ± 8.13 a	240.95 ± 29.77 ab	267.93 ± 19.97 b	169.84 ± 0.91 a	178.61 ± 13.92 a	167.32 ± 23.70 a
Total non-acylated	**496.94 ± 32.57 a**	**588.20 ± 58.37 b**	**679.85 ± 41.73 c**	**296.57 ± 7.58 a**	**324.41 ± 16.54 a**	**311.43 ± 47.42 a**
Delphinidin-3-acglc	10.42 ± 0.75 ab	9.93 ± 0.57 a	11.45 ± 0.48 b	6.66 ± 0.12 a	6.93 ± 0.66 a	6.94 ± 0.38 a
Cyanidin-3-acglc	3.84 ± 0.02 a	3.84 ± 0.01 a	3.79 ± 0.10 a	3.60 ± 0.02 a	3.62 ± 0.07 a	3.58 ± 0.01 a
Petunidin-3-acglc	6.86 ± 0.27 ab	6.79 ± 0.20 a	7.40 ± 0.33 b	5.57 ± 0.11 a	5.69 ± 0.39 a	5.57 ± 0.24 a
Peonidin-3-acglc	4.48 ± 0.08 a	4.97 ± 0.26 b	5.09 ± 0.05 b	3.85 ± 0.04 a	4.02 ± 0.17 ab	4.08 ± 0.06 b
Malvidin-3-acglc	11.71 ± 0.26 a	12.10 ± 0.15 a	12.45 ± 0.95 a	10.53 ± 0.42 a	10.37 ± 0.89 a	9.82 ± 0.58 a
Delphinidin-3-cmglc	16.28 ± 0.68 a	18.09 ± 1.21 a	22.30 ± 1.00 b	14.31 ± 0.38 a	14.62 ± 1.78 a	13.83 ± 1.16 a
Cyanidin-3-cmglc	6.21 ± 0.28 a	7.87 ± 0.68 b	8.59 ± 0.24 b	5.38 ± 0.17 a	5.79 ± 0.42 a	5.88 ± 0.30 a
Petunidin-3-cmglc	12.97 ± 0.26 a	14.34 ± 0.55 b	16.73 ± 0.81 c	12.47 ± 0.25 a	12.57 ± 0.99 a	11.37 ± 0.84 a
Peonidin-3-cmglc	8.27 ± 0.06 a	10.45 ± 0.58 b	11.36 ± 0.52 c	7.42 ± 0.07 a	8.08 ± 0.26 b	7.33 ± 0.34 a
Malvidin-3-*cis*-cmglc	4.55 ± 0.08 a	4.44 ± 0.14 a	4.64 ± 0.13 a	4.66 ± 0.21 a	4.53 ± 0.36 a	4.32 ± 0.16 a
Malvidin-3-*trans*-cmglc	36.74 ± 2.11 a	40.27 ± 2.57 ab	44.04 ± 2.89 b	51.03 ± 0.75 b	48.42 ± 4.48 b	39.29 ± 2.08 a
Malvidin-3-cfglc	4.21 ± 0.02 a	4.55 ± 0.30 a	4.24 ± 0.13 a	10.90 ± 1.42 a	9.95 ± 1.19 a	10.80 ± 1.06 a
Total acylated	**126.54 ± 1.10 a**	**137.63 ± 2.22 b**	**152.08 ± 6.68 c**	**136.37 ± 1.96 b**	**134.58 ± 5.10 b**	**122.53 ± 6.99 a**
Total anthocyanins	***623.48 ± 32.23 a***	***725.83 ± 58.85 b***	***831.93 ± 45.64 c***	***432.94 ± 9.42 a***	***458.99 ± 21.21 a***	***433.95 ± 53.95 a***

[a]Glc, glucoside; acglc, acetylglucoside; cmglc, *trans-p*-coumaroylglucoside; cfglc, caffeoylglucoside.
[b]All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

MeJ+Ur had more quecetin-3-glcU, kaempferol-3-glcU+3-glc, and isorhamnetin-3-glc than control grapes (Table 3). The only difference among MeJ and MeJ+Ur grapes was a higher content of laricitrin-3-glc in MeJ grapes than in MeJ+Ur grapes. No significant differences were found in total flavonols between control and treated grapes. These results agree with previous findings of a variable effect on flavonol content after MeJ foliar application, depending on variety, season, and time of application. Differences between the effect of MeJ foliar treatment among seasons can be explained by the meteorological dependence of this elicitor described previously (Paladines-Quezada et al. 2019). So, the differences in preharvest rainfalls between 2019 and 2020 could explain the different effects of MeJ and MeJ+Ur treatments. Overall, the foliar treatments did not enhance the content of flavonols in our study. The most abundant flavonol was myricetin-3-glc in both seasons, as reported previously in Tempranillo (Portu et al. 2017).

Influence of foliar MeJ and MeJ+Ur treatments on grape low molecular weight flavanols

In 2019, MeJ and MeJ+Ur grapes had less catechin, epicatechin-3-gallate, and epigallocatechin than control grapes (Table 3). Indeed, MeJ grapes had less total flavanol (18%) than control grapes. MeJ+Ur grapes had more procyanidin B1 than MeJ grapes. In 2019, catechin was the predominant low molecular weight flavanol, followed by epicatechin. Catechin and epicatechin accounted for ~67% of total flavanols in control, MeJ, and MeJ+Ur grapes. This effect of MeJ foliar treatment contrasts with previous reports (Portu et al. 2015a, 2016, 2018a), where flavanol content was not affected by MeJ foliar application.

In 2020, foliar treatments did not have a significant effect over individual low molecular weight flavanols; only the MeJ+Ur treatment produced grapes with significantly more epicatechin than control and MeJ grapes (Table 3). However, although there were no differences in individual low molecular weight flavonols, the MeJ and MeJ+Ur foliar treat-

Table 3 Content of flavonols, low molecular weight flavanols, phenolic acids, and stilbenes (mg/kg fresh weight) in grapes from control, methyl jasmonate (MeJ), and MeJ + Urea (MeJ+Ur) treatments in 2019 and 2020.

	2019			2020		
	Control	**MeJ**	**MeJ+Ur**	**Control**	**MeJ**	**MeJ+Ur**
Flavonols						
Myricetin-3-glcU[a]	27.15 ± 2.47 a[b]	23.86 ± 3.76 a	23.38 ± 3.03 a	16.26 ± 0.70 a	16.07 ± 0.98 a	15.16 ± 2.70 a
Myricetin-3-gal	35.08 ± 3.48 a	34.24 ± 2.26 a	37.78 ± 3.32 a	22.16 ± 1.58 a	23.29 ± 2.31 a	21.46 ± 4.08 a
Myricetin-3-glc	181.66 ± 15.36 a	179.26 ± 19.81 a	183.44 ± 4.32 a	82.27 ± 4.81 a	83.68 ± 8.37 a	80.40 ± 15.70 a
Quercetin-3-glcU	164.18 ± 15.66 b	122.47 ± 19.81 a	110.84 ± 6.94 a	24.60 ± 1.67 a	30.61 ± 2.69 ab	32.50 ± 15.70 b
Quercetin-3-glc	172.29 ± 14.90 a	157.59 ± 4.20 a	144.07 ± 27.94 a	32.82 ± 0.70 a	36.32 ± 6.50 a	37.40 ± 4.05 a
Laricitrin-3-glc	33.29 ± 3.44 a	30.37 ± 3.59 a	30.76 ± 1.28 a	30.31 ± 1.31 a	37.37 ± 4.05 b	29.19 ± 3.62 a
Kaempferol-3-gal	2.48 ± 0.23 b	1.89 ± 0.03 a	1.76 ± 0.20 a	0.46 ± 0.04 a	0.52 ± 0.06 a	0.48 ± 0.06 a
Kaempferol-3-glcU+3-glc	15.99 ± 1.83 a	14.55 ± 1.72 a	14.71 ± 1.87 a	2.17 ± 0.35 a	3.23 ± 0.28 b	3.66 ± 0.21 b
Isorhamnetin-3-glc	12.17 ± 1.18 ab	12.88 ± 0.33 b	10.44 ± 1.15 a	3.54 ± 0.21 a	3.86 ± 0.54 ab	4.96 ± 0.81 b
Syringetin-3-glc	21.88 ± 1.52 a	21.59 ± 2.39 a	21.14 ± 0.82 a	12.03 ± 0.94 a	14.02 ± 1.07 a	15.04 ± 2.24 a
Total flavonols	666.15 ± 33.09 b	598.69 ± 31.38 a	578.33 ± 24.51 a	226.61 ± 5.16 a	248.96 ± 13.50 a	240.25 ± 37.79 a
Low molecular weight flavanols						
Catechin	63.31 ± 3.37 b	48.32 ± 5.31 a	52.13 ± 6.94 a	11.06 ± 0.31 a	13.25 ± 3.05 a	13.85 ± 1.45 a
Epicatechin	39.10 ± 3.85 a	35.22 ± 1.24 a	34.75 ± 5.90 a	11.09 ± 0.43 a	14.28 ± 1.66 a	19.76 ± 2.47 b
Epicatechin-3-gallate	14.12 ± 2.12 b	10.90 ± 1.23 a	10.34 ± 0.98 a	8.24 ± 0.76 a	9.77 ± 2.04 a	8.21 ± 0.85 a
Epigallocatechin	4.42 ± 0.32 b	2.93 ± 0.49 a	2.64 ± 0.23 a	8.25 ± 0.91 a	9.30 ± 1.37 a	9.53 ± 1.01 a
Procyanidin B1	28.28 ± 3.57 ab	25.17 ± 3.86 a	31.99 ± 1.43 b	10.26 ± 1.10 a	10.61 ± 0.24 a	10.82 ± 0.78 a
Total flavanols	149.24 ± 9.65 b	122.53 ± 11.31 a	131.84 ± 13.63 ab	48.90 ± 1.71 a	57.20 ± 5.14 b	62.17 ± 2.77 b
Hydroxybenzoic acid						
Gallic acid	6.00 ± 0.80 a	5.18 ± 0.35 a	5.83 ± 0.71 a	5.20 ± 0.57 a	6.49 ± 0.80 b	6.12 ± 0.23 ab
Hydroxycinnamic acids (HCAs)						
trans-Caftaric acid	6.54 ± 0.09 a	5.81 ± 1.21 a	7.17 ± 0.65 a	1.51 ± 0.07 a	1.58 ± 0.19 a	1.76 ± 0.23 a
trans+*cis*-Coutaric acids	4.62 ± 0.40 c	1.87 ± 0.23 a	3.30 ± 0.47 b	0.17 ± 0.03 a	0.29 ± 0.00 b	0.94 ± 0.06 c
trans-Fertaric acid	1.78 ± 0.19 b	0.81 ± 0.07 a	1.62 ± 0.30 b	1.34 ± 0.23 b	1.57 ± 0.16 b	0.98 ± 0.10 a
Caffeic acid	0.43 ± 0.05 b	0.31 ± 0.04 a	0.25 ± 0.02 a	0.26 ± 0.03 a	0.27 ± 0.03 a	0.31 ± 0.03 a
p-Coumaric acid	0.36 ± 0.09 a	0.36 ± 0.00 a	0.27 ± 0.05 a	0.14 ± 0.01 a	0.19 ± 0.03 b	0.14 ± 0.02 a
Ferulic acid	2.27 ± 0.15 a	1.85 ± 0.34 a	1.88 ± 0.10 a	10.56 ± 1.65 ab	12.14 ± 0.92 b	9.58 ± 0.13 a
Total HCAs	15.99 ± 0.86 b	11.00 ± 1.08 a	14.49 ± 1.46 b	13.98 ± 1.36 ab	16.05 ± 1.12 b	13.71 ± 0.42 a
Stilbenes						
trans-Piceid	12.75 ± 1.06 a	12.43 ± 1.48 a	12.76 ± 0.33 a	5.37 ± 0.38 a	5.56 ± 0.59 a	5.70 ± 1.02 a
cis-Piceid	1.70 ± 0.24 a	1.60 ± 0.27 a	1.64 ± 0.04 a	1.13 ± 0.09 a	1.26 ± 0.19 a	2.32 ± 0.21 b
trans-Resveratrol	0.63 ± 0.05 b	0.58 ± 0.10 b	0.34 ± 0.04 a	0.11 ± 0.02 b	0.12 ± 0.02 b	0.04 ± 0.01 a
cis-Resveratrol	0.35 ± 0.03 a	0.40 ± 0.06 a	0.35 ± 0.03 a	0.20 ± 0.02 a	0.27 ± 0.05 a	0.39 ± 0.04 b
Total stilbenes	15.43 ± 1.30 a	15.01 ± 1.70 a	15.09 ± 0.28 a	6.82 ± 0.46 a	7.21 ± 0.79 a	8.45 ± 1.26 a

[a]GlcU, glucuronide; gal, galactoside; glc, glucoside.
[b]All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

ments produced more total flavanols than control grapes in 2020. Total flavanols increased 16% with MeJ and 27% with MeJ+Ur over the control. In this season, the content of catechin, epicatechin, and procyanidin B1 was very similar in all samples, with the exception of epicatechin in MeJ+Ur grapes. Catechin, epicatechin, and procyanidin B1 accounted for ~68% of total flavanols in all samples. These results agree partially with those that MeJ treatment did not have any effect on grape flavanols when compared to control grapes, as observed with the individual compounds (Portu et al. 2015a, 2016, 2018a), although it contrasts our increased total flavonols after foliar MeJ application. Flavanols are a family of phenolic compounds linked to bitter taste and astringency of grapes and can contribute to the stability of aged wines, so they are interesting compounds for wine quality (Santos-Buelga and Freitas 2009). Unfortunately, the effect of MeJ and MeJ+Ur foliar applications in our study is not clear. Once again, these differences among seasons can be explained by the climatic differences (Paladines-Quezada et al. 2019).

Influence of foliar MeJ and MeJ+Ur treatments on grape non-flavonoid phenolic compounds

Hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes are non-flavonoid phenolic compounds. They are colorless compounds, but can enhance and stabilize the color of red wines (Rentzsch et al. 2009). Gallic acid was the only hydroxybenzoic acid found in the samples (Table 3). In 2019, treatments did not affect gallic acid content in grapes. Previous studies found the same lack of effect of foliar MeJ on gallic acid (Portu et al. 2015a, 2018a). Thus, the foliar application of MeJ and MeJ+Ur did not improve the biosynthesis of gallic acid in grapevines. Nevertheless, in 2020, grapes from vines treated with MeJ had significantly more gallic acid (25%) than controls, with intermediate values in grapes from vines sprayed with MeJ+Ur (Table 3), in contrast to previous findings (Portu et al. 2016, 2018a).

The content of hydroxycinnamic acids in treated grapes was quite different among seasons (Table 3). In 2019, MeJ alone produced a widespread decline in hydroxycinnamic acids, except for *trans*-caftaric, *p*-coumaric, and ferulic acids. Thus, the total content of hydroxycinnamic acids in grapes sprayed with MeJ was 31% less than the control. MeJ+Ur grapes showed a decrease in *trans*+*cis*-coutaric and caffeic acid, while there were no significant differences in *trans*-caftaric acid, *trans*-fertaric acid, *p*-coumaric acid, ferulic acid, or total hydroxycinnamic acids. MeJ+Ur grapes had a higher content of *trans*+*cis*-coutaric acids, *trans*-fertaric acid, and total hydroxycinnamic acids than MeJ grapes (Table 3). These results contrast with previous reports (Portu et al. 2015a, 2016, 2018a).

In 2020, MeJ grapes had more *trans*+*cis*-coutaric acids and *p*-coumaric acid than control grapes, while MeJ+Ur grapes had more *trans*+*cis*-coutaric acids and less *trans*-fertaric acid than control grapes (Table 3). Control and treated grapes were not different in *trans*-caftaric and caffeic acids. MeJ grapes had more *trans*-fertaric, *p*-coumaric, and ferulic acids and less *trans*+*cis*-coutaric acids than MeJ+Ur grapes. Control and treated grapes were not statistically different in total hydroxycinnamic acids, but there were significant differences among treatments (Table 3). MeJ grapes had more total hydroxycinnamic acids than MeJ+Ur grapes. Thus, there did not appear to be a synergistic effect between MeJ and urea to enhance synthesis of hydroxycinnamic acids. Previous works reported no effect of MeJ treatment on hydroxycinnamic acid content (Portu et al. 2015a, 2016, 2018a). The most abundant hydroxycinnamic acid in 2019 was *trans*-caftaric acid, while in 2020, it was ferulic acid.

In 2019, only the MeJ+Ur treatment affected stilbenes: there was less *trans*-resveratrol than in the control or MeJ-treated grapes (Table 3). In 2020, MeJ+Ur increased *cis*-piceid and again decreased *trans*-resveratrol below that of control and MeJ grapes. The most abundant stilbene was *trans*-piceid in all samples. In both years, neither MeJ nor MeJ+Ur treatments altered the total stilbene concentration, showing that they did not improve stilbene synthesis overall. An increase in stilbenes would have been an interesting effect of foliar treatments because these antioxidant compounds are considered beneficial for human health. Stilbenes have antioxidant activity, antifungal and antibacterial effects, and cardioprotective and anticancer attributes (Guerrero et al. 2009, Gil-Muñoz et al. 2017), although stilbene content in grapes is generally low. Many studies suggest that a high intake of polyphenol-rich foods could have cardiovascular benefits (Ruiz-García and Gómez-Plaza et al. 2013). Our results contrast with previous reports of increased stilbenes after foliar MeJ application (Portu et al. 2015a, 2018a, 2018b), but are consistent with a different report that MeJ treatment did not increase stilbenes (Portu et al. 2016). Another study found more stilbenes after MeJ foliar applications and suggested that MeJ may favor stilbene biosyntheses, but the effect obtained varied with the variety and season (Gil-Muñoz et al. 2017). We also found that the effect of foliar MeJ treatment varied with the season.

Multifactor analysis of variance of general must chemistry and phenolic compounds in grapes

The effects of treatment, season, and their interaction on standard chemical measure were studied (Table 4). The treatment factor affected total phenols: both treatments significantly increased phenols, but there was no difference between treatments, so MeJ and MeJ+Ur both increased biosynthesis of phenolic compounds. The season factor affected weight of 100 berries, which was greater in 2020 than in 2019, while there was more total acidity, fructose, malic acid, and total phenols in 2019 than in 2020.

Our vineyard received less rain in August 2019 than in August 2020. The more abundant preharvest rainfall in 2020 increased berry weight due to greater absorption of water by the grapevines. Berry size is a grape quality factor, since the grape skin releases important compounds for wine and grape quality such as phenolic or aromatic compounds. Larger grapes have a lower skin to pulp ratio, which means that skin compounds will be more diluted in the must

(Paladines-Quezada et al. 2021). This is most likely why there were more phenolic compounds in 2019 must than in 2020 (Table 5). Vine water status influences the ratio of accumulation of phenolic compounds; however, the increased anthocyanins under water deficit is less because of increased synthesis of these compounds than because of smaller berry size (Koundouras et al. 2006). We did not determine vine water status, but reduced summer rain can reduce berry size (van Leeuwen and Destrac-Irvine 2017), as observed in 2019. Therefore, there was more acidity, fructose, malic acid, and total phenols in 2019, because in 2020 their contents were diluted. Treatment and season interaction did not affect any general must parameters (Table 4).

Treatment factor affected all anthocyanins, except for delphinidin-3-acglc, cyanidin-3-acglc, petunidin-3-acglc, malvidin-3-acglc, malvidin-3-*cis*-cmglc, malvidin-3-*trans*-cmglc, malvidin-3-cfglc, and total acylated anthocyanins (Table 5). MeJ+Ur affected the content of all non-acylated anthocyanins with respect to the control, while MeJ alone did not affect the content of petunidin-3-glc or malvidin-3-glc (Table 5). Among the individual acylated anthocyanins, both treatments increased peonidin-3-acglc, cyanidin-3-cmglc, and peonidin-3-cmglc. MeJ+Ur also increased delphinidin-3-cmglc and petunidin-3-cmglc. Therefore, total non-acylated and total anthocyanin content was increased by the treatments. Multifactorial analysis showed both MeJ and MeJ+Ur increase anthocyanin synthesis, in agreement with a previous report (Portu et al. 2018a).

Season also affected the content of all anthocyanins but malvidin-3-*cis*-cmglc (Table 5). Thus, the content of all anthocyanins but malvidin-3-*cis*-cmglc, malvidin-3-*trans*-cmglc, and malvidin-3-cfglc, and the total content of non-acylated and acylated anthocyanins, was greater in 2019 grapes than in 2020 grapes. In agreement with the dilution effect produced in 2020 by the greater water absorption undergone by grapevines due to the higher preharvest rainfall, a bigger berry size diluted skin compounds in the must (Paladines-Quezada et al. 2021), except for malvidin-3-*trans*-cmglc and malvidin-3-cfglc, which were more abundant in 2020 grapes. Water stress applied to Tempranillo grapes postveraison improved berry quality by increasing soluble solids content and polyphenol and anthocyanin concentrations in the must (Girona et al. 2009). The lower rainfall in 2019 could contribute to the accumulation of polyphenolic compounds in grapes (Koundouras et al. 2006). Treatment and season interaction affected the content of several anthocyanidins and its total content except for malvidin-3-glc, cyanidin-3-acglc, petunidin-3-acglc, peonidin-3-acglc, malvidin-3-acglc, malvidin-3-*cis*-cmglc, and malvidin-3-cfglc (Table 5).

Among flavonols, both treatments decreased quercetin-3-glcU and kaempferol-3-gal (Table 5). MeJ grapes were not significantly different from control grapes in total flavonols, while MeJ+Ur grapes had significantly less total flavonols. Therefore, foliar application of MeJ and MeJ+Ur did not enhance flavonols. Our findings contrast with earlier findings that MeJ increased total flavonols (Portu et al. 2018a). Season affected the content of all flavonols but laricitrin-2-glc. Content of individual and total flavonols was greater in 2019 than in 2020, except for laricitrin-3-glc, which was not different among seasons. This result is explained by the preharvest rains in 2020 and their dilution of polyphenols in must (Paladines-Quezada et al. 2021). Treatment and season interaction affected the content of quercetin-3-glcU, laricitrin-3-glc, kaempferol-3-gal, isorhamnetin-3-glc, and total flavonols.

Among low molecular weight flavanols, the two treatments affected catechin and epicatechin-3-gallate content differently (Table 5). MeJ reduced catechin, while MeJ+Ur reduced epicatechin-3-gallate. There was more procyanidin B1 in MeJ+Ur than in MeJ samples (Table 5). There were no differences in total flavanols among control and treated grapes. Therefore, foliar application of MeJ and MeJ+Ur to vineyard did not enhance low molecular weight flavanols content of grapes. A similar lack of effect of MeJ on flavanol content has been reported previously (Portu et al. 2018a). Season affected both individual and total flavanol concentrations, but there was more epigallocatechin in 2019 than in 2020. These differences were again due to the dilution effect of rain (Paladines-Quezada et al. 2021). Treatment and

Table 4 Multifactor analysis of variance of general parameters of the musts with the two factors studied: treatment (Control, methyl jasmonate [MeJ], and MeJ + Urea [MeJ+Ur]) and season (2019 and 2020) and their interaction (treatment × season). TSS, total soluble solids.

	Weight of 100 berries (g)	TSS (Brix)	Potential alcoholic strength (% v/v)	pH	Total acidity (g/L)	Glu+Fru (g/L)	Glu (g/L)	Fru (g/L)	Malic acid (g/L)	Total phenols (mg/L)
Treatment (T)										
Control	156.63 a[a]	23.50 a	13.80 a	3.79 a	4.37 a	233.14 a	113.74 a	119.39 a	1.73 a	863.47 a
MeJ	174.74 a	22.20 a	12.91 a	3.74 a	4.87 a	217.06 a	104.48 a	112.58 a	2.04 a	954.82 b
MeJ+Ur	177.18 a	22.90 a	13.39 a	3.76 a	4.47 a	227.76 a	110.27 a	117.50 a	1.94 a	965.00 b
Season (S)										
2019	129.00 a	23.32 a	13.68 a	3.80 a	4.98 b	230.68 a	110.16 a	120.52 b	2.41 b	1281.24 b
2020	210.02 b	22.41 a	13.51 a	3.72 a	4.17 a	221.30 a	108.83 a	112.46 a	1.39 a	574.28 a
Interaction										
T x S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[a]For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). N.S., not significant ($p > 0.05$).

Table 5 Multifactor analysis of variance of grape phenolic compounds (expressed as mg/kg fresh weight). MeJ, methyl jasmonate; MeJ + Urea, MeJ+Ur.

	Treatment (T)			Season (S)		
	Control	**MeJ**	**MeJ+Ur**	**2019**	**2020**	**Interaction (T x S)**
Anthocyanins						
Delphinidin-3-glc[a]	87.18 a[b]	102.73 b	114.04 b	148.04 b	54.58 a	**[c]
Cyanidin-3-glc	17.14 a	27.55 b	28.22 b	38.54 b	10.07 a	***
Petunidin-3-glc	66.89 a	75.16 ab	85.20 b	100.30 b	51.19 a	*
Peonidin-3-glc	32.75 a	41.09 b	50.56 c	59.90 b	23.03 a	***
Malvidin-3-glc	192.80 a	209.78 ab	217.63 b	241.55 b	171.93 a	N.S.
Total non-acylated	396.75 a	456.30 b	495.64 b	583.33 b	310.80 a	**
Delphinidin-3-acglc	8.54 a	8.42 a	9.04 a	10.60 b	6.74 a	*
Cyanidin-3-acglc	3.72 a	3.72 a	3.69 a	3.82 b	3.60 a	N.S.
Petunidin-3-acglc	6.21 a	6.24 a	6.49 a	7.02 b	5.61 a	N.S.
Peonidin-3-acglc	4.16 a	4.50 b	4.59 b	4.85 b	3.98 a	N.S.
Malvidin-3-acglc	11.12 a	11.23 a	11.13 a	12.08 b	10.24 a	N.S.
Delphinidin-3-cmglc	15.29 a	16.36 a	18.07 b	18.89 b	14.25 a	***
Cyanidin-3-cmglc	5.79 a	6.83 b	7.24 b	7.56 b	5.68 a	**
Petunidin-3-cmglc	12.72 a	13.45 ab	14.06 b	14.68 b	12.14 a	***
Peonidin-3-cmglc	7.85 a	9.27 b	9.35 b	10.03 b	7.61 a	***
Malvidin-3-*cis*-cmglc	4.61 a	4.49 a	4.48 a	4.54 a	4.50 a	N.S.
Malvidin-3-*trans*-cmglc	43.89 a	44.34 a	41.67 a	40.35 a	46.25 b	***
Malvidin-3-cfglc	7.55 a	7.25 a	7.52 a	4.33 a	10.55 b	N.S.
Total acylated	131.46 a	136.11 a	137.31 a	138.75 b	131.16 a	***
Total anthocyanins	528.21 a	592.41 b	632.94 b	727.08 b	441.96 a	**
Flavonols						
Myricetin-3-glcU	21.70 a	19.96 a	19.27 a	24.79 b	15.83 a	N.S.
Myricetin-3-gal	28.62 a	28.76 a	29.62 a	35.70 b	22.30 a	N.S.
Myricetin-3-glc	131.97 a	131.47 a	131.92 a	181.46 b	82.12 a	N.S.
Quercetin-3-glcU	94.39 b	76.54 a	71.67 a	132.50 b	29.23 a	***
Quercetin-3-glc	102.55 a	96.95 a	90.73 a	157.98 b	35.51 a	N.S.
Laricitrin-3-glc	31.80 a	33.87 a	29.98 a	31.47 a	32.29 a	*
Kaempferol-3-gal	1.47 b	1.21 a	1.12 a	2.04 b	0.49 a	***
Kaempferol-3-glcU+3-glc	9.08 a	8.90 a	9.19 a	15.08 b	3.02 a	N.S.
Isorhamnetin-3-glc	7.85 a	8.37 a	7.70 a	11.83 b	4.12 a	**
Syringetin-3-glc	16.95 a	17.80 a	17.09 a	21.54 b	13.69 a	N.S.
Total flavonols	446.38 b	423.83 ab	409.29 a	614.39 b	238.61 a	*
Low molecular weight flavanols						
Catechin	37.19 b	30.78 a	32.99 ab	54.58 b	12.72 a	**
Epicatechin	25.10 a	24.75 a	27.26 a	36.36 b	15.04 a	*
Epicatechin-3-gallate	11.18 b	10.33 ab	9.27 a	11.78 b	8.74 a	*
Epigallocatechin	6.34 a	6.11 a	6.09 a	3.33 a	9.03 b	*
Procyanidin B1	19.27 ab	17.89 a	21.40 b	28.48 b	10.56 a	N.S.
Total flavanols	99.07 a	89.87 a	97.01 a	134.54 b	56.09 a	**
Hydroxybenzoic acid						
Gallic acid	5.60 a	5.83 a	5.97 a	5.67 a	5.94 a	*
Hydroxycinnamic acids (HCAs)						
trans-Caftaric acid	4.02 ab	3.70 a	4.47 b	6.51 b	1.62 a	N.S.
trans+*cis*-Coutaric acids	2.39 b	1.08 a	2.12 b	3.26 b	0.47 a	***
trans-Fertaric acid	1.56 b	1.19 a	1.30 a	1.40 a	1.30 a	***
Caffeic acid	0.35 b	0.29 a	0.28 a	0.33 b	0.28 a	***
p-Coumaric acid	0.25 ab	0.27 b	0.21 a	0.33 b	0.16 a	N.S.
Ferulic acid	6.42 ab	6.99 b	5.73 a	2.00 a	10.76 b	*
Total HCAs	14.99 b	13.52 a	14.10 ab	13.83 a	14.58 a	***
Stilbenes						
trans-Piceid	9.06 a	9.00 a	9.23 a	12.65 b	5.54 a	N.S.
cis-Piceid	1.42 a	1.43 a	1.98 b	1.65 a	1.57 a	***
trans-Resveratrol	0.37 b	0.35 b	0.19 a	0.52 b	0.09 a	**
cis-Resveratrol	0.28 a	0.34 b	0.37 b	0.37 b	0.29 a	**
Total stilbenes	11.12 a	11.11 a	11.77 a	15.18 b	7.49 a	N.S.

[a]Glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-*p*-coumaroylglucoside; cfglc, caffeoylglucoside; glcU, glucuronide; gal, galactoside.
[b]For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$).
[c]*, **, ***, and N.S. indicate significant differences at $p \leq 0.05$, 0.01, 0.001, and not significant, respectively.

season interaction affected catechin, epicatechin, epicatechin-3-gallate, epigallocatechin, and total flavanols content (Table 5). Neither treatment nor season affected the gallic acid content, but their interaction did.

Treatment factor affected the hydroxycinnamic acids *trans*-fertaric acid and caffeic acid, in addition to total hydroxycinnamic acids (Table 5). MeJ reduced *trans*+*cis*-coutaric acids, *trans*-fertaric acid, and caffeic acid content below those of the control grapes, while MeJ+Ur only had less *trans*-fertaric acid and caffeic acid. Neither MeJ nor MeJ+Ur increased the total hydroxycinnamic acids and in the MeJ treatment, there were less total hydroxycinnamic acids. This contrasts with reports of no differences in hydroxycinnamic acids after foliar MeJ application (Portu et al. 2015a). Season affected the content of all hydroxycinnamic acids but *trans*-fertaric acid. The content of most hydroxycinnamic acids were greater in 2019 than in 2020, but there was more ferulic acid in 2020. Nevertheless, hydroxycinnamic acids were the only family of phenolic compounds with no significant differences in total content between seasons (Table 5). Treatment and season interaction affected the content of all hydroxycinnamic acids except *trans*-caftaric and *p*-coumaric acids.

Among the stilbenes, treatment factor affected the content of *cis*-piceid, *trans*-resveratrol, and *cis*-resveratrol (Table 5). MeJ grapes had more *cis*-resveratrol than control grapes. MeJ+Ur increased *cis*-piceid and *cis*-resveratrol and decreased *trans*-resveratrol. A lack of effect of foliar MeJ on total stilbenes has been reported previously (Portu et al. 2016). Season affected the content of all stilbenes but *cis*-piceid, including total stilbenes, which were more abundant in 2019 than in 2020. The increased stilbenes in 2019 was due to dilution by the greater preharvest rainfall in 2020 (Paladines-Quezada et al. 2019). Treatment and season interaction did not affect either the *trans*-piceid or total stilbenes content (Table 5).

Discriminant analysis of phenolic compounds in grapes

A discriminant analysis of the phenolic compounds data from control and treated samples of the two seasons studied was performed (Figure 1). In 2019 (Figure 1A), Function 1 explained 99.9% and Function 2 explained 0.1%, so the total variance explained was 100%. The variables that contributed most to the discriminant model were peonidin-3-cmlg, *trans*- + *cis*-coutaric acids, and epigallocatechin (Function 1), and *trans*- + *cis*-coutaric acids, peonidin-3-cmlg, and syringetin-3-glc (Function 2). So, anthocyanins (peonidin-3-cmlg), hydroxycinnamic acids (*trans*- + *cis*-coutaric acids), flavanols (epigallocatechin), and flavonols (syringetin-3-glc) contributed most to discriminating the treatments. The discriminant model showed a very good separation among treatments in this season (Figure 1A). MeJ grapes were placed closer to the control grapes than MeJ+Ur grapes. This can be explained because MeJ+Ur grapes had the most peonidin-3-cmglc.

In 2020, Function 1 explained 99.8% and Function 2 explained 0.2% (total variance: 100%; Figure 1B). The variables that contributed most to the discriminant model were epicatechin (flavanol), *p*-coumaric acid (hydroxycinnamic acid),

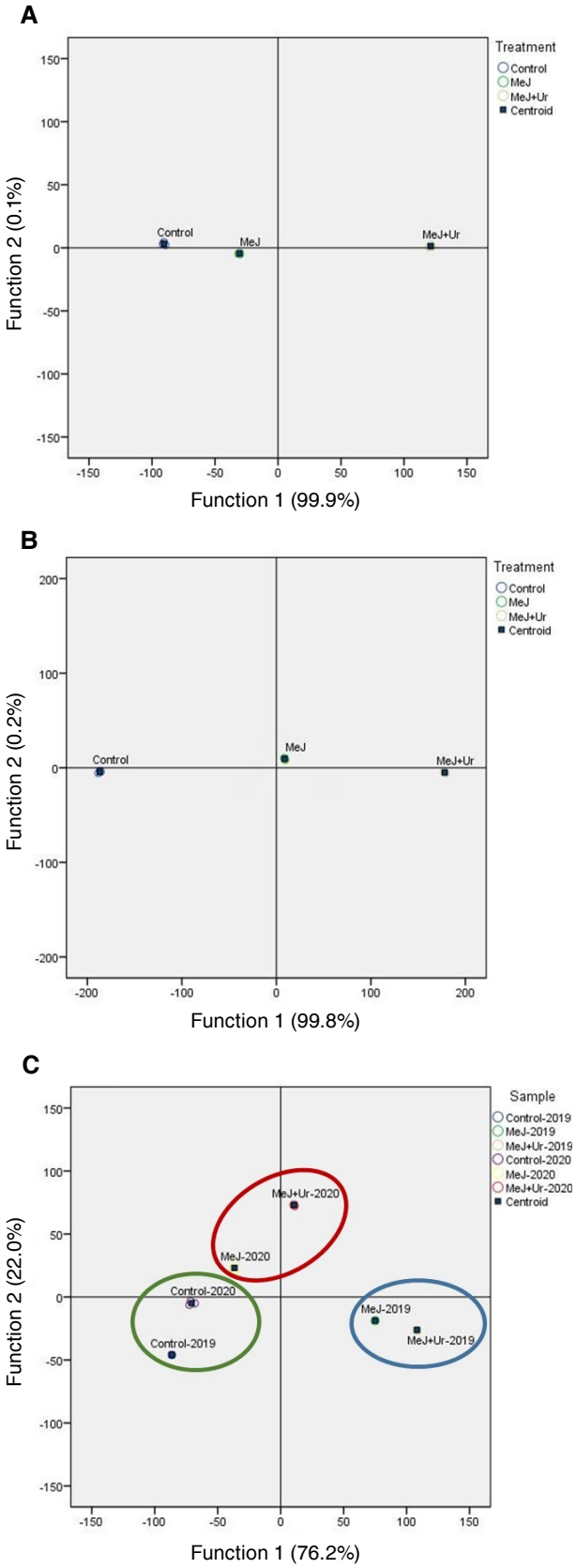

Figure 1 Discriminant analysis of phenolic compound content (mg/kg) in grapes from control, methyl jasmonate (MeJ), and MeJ+Urea (MeJ+Ur) treatments in (**A**) 2019, (**B**) 2020, and (**C**) 2019 and 2020 seasons.

and kaempferol-3-glcU+glc (flavonol) for Function 1 and *p*-coumaric acid, kaempferol-3-glcU+glc, and epicatechin for Function 2. There was good separation of treatments in this year also. MeJ+Ur samples were located on the positive side of Function 1 because they had the most epicatechin, control samples were located on the negative side because they had the least kaempferol-3-glcU+glc, and MeJ grapes had intermediate placement and concentrations of these compounds.

When samples from both vintages were combined, Function 1 explained 76.2% and Function 2 explained 22.0% (total variance: 98.2%; Figure 1C). The variables that contributed the most to the discriminant model were myricetin-3-glc (flavonol), catechin (flavanol), quercetin-3-glc (flavonol), *cis*-piceid (stilbene), and peonidin-3-*trans*-*p*-cmglc (anthocyanin) to Function 1, and myricetin-3-glc, *cis*-piceid, and peonidin-3-*trans*-*p*-cmglc to Function 2. Control samples from both seasons grouped together, while treated grapes grouped according to season. In the multifactorial analysis, the strong effect of season on phenolic compounds was confirmed, probably due to the differences in preharvest rainfall. Therefore, foliar application of MeJ and MeJ+Ur differentiated these treated grapes from control grapes in both years, but the MeJ and MeJ+Ur grapes separated by season instead of by treatment. This seasonal effect probably occurred because the phenolic content in grapes are strongly influenced by seasonal changes in weather, crop management techniques, or biotic or abiotic stresses to which the vineyard is exposed (Portu et al. 2018a, Paladines-Quezada et al. 2021). The preharvest rainfall in 2020 was greater than in 2019. This difference had a stronger effect on phenolic content than that of foliar treatments in each vintage.

Conclusions

The influence of foliar MeJ and MeJ+Ur on grape phenolic composition was studied in two consecutive seasons. Their effects were different in each season due to differences in climatic conditions. In 2019, foliar MeJ and MeJ+Ur both reduced sugar content and MeJ musts had a lower TSS and potential alcoholic strength than the control. Overall, MeJ and MeJ+Ur increased anthocyanins or had no effect; neither flavonols nor hydroxycinnamic acids were affected. MeJ treatment decreased total flavonols in 2019, but increased them in 2020. MeJ+Ur foliar treatment did not affect total flavonol or stilbene concentrations.

This study is the first to our knowledge to examine the combination of MeJ and urea for their effect on grape phenols. In 2019, there was a stronger effect on anthocyanin content when these compounds were applied together instead of MeJ alone. However, season had a greater effect than treatments on phenolic compounds. Foliar application of both treatments increased total phenols and total anthocyanidin, but there was no consistent effect among seasons on anthocyanins, flavonols, flavanols, hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes. Thus, season and weather conditions have a strong influence on the effect of foliar applications of MeJ and MeJ+Ur on the phenolic content of grapes and further research is required to clarify this interaction in the vineyard.

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