Methyl jasmonate treatment to increase grape and wine phenolic content in Tempranillo and Graciano varieties during two growing seasons

Javier Portu, Rosa López, Pilar Santamaría, Teresa Garde-Cerdán*

Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja, CSIC, Universidad de La Rioja). Carretera de Burgos Km. 6. 26071 Logroño, Spain. *Teresa.GardeCerdan@gmail.com; teresa.garde@icvv.es

Abstract

Phenolic compounds include a heterogeneous group of secondary metabolites that play diverse biological functions. Moreover, these compounds play a key role in grape and wine organoleptic and health promoting properties. Therefore, these compounds have been the subject of recent studies aimed at increasing their concentration in both grape and wine. The exogenous application of elicitors, like methyl jasmonate, stands out among these practices. We aimed to contribute to this growing area of research by carrying out this practice with two different grape varieties, Tempranillo and Graciano, during two growing seasons, providing therefore relevant information of the effect of this practice on the grape and wine phenolic composition. Despite the huge influence of the growing season and grape variety, a significant influence of MeJ treatment was found in grape phenolic composition, especially in anthocyanins, flavonols, and stilbenes. Moreover, certain wine chromatic parameters were also significantly improved by MeJ treatment. In conclusion, MeJ foliar application led to obtain grapes with a higher concentration of phenolic compounds.

Keywords: elicitation, methyl jasmonate, phenolic, anthocyanins, flavonols, stilbenes, viticulture

1. Introduction

Phenolic compounds comprise a heterogeneous group of compounds that are formed through the phenylpropanoid pathway, starting with the amino acid phenylalanine. These secondary metabolites are divided according to their structure in non-flavonoids (i.e. phenolic acids and stilbenes) and flavonoids (i.e. anthocyanins, flavonols, and flavanols). Together with the volatile compounds, the phenolic compounds are the major responsible for grape and wine quality, taking part in color, mouthfeel properties and wine aging potential. Moreover, phenolic compounds have drawn the attention during the last decade of many studies, given their role in the beneficial health properties related to the moderate consumption of wine. In this respect, it is noteworthy their antioxidant properties, as well as biological activities like anticarcinogenic or cardioprotection (Xia et al., 2010), which could depend on the gut microbiota composition (Espín et al., 2017).

In view of the foregoing reasons, various studies have evaluated different tools to increase grape and wine phenolic content. However, grape phenolic composition depends on many factors that include the grape variety (Mazza et al., 1999), climate factors (Carbonell-Bejerano et al., 2014), biotic factors (Romero-Pérez et al., 2001), as well as viticultural practices such as early leaf removal (Diago et al., 2012), cluster thinning (Avizcuri-Inac et al., 2013) or the establishment of vegetal ground cover crops (Bouzas-Cid et al., 2016).

Among the viticultural practices aimed at improving grape phenolic composition, the application of elicitors has drawn the attention of different studies in recent years (Ruiz-García and Gómez-Plaza, 2013). Previous works have demonstrated that exogenous application of substances known as elicitors may induce plant defense mechanisms. Thus, plants could react to elicitor application by inducing the

phenylpropanoid pathway and accumulating phenolic compounds (Dixon et al., 2002). In this respect, *in vitro* and *in vivo* studies have shown that the elicitor methyl jasmonate could improve grape and wine phenolic content in grape varieties like Tempranillo or Monastrell (Portu et al., 2016; Ruiz-García et al., 2012).

In addition, an improvement in grape phenolic composition has a special relevance in the current context of the climate change, which is known to accelerate grapevine phenology (Trought et al., 2015) and, in consequence, the challenge in warm areas is nowadays to get grapes with an optimal phenolic ripeness but not too high sugar levels.

Therefore, the aim of this study was to evaluate the foliar application of methyl jasmonate, as a promising tool to improve grape and wine phenolic composition, by studying the detailed grape and wine phenolic composition. We aim to contribute to this growing area of research by carrying out this practice with two different grape varieties, Tempranillo and Graciano, both originated in Rioja wine region, a region susceptible to climate change impact. Moreover, this work has also been conducted during two growing seasons, providing therefore relevant information of this strategy.

2. Materials and methods

2.1. Vineyard site and experimental layout

This study was conducted during two growing seasons (2015 and 2016) with two different *Vitis vinifera* grape varieties: Tempranillo and Graciano.

Tempranillo commercial vineyard was located in Alfaro (Rioja Baja, Spain) at an altitude of 335 meters above sea level (m.a.s.l.). The exact locations was 42° 10´ 2" north latitude; 1° 49´ 53" west longitude. Vines were planted in 1999 in north–south

rows 2.80 m apart, with 1.20 m within-row spacing, resulting in a plant density of 3,000 plants ha^{-1}, and grafted onto rootstock 1103-Paulsen.

As for Graciano grape variety, in 2015 the experimental site was located in Alfaro at an altitude of 345 m.a.s.l. The exact location was 42° 9´ 36" north latitude; 1° 50´ 6" west longitude. Vines were planted in 1997 in east-west rows 3.00 m apart, with a 1.28 m within-row spacing, resulting in a plant density of 2,600 plants ha^{-1}. In 2016, Graciano trial was moved to a nearby vineyard, also located in Alfaro at an altitude of 465 m.a.s.l. The exact location was 42º 7' 36" north latitude; 1º 52' 52" west longitude. Vines were planted in 2002 in northwest-southeast rows 2.90 m apart with a 1.20 m within-row spacing, resulting in a plant density of 2,900 plants ha^{-1}.

All vineyards were trained to a VSP (vertical shoot positioned) trellis system and managed according to the standard viticultural practices for the cultivars and region. Climatic conditions were recorded by a local weather station belonging to the Agroclimatic Information Service of La Rioja (SIAR). The growing season in 2015 was drier and slightly warmer than 2016. In this respect, annual rainfall in 2015 was 301 mm and average annual temperature was 14.1 ºC. In 2016, annual rainfall was 386 mm while average annual temperature was 13.9 ºC. Climatic conditions during vegetative growth period (i.e. from April to the end of September) followed a similar pattern: accumulated rainfall and average temperature during this period were, respectively, 128 mm and 19.5 ºC in 2015; 145 mm and 19.1 ºC in 2016.

The experimental design was set up as a completely randomized block design with three replicates of ten vines. The methyl jasmonate (MeJ) solution was prepared according to Portu et al. (2015b) at a concentration of 10 mM; 200 mL per plant were applied using Tween 80 as the wetting agent (0.1 % v/v). Control plants were sprayed

with Tween 80 aqueous solution. The treatments were carried out twice, at veraison and one week later.

2.2. Harvest and must parameters

Grapes were harvested when they reached an average ºBrix between 22.5 and 24. Harvest dates for Tempranillo were 17th of September in 2015 and 9th of September in 2016. Harvest dates for Graciano were 10th of September in 2015 and 6th of October in 2016. From each replicate, about 150 berries were separated and frozen at -20 °C in order to determine grape monomeric phenolic composition. Another set of 400 berries per replicate was separated and crushed in order to determine must parameters.

ºBrix was determined by refractometry. pH, total acidity, and potassium were analyzed in musts according to the International Organization of Vine and Wine (2013), while the tartaric acid was determined following the Rebelein method (Lipka and Tanner, 1974). An automatic analyser (Miura One, TDI, Barcelona, Spain) was used to determine malic acid.

Since treatments were performed in triplicate, the results of these parameters are the average of the analyses of three samples (n = 3).

2.3. Vinification and wine parameters

Grapes from each field replicate were destemmed and crushed and vinified in the experimental winery of the Instituto de Ciencias de la Vid y del Vino (ICVV, Logroño, Spain). Vinifications were performed at room temperature and potassium metabisulfite was added to the samples to give a final total SO_2 concentration of 50 mg L^{-1} and then the musts were inoculated with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand, St Simon, France) (20 g hL^{-1}). Caps were punched down

daily and fermentation activity was followed by determining must temperature and ºBrix decrease.

Once the alcoholic fermentation was finished, wines were pressed and inoculated with the commercial *Oenococcus oeni* strain Uvaferm α (Lallemand) (1 g hL^{-1}) in order to perform the malolactic fermentation (MLF) under controlled conditions at 20 ºC. The evolution of the MLF was followed by analyzing malic acid content. Once the MLF was finished, aliquots of each wine were frozen and stored at −20 °C until the analyses of monomeric phenolic compounds were carried out. Wines were then characterized by measuring the alcoholic degree, pH, total acidity, hue and color intensity (CI) according to the International Organization of Vine and Wine (2013). Tartaric acid was determined by Rebelein method (Lipka and Tanner, 1974). Miura One (TDI) was used to determine Folin-Ciocalteu index and the concentration of malic and lactic acids. Total phenolics were determined as total polyphenol index (TPI) by spectrophotometric absorbance at 280 nm after previous dilution of samples (Ribéreau-Gayon and Stonestreet, 1965). Ionised anthocyanins were determined according to Glories (1978) and polymerization index was calculated according to Ruiz (1999). The total antioxidant activity in wines was determined according to the DPPH method following the methodology described by Nixdorf and Hermosín-Gutiérrez (2010). Spectrophotometric analyses were carried out with the following spectrophotomers: Helios Omega (Thermo Fisher Scientific, Waltham, USA) for IC, hue and TPI; DR 5000 (Hach, Dusseldorf, Germany) for ionized anthocyanins and polymerization index; Cary 60 (Agilent, Palo Alto, USA) for the total antioxidant activity.

Since field treatments were performed in triplicate and one vinification was performed from each replicate, the results of wine parameters correspond to the average of the analyses of three samples (n = 3).

150 *2.4. Determination of grape and wine low molecular weight phenolic compounds*

151 *2.4.1. Sample preparation*

152 Phenolic compounds were extracted from grape berries according to the method
153 described by Portu et al. (2016). Moreover, in order to isolate grape and wine non-
154 flavonoid compounds, a purification step by solid phase extraction (SPE) was
155 performed using PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent) placed
156 in a Visiprep^{TM} Vacuum Manifold extraction system (Sigma-Aldrich, San Luis, USA)
157 (Portu et al., 2015a). The anthocyanin-free fraction was used to analyze flavonols,
158 flavanols, hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes.

159

160 *2.4.2. Analysis of phenolic compounds by HPLC-DAD*

161 Phenolic compounds but stilbenes were analyzed using an Agilent 1260 Infinity
162 chromatograph, equipped with a diode array detector (DAD). The chromatographic
163 procedure was as described by Portu et al. (2016) using a Licrospher® 100 RP-18
164 reversed-phase column (250 × 4.0 mm; 5 μm packing; Agilent) with pre-column
165 Licrospher® 100 RP-18 (4 × 4 mm; 5 μm packing; Agilent), both thermostated at 40 °C.
166 For the analysis of anthocyanins, 10 μL of the grape extract or wine were injected into
167 the system. For the analysis of non-anthocyanin phenolic compounds fractions, the
168 injection volume was 20 μL. Flow rate was set at 0.630 mL min^{-1}. For anthocyanin
169 analysis, a gradient solvent system consisting of acetonitrile–water–formic acid
170 (3:88.5:8.5, v/v/v) (eluent A) and acetonitrile–water–formic acid (50:41.5:8.5, v/v/v)
171 (eluent B) was used as follows: 0 min, 6 % B; 15 min, 30 % B; 30 min: 50 % B; 35 min,
172 60 % B; 38 min, 60 % B; 46 min, 6 % B. For the analysis of flavonols, flavanols,
173 hydroxybenzoic acids and hydroxycinnamic acids, a gradient solvent system consisting
174 of acetonitrile–water–formic acid (3:88.5:8.5, v/v/v) (eluent A), acetonitrile–water–

formic acid (50:41.5:8.5, v/v/v) (eluent B) and methanol–water–formic acid (90:1.5:8.5, v/v/v) (eluent C) was used as follows: 0 min, 4 % B and 0 % C; 7 min, 4 % B and 0 % C; 38 min, 17 % B and 13 % C; 52 min, 30 % B and 20 % C; 52.5 min, 40 % B and 30 % C; 57 min, 50 % B and 50 % C; 58 min, 50 % B and 50 % C; 65 min, 4 % B and 0 % C.

Stilbene determination was performed in 2015 by UHPLC–DAD using an Agilent 1290 Infinity chromatograph. The procedure was as described by Portu et al. (2018). In 2016, stilbenes were analyzed by HPLC–DAD by adapting the former methodology to an Agilent 1260 Infinity chromatograph. Briefly, samples were injected into a Licrospher® 100 RP-18 reversed-phase column (Agilent) with pre-column Licrospher® 100 RP-18 (Agilent), both thermostated at 40 °C. Flow rate was set at 0.500 mL min^{-1} and 20 μL of sample were injected into the system. Water–acetonitrile–formic acid (100:10:0.1 v/v/v) was used as solvent A and acetonitrile was used as solvent B. Linear solvent gradient was as follows: 0 min, 0% B; 20.8 min, 16% B; 32.8 min, 16% B; 49.4 min, 42% B; 60 min, 0% B.

Phenolic compounds were identified according to the retention times of the available pure compounds and the UV–Vis data obtained from authentic standards and/or published in previous studies (Castillo-Muñoz et al., 2009). Anthocyanins were quantified at 520 nm as malvidin-3-*O*-glucoside (Extrasynthèse, Genay, France); flavonols were quantified at 360 nm as quercetin-3-*O*-glucoside (Sigma-Aldrich); hydroxycinnamic acids were quantified at 320 nm as *trans*-caftaric acid (Extrasynthèse); *cis*-piceid and *cis*-resveratrol were quantified at 305 nm as their corresponding *trans* isomers; gallic acid (Sigma-Aldrich) was quantified at 280 nm; flavanols were quantified at 280 nm using catechin (Sigma-Aldrich) for catechin and procyanidins B1 and B2, while epicatechin (Sigma-Aldrich) was used for epicatechin,

epigallocatechin, and epicatechin-3-gallate quantification. Concentrations in grape samples were expressed as milligrams per weight of grape (mg kg^{-1}), while concentrations in wines were expressed as milligrams per liter of wine (mg L^{-1}).

Since treatments were performed in triplicate and one vinification was performed from each field replicate, the results for phenolic compounds are the average of the analyses of three samples (n = 3).

2.5. Statistical analysis

The statistical procedure was carried out with SPSS Version 21.0 statistical package for Windows (Chicago, USA). The data for the different determinations were processed using the variance analysis (ANOVA).

3. Results

3.1. Yield and grape parameters

Results of yield and grape parameters are shown in Table 1. The results showed that grapes had a balanced physico-chemical composition among the standards of the region, being adequate for vinification. Moreover, it was noticed that Graciano's grapes were characterized by a lower pH and a higher acidity than Tempranillo's, which are distinctive qualities of this grape variety. It was also noticeable that Tempranillo vineyard in 2015 had an unusual higher yield and weight of 100 berries as well as a lower probable alcohol, in comparison with the rest of the samples.

In 2015, there were no significant differences between control and MeJ in any grape variety but the tartaric acid content in Graciano, which was at higher level in MeJ samples (Table 1). In contrast, in 2016, more significant differences were observed, although these varied according to the grape variety. On the one hand, significant

differences in Tempranillo were related to the weight of 100 berries, malic acid, and potassium, all of them with lower values in samples from MeJ treatment than in control. On the other hand, parameters affected by MeJ treatment in Graciano concerned pH, total acidity, and tartaric acid. In this respect, the application of MeJ resulted in grapes with higher acidity given the higher values of total acidity and tartaric acid as well as the lower pH. In addition, the statistical analysis from Table 2 showed that the percentage of variation attributable to MeJ treatment was statistically significant in the case of parameters related to grape acidity, like tartaric acid ($\rho \leq 0.01$), total acidity and malic acid ($\rho \leq 0.05$).

3.2. Anthocyanins composition

Results of the HPLC analysis of anthocyanins in grape are outlined in Table 3. The results reflected differences between both grape varieties. In this respect, grapes from Tempranillo had the following anthocyanin profile: malvidin-type (55 %), delphinidin-type (20 %), petunidin-type (15 %), peonidin-type (5 %), and cyanidin-type (5 %). On the other hand, grapes from Graciano had a similar percentage of malvidin- and cyanidin-type anthocyanins but, contrary to Tempranillo, this grape variety was characterized by a higher percentage of peonidin-type anthocyanins (20 %) and lower percentages of delphinidin- and petunidin-type anthocyanins (10 % each).

As it can be seen from Table 3, the application of MeJ had a significant impact on grape anthocyanins content. In the case of Tempranillo in 2015, the application of MeJ increased the concentration of all non-acylated anthocyanins. Among acylated anthocyanins, the treatment increased the concentration of delphinidin-3-acetylglucoside, cyanidin-3-acetylglucoside, cyanidin-3-coumaroylglucoside, and peonidin-3-coumaroylglucoside. Overall, there was a significant increase of total non-

250 acylated anthocyanins, while the total amount of the acylated-type was not affected. In
251 2016, however, acylated anthocyanins were clearly increased by the application of MeJ
252 (Table 3). In detail, all acylated forms but cyanidin-3-acetylglucoside, peonidin-3-
253 acetylglucoside, and malvidin-3-*cis*-coumaroylglucoside, were enhanced by MeJ
254 treatment. Moreover, the total amount of acylated anthocyanins was also significantly
255 higher in the grapes from MeJ application when compared to the control. Regarding
256 non-acylated anthocyanins, MeJ treatment increased as well the concentration of
257 delphinidin-3-glucoside and petunidin-3-glucoside.

258 Moreover, the results showed that MeJ application increased the concentration
259 of total anthocyanins in both growing seasons: 2015 ($\rho \leq 0.05$) and 2016 ($\rho \leq 0.10$).
260 Nonetheless, it was observed that the treatment had a greater influence in non-acylated
261 anthocyanins in 2015 while the effect was greater in acylated-forms in 2016.

262 On another note, the HPLC analysis of Graciano grapes samples (Table 3)
263 showed only slight significant differences. In this respect, there were no significant
264 differences in 2015 for any compound. In 2016, as seen for Tempranillo, acylated
265 anthocyanins were slightly more influenced by MeJ application than non-acylated
266 anthocyanins. In this respect, the application of MeJ increased the concentration of
267 peonidin-3-coumaroylglucoside and malvidin-3-*trans*-coumaroylglucoside. The absence
268 of significant differences regarding total anthocyanins was in contrast with the results
269 above mentioned for Tempranillo.

270

271 *3.3. Flavonols composition*

272 Table 4 shows the results of the HPLC analysis of grape flavonols. Grapes from
273 Tempranillo had higher concentrations of myricetin- and kaempferol-type flavonols in

comparison with Graciano. In contrast, Graciano grapes had higher proportion of isorhamnetin- and syringetin-type flavonols.

Significant differences between control and treatment regarding grape flavonol content were found especially in 2016 rather than in 2015 (Table 4). In 2015, only syrigentin-3-glucoside was increased by the application of MeJ to Tempranillo grapevines, while no significant differences were observed in Graciano. In contrast, several significant differences occurred in 2016 in both grape varieties. In this respect, the concentration of quercetin-3-glucuronide, quercetin-3-glucoside, kaempferol-3-galactoside, and kaempferol-3-glucoside was increased after the application of MeJ in both Tempranillo and Graciano grape varieties. In addition, the sum of myricetin-3-glucuronide and myricetin-3-galactoside and isorhamnetin-3-glucoside were also increased in Graciano. Moreover, the foliar treatement with MeJ also increased the total amount of flavonols in both grape varieties ($\rho \leq 0.10$).

3.4. Flavanols, phenolic acids and stilbenes composition

Results of the HPLC analysis of flavanol monomers are shown in Table 5. Catechin and epicatechin were the major flavanols in Tempranillo and Graciano, respectively. Moreover, it was observed that the total amount of flavanol monomers was higher in the case of Graciano variety. None significant differences were found in flavanol composition for any grape variety or growing season. Similar results were found for grape phenolic acids (hydroxybenzoic and hydroxycinnamic acids), since no differences were found for any of these compounds, but *trans*-fertaric acid content in Graciano 2016, which was significantly higher in the samples from MeJ treatment (Table 5).

In contrast, stilbenes were hugely increased by the foliar treatment with MeJ. In Tempranillo, it was observed that the application of MeJ enhanced the total stilbene amount during the two growing seasons of the study: 2015 ($\rho \leq 0.05$) and 2016 ($\rho \leq 0.10$) (Table 5). A more detailed view reflects that, in 2015, all the stilbenes were individually increased while, in 2016, only *trans*-piceid was increased by the treatment. As for Graciano grape variety, the stilbenes total content was also higher in MeJ wines than in control, both in 2015 ($\rho \leq 0.10$) and 2016 ($\rho \leq 0.05$). In 2015, the amount of *cis*-piceid and viniferin was increased by the treatment in 2015, while in 2016, there was an individual increase of *trans*-piceid and *trans*-resveratrol due to the treatment.

3.5. Main factors of variability on the grape phenolic composition

Table 6 shows the main factors of variability (treatment, growing season, and variety) on the total amounts of the main classes of phenolic compounds. In general, the percentage of variability explained by MeJ treatment was lower than the other two factors (growing season and variety), although it was statistically significant for every parameter but hydroxycinnamic acids. In this respect, the greatest statistically significance was observed in stilbenes ($p \leq 0.001$), followed by acylated anthocyanins and flavonols ($p \leq 0.01$), total anthocyanins, non-acylated anthocyanins and flavanols ($p \leq 0.05$). Moreover, the greatest percentage of variability explained by the treatment was observed in the case flavonols and anthocyanins. The influence of MeJ treatment was in general independent from the other factors, since there was only a significant interaction between MeJ treatment and the growing season factor for total stilbenes ($p \leq 0.01$).

Regarding the growing season factor, this explained the greatest variability in the following parameters: anthocyanins (both acylated and non-acylated forms) and flavonols (Table 6). The grape variety explained the greatest variability in the case of

flavanols and stilbenes. As for the variability in hydroxycinnamic acids, this was mostly explained by the interaction between growing season and variety factors.

3.6. Wine analyses

The analyses of wine general parameters are shown in Table S1. No significant differences were found in alcohol content and pH for any grape variety and growing season. In Tempranillo in 2016, MeJ wines had lower total acidity than control wines. However, in the case of Graciano in the same year, MeJ wines showed higher levels of tartaric and lactic acids when compared to the control. Regarding the parameters related to the wine phenolic content, the application of MeJ had a significant effect on ionization index, which was increased in Tempranillo (2015 and 2016) and in Graciano (2016). This result suggests that MeJ foliar application increased the percentage of anthocyanins that contribute to the wine color. In relation to the latter result, the application of MeJ also improved color intensity in 2016 in Graciano. In the case of the same grape variety and year, hue value was decreased by the MeJ treatment in 2016, indicating that MeJ wine was less oxidized than control wine. In addition, MeJ wine also had higher antioxidant capacity. Moreover, according to the statistical analysis shown in Table S2, the percentage of variability attributable to the treatment was significant in the case of color intensity and ionization index ($\rho \leq 0.001$), followed by hue ($\rho \leq 0.01$) and tartaric acid ($\rho \leq 0.05$).

However, minor significant differences were found concerning the analysis of wine monomeric phenolic compounds (Tables S3-S5). In general, the greatest differences were observed for Graciano in 2016, since wines made from the treatment had higher concentrations of several phenolic compounds, including non-acylated anthocyanins and total anthocyanins (Table S3), flavonols (Table S4), and stilbenes

348 (Table S5) than control wines. Notwithstanding, it is noteworthy to mention that,
349 although differences in stilbene composition were not significant in the rest of cases,
350 these were important in terms of percentages (30 % and 13 % higher in MeJ
351 Tempranillo wines in 2015 and 2016, respectively; 21 % higher in MeJ Graciano wines
352 in 2015).

353 These results were confirmed by the statistical analysis from Table S6. In this
354 respect, the percentage of variability explained by MeJ treatment was not significant
355 when compared to the growing season and variety factors. Only the variability found in
356 wine stilbenes was significantly attributable to the foliar treatment ($p \leq 0.01$).

357

358 **4. Discussion**

359 Overall, only slight differences have been found in the current work regarding
360 must parameters, although this observation depended on the year. This finding was also
361 reported by Ruiz-García et al. (2012), who studied the application of MeJ to Monastrell
362 clusters during two growing seasons and reported that significant differences on grape
363 physiochemical characteristics depended on the year. In this respect, in their first year of
364 the study, the application of MeJ increased must total acidity and tartaric acid content.
365 However, in their second year of the study, no significant differences were observed
366 regarding these former parameters while the authors found that other parameters (i.e. pH
367 and malic acid content) were increased by the treatment. Moreover, in our previous
368 studies, we did not find significant differences regarding must parameters from
369 Tempranillo grapes (Portu et al., 2016, 2015b). This is partly in agreement with Ju et al.
370 (2016), who applied three different concentrations of MeJ to Cabernet Sauvignon
371 grapes and found that the application of 800 μmol L^{-1} of MeJ decreased must pH while
372 the application of 200 μmol L^{-1} increased must sugar content. In contrast, no differences

were observed after the application of 50 µmol L^{-1}. In addition, Ruiz-García et al. (2013) reported that MeJ treatment only had a slight effect on grape parameters of different clones of Monastrell. Recently, D'Onofrio et al. (2018) reported an interesting observation since they found that MeJ application slowed down ripening in Sangiovese grapes, delaying by 10 days the technology maturity (i.e. º Brix and pH). Although the latter results could be of great interest regarding the climate change challenge, these were not confirmed in the works previously described. Taking into consideration our results and those from the literature, it could be suggested that MeJ does not normally modify grape physico-chemical parameters.

Results from anthocyanin analyses showed that MeJ treatment had a big influence on grape anthocyanin composition. In particular, the treatment exerted a huge impact on Tempranillo grape variety. However, the influence of the treatment seemed independent of the type of anthocyanins, given the fact that the treatment had a greater influence in non-acylated anthocyanins in 2015 while the effect was greater in acylated-forms in 2016. Previous works conducted in Monastrell showed a certain trend that non-acylated anthocyanins are influenced in a greater extent than acylated derivatives (Ruiz-García et al., 2013, 2012). In any case, the results here presented provide strong evidence that foliar treatment with MeJ improved grape anthocyanin composition in Tempranillo. This is in agreement with previous works in Tempranillo (Portu et al., 2016, 2015b) but the current work is the first to prove this fact during two growing seasons.

In addition, MeJ application exerted a bigger impact on Tempranillo's anthocyanin composition than in Graciano's. Previous works have also demonstrated that MeJ application increased anthocyanins content in several grape varieties such as Garnacha (Portu et al., 2017) or Monastrell (Ruiz-García et al., 2012). Nonetheless, the

latter authors have also recently reported that anthocyanin accumulation after MeJ application depended on the grape variety, since they found that anthocyanin concentration in the treated grapes at the end of ripening was higher than that of the control grapes for Merlot and Monastrell, but no in the case of Syrah (Gómez-Plaza et al., 2017).

In this respect, Graciano is a grape variety traditionally associated with colorful wines. Therefore, it could be hypothesized that the influence of MeJ could be less intense in Graciano than in grape varieties that have less anthocyanins, as it has been suggested previously by Portu et al. (2017) and it has also been demonstrated with other technologies aimed at improving grape phenolic content (López-Giral et al., 2015).

Treatment with MeJ has been reported to promote anthocyanin biosynthesis in several vegetables. For example, Wei et al. (2017) showed that MeJ application to peach fruits increased the expression of genes that codify enzymes involved in the anthocyanins metabolic pathway: *CHS* (chalcone synthase), *UFGT* (UDP-glycose: flavonoid glycosyltransferase), *F3H* (flavanone 3-hydroxylase) and *DFR* (dihydroflavonol 4-ammonia lyase). Similar results were reported by Sun et al. (2017), who studied the effect of MeJ on the accumulation of anthocyanins in callus material induced from apple leaves and showed that MeJ upregulated the expression of the anthocyanin structural genes *CHS*, *F3H* and *UFGT*. Both studies also showed that MeJ induced upregulated the expression of MYB genes, which are important regulator factors in the synthesis of anthocyanins. In apple fruits, Feng et al. (2017) observed that MeJ may lead to important increases in anthocyanin content but the effect was dose dependent. In this respect, fruits treated with 10 mM inhibited anthocyanin synthesis while treatments with 1 and 0.1 mM promoted anthocyanin accumulation by

upregulating genes involved in anthocyanin biosynthesis (*UFGT* and flavonol synthase (*FLS*).

Regarding the influence of the treatment on grape flavonol composition, the results showed that it varied according to the growing season, since greater differences were found in 2016 than in 2015. Results from previous studies have shown contradictory results regarding the effect of MeJ application on grape flavonols. On the one hand, several works have reported that grape flavonol content at harvest is normally unaffected by preharvest treatments with MeJ to varieties like Syrah or Monastrell (Gómez-Plaza et al., 2017) as well as Tempranillo (Portu et al., 2016, 2015b). Even more, Gómez-Plaza et al. (2017) reported that grapes treated with MeJ had less flavonols than control grapes at harvest in Merlot. On the other hand, foliar application of MeJ has been reported to enhance flavonols content in Garnacha grape variety (Portu et al., 2017).

In this respect, Gómez-Plaza et al. (2017) suggested that there were great differences throughout the ripening period, concluding that the most pronounced differences in flavonol concentration between treated and control grapes were observed three or four weeks after the treatment. Therefore, these authors suggested that elicitors should be applied as close as possible to the harvest date in order to obtain the strongest effect on this kind of compounds. Similar observations were previously reported by (Villangó et al., 2015) with respect to the effect of a commercial elicitor on grape stilbene accumulation. This idea could explain the results obtained in our study regarding Tempranillo, since in 2016 the time between the first application and harvest was 20 days shorter than in 2015. In Graciano, however, this period was 8 days longer in 2016 although it is worth to notice that the experimental site was not the same since, in 2016, this was located at an altitude of 120 m.a.s.l. higher than in 2015, explaining

447 therefore the delay in the ripening period. Overall, although promising results were only
448 found in one of the two years of the study, the results here reported are of especial
449 relevance given the key role that these compounds play in wine organoleptic properties,
450 especially in wine color stabilization by means of copigmentation reactions (Boulton,
451 2001).

452 Anthocyanins and flavonols are both flavonoid compounds and are formed
453 through the flavonoid biosynthetic pathway (Flamini et al., 2013). As abovementioned,
454 anthocyanin accumulation after MeJ application has been extensively reported for many
455 fruits and vegetables. Concerning flavonols, Flores and Ruiz del Castillo (2016)
456 reported that pre-harvest treatment with MeJ increased total flavonol content in black
457 currant. De la Peña Moreno et al. (2010) observed that post-harvest treatments with MeJ
458 favored flavonol accumulation in red raspberry, promoting the activity of the enzymes
459 FLS and flavanone 3β-hydroxylase (FHT). In addition, Król et al. (2015) observed that
460 seedlings grown from seeds soaked with 0.1 mM MeJ had higher concentrations of
461 kaempferol and quercetin. These authors also found that phenylalanine ammonia lyase
462 (*PAL*), *CHS* and *FLS* genes were upregulated after the elicitor treatment.

463 As for the effect of methyl jasmonate on flavanols, phenolic acids and stilbenes,
464 on the one hand, the treatment had a very limited impact on flavanols and phenolic acids
465 composition. On the other hand, however, grape stilbene composition was clearly
466 enhanced by the treatment in both varieties and growing seasons. These results are in
467 accordance with previous works that have suggested that the application of MeJ exerts a
468 big impact on stilbenes (Belhadj et al., 2006), although this effect could depend on the
469 grape variety (Gil-Muñoz et al., 2017). For example, Tempranillo has been reported to
470 be less sensitive than Monastrell (Gil-Muñoz et al., 2017). Moreover, it is noteworthy
471 that grapes from Graciano had a much higher concentration of stilbenes than

Tempranillo, being in general around ten-fold higher. This fact shows the huge influence of grape variety, and confirms the potential of Graciano grape cultivar as an excellent source of stilbenes.

Grape stilbenes are synthesized from 4-coumaroyl-CoA and three molecules of malonyl-CoA by the enzyme stilbene synthase (STS) (Flamini et al., 2013). Different works performed in *Vitis vinifera* cell suspension cultures have shown that MeJ treatments promotes stilbenes accumulation by upregulating the gene *STS* (Andi et al., 2018; Xu et al., 2015). Moreover, Belhadj et al. (2006) found that MeJ treatment upregulated *STS* and led to the accumulation of resveratrol, piceid, viniferins and pterostilbene in leaves, which in turn increased protection against the fungal disease *Erysiphe necator*.

Overall, MeJ application led to a significant improvement of grape anthocyanin and stilbene composition, followed by flavonol's. Flavanols and hydroxycinnamic acids were barely affected by the treatment. In any case, both grape variety and growing seasons factors had a higher impact on grape phenolic composition than the treatment with MeJ.

In the case of wine parameters analyses, the results indicated that MeJ application improved the wine chromatic parameters, especially color intensity and ionization index. However, there were only slight significant differences in the phenolic composition between control wines and those from the treatment. The absence of significant differences between control and MeJ wines regarding compounds like anthocyanins and flavonols in Tempranillo is in contrast with previous works from our group (Portu et al., 2016, 2015b). Nonetheless, it must be taken into account that in the previous studies, wine samples were collected after the alcoholic fermentation while, in the current work, malolactic fermentation was performed. This could indicate that

497 significant differences were minimized throughout the fermentation process. Therefore,
498 given the fact that grapes from MeJ had a higher phenolic content, the absence of
499 significant differences in wine could be due to a lower release of phenolic compounds
500 during vinification. This fact could be explained by recent results reported by Quezada-
501 Paladines et al. (2017), who found that MeJ application can also increase protein
502 concentration in the skin cell wall, resulting in a more rigid cell wall structure, which
503 could hinder the release of phenolic compounds during winemaking (Ortega-Regules et
504 al., 2006). Given this hypothesis, special care should be taken during winemaking in
505 terms of extraction and maceration in order to make the most of the phenolic content of
506 elicited grapes.
507
508 **5. Conclusions**
509 To authors' knowledge, this is the first work that studies the foliar application of
510 MeJ to two grape varieties during two growing seasons, evaluating its influence on the
511 grape and wine phenolic composition. The results here presented revealed that, despite
512 the huge influence of the growing season and the grape variety, the treatment with MeJ
513 had a significant influence on grape phenolic composition. In this respect, grape
514 anthocyanins, flavonols and stilbenes were influenced by the foliar treatment. In
515 contrast, the positive results observed in grape were not completely correlated with
516 wine's. However, it is also noteworthy that certain wine chromatic parameters, in
517 particular color intensity and ionization index, were significantly improved by MeJ
518 treatment. In conclusion, this study demonstrates that field applications of MeJ led to
519 obtain grapes with a higher concentration of phenolic compounds, independently from
520 the great influence of the variety and growing season factors.
521

Aknowledgements

The financial support was given by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)-Gobierno de La Rioja under the RTA2013-00053-C03-01 project. J. P. and T. G.-C. thank INIA-Gobierno de La Rioja and the European Social Fund for funding their contracts through the FPI-INIA and DOC-INIA programs, respectively. T. G.-C. also thanks MINECO for her Ramón y Cajal contract.

References

Andi, S.A., Gholami, M., Ford, C.M., 2018. The effect of methyl jasmonate and light irradiation treatments on the stilbenoid biosynthetic pathway in *Vitis vinifera* cell suspension cultures. Nat. Prod. Res. 32, 909–917.

Avizcuri-Inac, J.-M., Gonzalo-Diago, A., Sanz-Asensio, J., Martínez-Soria, M.-T., López-Alonso, M., Dizy-Soto, M., Echávarri-Granado, J.-F., Vaquero-Fernández, L., Fernández-Zurbano, P., 2013. Effect of cluster thinning and prohexadione calcium applications on phenolic composition and sensory properties of red wines. J. Agric. Food Chem. 61, 1124–1137.

Belhadj, A., Saigne, C., Telef, N., Cluzet, S., Bouscaut, J., Corio-Costet, M.-F., Mérillon, J.-M., 2006. Methyl jasmonate induces defense responses in grapevine and triggers protection against Erysiphe necator. J. Agric. Food Chem. 54, 9119–9125.

Boulton, R., 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. Am. J. Enol. Vitic. 52, 67–87.

Bouzas-Cid, Y., Portu, J., Pérez-Álvarez, E.P., Gonzalo-Diago, A., Garde-Cerdán, T., 2016. Effect of vegetal ground cover crops on wine anthocyanin content. Sci. Hortic. 211, 384–390.

547 Carbonell-Bejerano, P., Diago, M.-P., Martínez-Abaigar, J., Martínez-Zapater, J.M.,
548 Tardáguila, J., Núñez-Olivera, E., 2014. Solar ultraviolet radiation is necessary to
549 enhance grapevine fruit ripening transcriptional and phenolic responses. BMC
550 Plant Biol. 14, 183. https://doi.org/10.1186/1471-2229-14-183.
551 Castillo-Muñoz, N., Fernández-González, M., Gómez-Alonso, S., García-Romero, E.,
552 Hermosín-Gutiérrez, I., 2009. Red-color related phenolic composition of Garnacha
553 Tintorera (*Vitis vinifera* L.) grapes and red wines. J. Agric. Food Chem. 57, 7883–
554 7891.
555 D'Onofrio, C., Matarese, F., Cuzzola, A., 2018. Effect of methyl jasmonate on the
556 aroma of Sangiovese grapes and wines. Food Chem. 242, 352–361.
557 de la Peña Moreno, F., Blanch, G.P., Ruiz Del Castillo, M.L., 2010. (+)-Methyl
558 jasmonate-induced bioformation of myricetin, quercetin and kaempferol in red
559 raspberries. J. Agric. Food Chem. 58, 11639–11644.
560 Diago, M.P., Ayestarán, B., Guadalupe, Z., Garrido, Á., Tardaguila, J., 2012. Phenolic
561 composition of Tempranillo wines following early defoliation of the vines. J. Sci.
562 Food Agric. 92, 925–934.
563 Dixon, R.A., Achnine, L., Kota, P., Liu, C.-J., Reddy, M.S.S., Wang, L., 2002. The
564 phenylpropanoid pathway and plant defence - A genomics perspective. Mol. Plant
565 Pathol. 3, 371–390.
566 Espín, J.C., González-Sarrías, A., Tomás-Barberán, F.A., 2017. The gut microbiota: A
567 key factor in the therapeutic effects of (poly)phenols. Biochem. Pharmacol. 139,
568 82–93.
569 Feng, S., Sun, J., Sun, S., Wang, Y., Tian, C., Sun, Q., Chen, X., 2017. Transcriptional
570 Profiles Underlying the Effects of Methyl Jasmonate on Apple Ripening. J. Plant
571 Growth Regul. 36, 271–280.

572 Flamini, R., Mattivi, F., De Rosso, M., Arapitsas, P., Bavaresco, L., 2013. Advanced
573 knowledge of three important classes of grape phenolics: Anthocyanins, stilbenes
574 and flavonols. Int. J. Mol. Sci. 14, 19651–19669.
575 Flores, G., Ruiz del Castillo, M.L., 2016. Accumulation of anthocyanins and flavonols
576 in black currants (*Ribes nigrum* L.) by pre-harvest methyl jasmonate treatments 96,
577 4026–4031.
578 Gil-Muñoz, R., Fernández-Fernández, J.I., Crespo-Villegas, O., Garde-Cerdán, T.,
579 2017. Elicitors used as a tool to increase stilbenes in grapes and wines. Food Res.
580 Int. 98, 34–39.
581 Glories, Y., 1978. Recherches sur la Matière Colorantes des Vins Rouges. Ph.D. Thesis.
582 Université de Bourdeaux II, France.
583 Gómez-Plaza, E., Bautista-Ortín, A.B., Ruiz-García, Y., Fernández-Fernández, J.I., Gil-
584 Muñoz, R., 2017. Effect of elicitors on the evolution of grape phenolic compounds
585 during the ripening period. J. Sci. Food Agric. 97, 977–983.
586 International Organization of Vine and Wine, 2013. Compendium of International
587 Methods of Wine and Must Analysis. Paris.
588 Ju, Y.-L., Liu, M., Zhao, H., Meng, J.-F., Fang, Y.-L., 2016. Effect of exogenous
589 abscisic acid and methyl jasmonate on anthocyanin composition, fatty acids, and
590 volatile compounds of cabernet sauvignon (*Vitis vinifera* L.) grape berries.
591 Molecules 21, 1354.
592 Król, P., Igielski, R., Pollmann, S., Kepczyńska, E., 2015. Priming of seeds with methyl
593 jasmonate induced resistance to hemi-biotroph *Fusarium oxysporum* f.sp.
594 *lycopersici* in tomato via 12-oxo-phytodienoic acid, salicylic acid, and flavonol
595 accumulation. J. Plant Physiol. 179, 122–132.
596 Lipka, Z., Tanner, H., 1974. Une nouvelle méthode de dosage du acide tartarique dans

597 les moûts, les vins et autres boissons (selon Rebelein). Rev. Suisse Vitic. Arboric.
598 Hortic. 6, 5–10.
599 López-Giral, N., González-Arenzana, L., González-Ferrero, C., López, R., Santamaría,
600 P., López-Alfaro, I., Garde-Cerdán, T., 2015. Pulsed electric field treatment to
601 improve the phenolic compound extraction from Graciano, Tempranillo and
602 Grenache grape varieties during two vintages 28, 31–39.
603 Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., Ewert, B., 1999. Anthocyanins,
604 phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British
605 Columbia. J. Agric. Food Chem. 47, 4009–4017.
606 Nixdorf, S.L., Hermosín-Gutiérrez, I., 2010. Brazilian red wines made from the hybrid
607 grape cultivar Isabel: Phenolic composition and antioxidant capacity. Anal. Chim.
608 Acta 659, 208–215.
609 Ortega-Regules, A., Romero-Cascales, I., Ros-García, J.M., López-Roca, J.M., Gómez-
610 Plaza, E., 2006. A first approach towards the relationship between grape skin cell-
611 wall composition and anthocyanin extractability. Anal. Chim. Acta 563, 26–32.
612 Portu, J., López-Alfaro, I., Gómez-Alonso, S., López, R., Garde-Cerdán, T., 2015a.
613 Changes on grape phenolic composition induced by grapevine foliar applications
614 of phenylalanine and urea. Food Chem. 180, 171–180.
615 Portu, J., López, R., Baroja, E., Santamaría, P., Garde-Cerdán, T., 2016. Improvement
616 of grape and wine phenolic content by foliar application to grapevine of three
617 different elicitors: Methyl jasmonate, chitosan, and yeast extract. Food Chem. 201,
618 213–221.
619 Portu, J., López, R., Ewald, P., Santamaría, P., Winterhalter, P., Garde-Cerdán, T.,
620 2018. Evaluation of Grenache, Graciano and Tempranillo grape stilbene content
621 after field applications of elicitors and nitrogen compounds. J. Sci. Food Agric. 98,

622 1856-1862.
623 Portu, J., López, R., Santamaría, P., Garde-Cerdán, T., 2017. Elicitation with methyl
624 jasmonate supported by precursor feeding with phenylalanine: Effect on Garnacha
625 grape phenolic content. Food Chem. 237, 416–422.
626 Portu, J., Santamaría, P., López-Alfaro, I., López, R., Garde-Cerdán, T., 2015b. Methyl
627 jasmonate foliar application to tempranillo vineyard improved grape and wine
628 phenolic content. J. Agric. Food Chem. 63, 2328–2337.
629 Quezada-Paladines, D.F., Fernández-Fernández, J.I., Bautista-Ortín, A.B., Gómez-
630 Plaza, E., Bleda-Sánchez, J.A., Gil-Muñoz, R., 2017. Influence of the use of
631 elicitors over the composition of cell wall grapes, in: Escribano-Bailón, M.T.,
632 García Estévez, I., González Paramás, A.M., Dueñas Patón, M. (Eds.), Book of
633 Abstracts IVAS 2017. Fundación General Universidad Salamanca, Salamanca.
634 Ribéreau-Gayon, P., Stonestreet, E., 1965. Determination of anthocyanins in red wine.
635 Bull. Soc. Chim. Fr. 9, 2649–2652.
636 Romero-Pérez, A.I., Lamuela-Raventós, R.M., Andrés-Lacueva, C., De La Carmen
637 Torre-Boronat, M., 2001. Method for the quantitative extraction of resveratrol and
638 piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene
639 content. J. Agric. Food Chem. 49, 210–315.
640 Ruiz-García, Y., Gómez-Plaza, E., 2013. Elicitors: a tool for improving fruit phenolic
641 content. Agriculture 3, 33–52.
642 Ruiz-García, Y., Romero-Cascales, I., Bautista-Ortín, A.B., Gil-Muñoz, R., Martínez-
643 Cutillas, A., Gómez-Plaza, E., 2013. Increasing bioactive phenolic compounds in
644 grapes: Response of six monastrell grape clones to benzothiadiazole and methyl
645 jasmonate treatments. Am. J. Enol. Vitic. 64, 459–465.
646 Ruiz-García, Y., Romero-Cascales, I., Gil-Muñoz, R., Fernández-Fernández, J.I.,

López-Roca, J.M., Gómez-Plaza, E., 2012. Improving grape phenolic content and wine chromatic characteristics through the use of two different elicitors: Methyl jasmonate versus benzothiadiazole. J. Agric. Food Chem. 60, 1283–1290.

Ruiz, M., 1999. La Crianza del Vino Tinto desde la Perspectiva Vitícola, La Crianza del Vino Tinto desde la Perspectiva Vitícola. AMV Ediciones, Madrid, Spain.

Sun, J., Wang, Y., Chen, X., Gong, X., Wang, N., Ma, L., Qiu, Y., Wang, Y., Feng, S., 2017. Effects of methyl jasmonate and abscisic acid on anthocyanin biosynthesis in callus cultures of red-fleshed apple (*Malus sieversii* f. *niedzwetzkyana*). Plant Cell. Tissue Organ Cult. 130, 227–237.

Trought, M.C.T., Parker, A.K., Van Leeuwen, C., 2015. Can a change in vineyard practice mitigate warming due to climate change? Acta Hortic. 1082, 397–402.

Villangó, S., Pásti, G., Kállay, M., Leskó, A., Balga, I., Donkó, A., Ladányi, M., Pálfi, Z., Zsófi, Z., 2015. Enhancing phenolic maturity of Syrah with the application of a new foliar spray. South African J. Enol. Vitic. 36, 304–315.

Wei, J., Wen, X., Tang, L., 2017. Effect of methyl jasmonic acid on peach fruit ripening progress. Sci. Hortic. 220, 206–213.

Xia, E.-Q., Deng, G.-F., Guo, Y.-J., Li, H.-B., 2010. Biological activities of polyphenols from grapes. Int. J. Mol. Sci. 11, 622–646.

Xu, A., Zhan, J.-C., Huang, W.-D., 2015. Effects of ultraviolet C, methyl jasmonate and salicylic acid, alone or in combination, on stilbene biosynthesis in cell suspension cultures of *Vitis vinifera* L. cv. Cabernet Sauvignon. Plant Cell. Tissue Organ Cult. 122, 197–211.

Table 1. Yield and must parameters from control vines and from vines treated with methyl jasmonate (MeJ) over two growing seasons (2015 and 2016) in two different grape varieties (Tempranillo and Graciano).

	Tempranillo				Graciano			
	2015		2016		2015		2016	
	Control	MeJ	Control	MeJ	Control	MeJ	Control	MeJ
Yield (kg plant^{-1})	6.01±0.67	5.80±1.41	3.44±0.89	3.70±0.42	3.24±0.48	3.50±0.90	4.21±0.74	4.25±0.13
Weight of 100 berries (g)	213.45±3.89	216.00±8.94	139.72±3.31b	127.87±4.30a	149.10±22.87	132.23±5.23	140.03±5.01	136.23±2.55
ºBrix	22.4±0.1	22.6±1.6	23.7±0.4	23.8±0.5	23.6±0.9	23.4±0.8	24.2±0.7	23.5±0.9
Probable alcohol (% v/v)	13.0±0.1	13.2±1.1	13.9±0.3	14.0±0.4	13.9±0.6	13.7±0.5	14.3±0.5	13.8±0.6
pH	3.46±0.06	3.43±0.06	3.83±0.06	3.78±0.03	3.31±.04	3.37±0.04	3.19±0.02B	3.15±0.03A
Total acidity (g L^{-1})[a]	4.60±0.13	4.78±0.18	3.90±0.13	3.96±0.06	7.23±0.23	7.06±0.37	7.10±0.05a	7.72±0.02b
Tartaric acid (g L^{-1})	6.93±0.23	6.86±0.05	6.63±0.07	6.72±0.12	6.98±0.22A	7.87±0.57B	9.94±0.12a	10.55±0.25b
Malic acid (g L^{-1})	1.46±0.18	1.35±0.21	1.76±0.06b	1.52±0.05a	1.91±0.27	1.79±0.12	1.05±0.15	0.92±0.13
Potassium (mg L^{-1})	1434±200	1399±85	1665±15b	1538±48a	1537±40	1545±37	1346±57	1254±92

All parameters are listed with their standard deviation (n = 3). For each parameter, growing season and variety, different letters indicate significant differences between the samples at $p \leq 0.05$ (lower case) and $p \leq 0.10$ (upper case). [a]As g L^{-1} tartaric acid.

Table 2. Percentage of variability attributable to each factor (treatment, growing season and variety) and the interaction between them on the grape general parameters.

	Treatment	Growing season	Variety	Treatment X Growing season	Treatment X Variety	Growing season X Variety	Treatment X Growing season X Variety	Residual
Yield (kg plant^{-1})	0.14 NS	9.69*	15.62**	0.06 NS	0.07 NS	45.48***	0.54 NS	28.41
Weight of 100 berries (g)	1.16 NS	36.00***	25.12***	0.00 NS	0.17 NS	31.76***	0.97 NS	4.81
^{o}Brix	0.80 NS	20.74*	8.75 NS	0.80 NS	3.02 NS	6.19 NS	0.37 NS	59.35
Probable alcohol (% v/v)	0.73 NS	20.98*	8.86 NS	0.78 NS	3.07 NS	6.05 NS	0.34 NS	59.18
pH	0.08 NS	4.24***	61.20***	0.53 NS	0.22 NS	31.34***	0.18 NS	2.21
Total acidity (g L^{-1})[a]	0.32*	0.65**	94.43***	0.30*	0.02 NS	2.80***	0.56**	0.91
Tartaric acid (g L^{-1})	1.66**	19.47***	48.51***	0.01 NS	1.58**	26.74***	0.14 NS	1.89
Malic acid (g L^{-1})	4.40*	19.79***	2.21 NS	0.26 NS	0.11 NS	60.09***	0.24 NS	12.89
Potassium (mg L^{-1})	5.00 NS	1.03 NS	10.37**	3.07 NS	0.51 NS	60.29***	0.00 NS	19.73

Statistically significant at: *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$, respectively. NS, not significant. [a]As g L^{-1} tartaric acid.

Table 3. Anthocyanins content (mg kg^{-1}) in control grapes and grapes from vines treated with methyl jasmonate (MeJ) over two growing seasons (2015 and 2016) in two different grape varieties (Tempranillo and Graciano).

	Tempranillo				Graciano			
	2015		2016		2015		2016	
	Control	MeJ	Control	MeJ	Control	MeJ	Control	MeJ
Dp-3-glc	309.2±13.9a	422.0±11.8b	200.2±30.9A	266.0±38.9B	240.6±86.1	222.2±64.7	155.4±24.2	152.5±19.5
Cn-3-glc	35.32±7.95A	56.37±9.12B	23.18±5.57	35.71±11.08	85.46±28.92	69.98±21.56	30.79±1.60	28.20±4.93
Pt-3-glc	223.4±7.7a	301.9±8.1b	153.5±21.1A	196.8±25.8B	200.0±64.2	196.0±53.2	143.6±20.8	145.9±18.4
Pn-3-glc	73.77±15.00A	112.6±14.3B	49.88±11.17	69.42±17.27	520.7±115.2	507.0±121.6	285.7±5.2	305.0±34.1
Mv-3-glc	707.9±32.1a	903.4±32.3b	420.3±42.8	480.4±41.3	921.5±108.2	1029±123	844.3±53.0	900.6±39.3
∑ non-acylated	1350±77a	1796±70b	847±111	1048±134	1968±400	2024±368	1460±322	1532±111
Dp-3-acglc	30.68±1.02a	36.87±1.71b	17.97±1.65a	22.57±2.25b	38.01±7.05	38.48±5.92	22.74±2.60	22.36±2.14
Cn-3-acglc	6.42±0.30A	6.96±0.17B	3.86±0.26	4.42±0.50	11.81±2.25	10.49±2.03	4.68±0.22	4.59±0.31
Pt-3-acglc	18.21±0.46	19.24±0.55	12.62±0.67a	15.19±1.23b	22.47±4.49	20.69±4.09	12.52±1.39	11.88±0.87
Pn-3-acglc	6.04±0.48	6.63±0.28	4.24±0.27	4.95±0.53	44.65±6.67	41.60±8.97	17.04±0.65	18.09±1.03
Mv-3-acglc	51.58±1.76	52.14±3.32	41.67±1.31A	45.87±2.36B	112.2±4.2	112.2±8.2	86.02±3.47	90.93±7.38
Dp-3-cmglc	74.43±0.73	82.67±5.91	56.53±1.09a	71.39±4.26b	25.40±7.75	24.64±3.25	16.63±2.29	16.80±0.86
Cn-3-cmglc	11.87±0.80A	14.22±0.95B	8.41±0.83A	11.60±2.11B	13.40±3.30	11.79±1.88	5.60±0.27	5.62±0.35
Pt-3-cmglc	62.53±0.60	67.60±4.18	49.62±0.78a	60.11±2.47b	26.21±6.09	26.81±2.85	19.04±2.54	19.71±1.13
Pn-3-cmglc	28.20±2.21A	33.26±1.43B	18.21±1.35A	22.35±2.46B	113.4±16.6	116.0±12.4	56.60±1.98a	64.49±1.51b
Mv-3-*cis*-cmglc	10.50±0.47	9.47±0.53	7.63±0.53	7.96±0.37	7.57±0.78	7.89±1.09	7.65±0.74	8.12±0.38
Mv-3-*trans*-cmglc	282.0±1.3	302.9±24.0	218.2±7.8A	234.4±7.5B	200.1±2.9	226.5±28.0	236.0±22.2A	266.2±7.9B
Mv-3-cfglc	5.03±0.14	5.20±0.03	5.62±0.22B	5.02±0.38A	6.36±0.51	6.05±0.22	4.71±0.46	4.58±0.21
∑ acylated	587±10	635±36	445±11a	506±18b	622±54	643±21	489±34	533±15
∑ anthocyanins	1937±87a	2432±68b	1292±119A	1554±151B	2590±454	2667±389	1949±130	2066±121

Nomenclature abbreviations: Dp, delphinidin; Cn, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-p-coumaroylglucoside; cfglc, caffeoylglucoside.

All parameters are listed with their standard deviation (n = 3). For each parameter, growing season and variety, different letters indicate significant differences between the samples at $p \leq 0.05$ (lower case) and $p \leq 0.10$ (upper case).

686 **Table 4.** Flavonols content (mg kg^{-1}) in control grapes and grapes from vines treated with methyl jasmonate (MeJ) over two growing seasons (2015 and 2016) in two different
687 grape varieties (Tempranillo and Graciano).

	Tempranillo				Graciano			
	2015		2016		2015		2016	
	Control	MeJ	Control	MeJ	Control	MeJ	Control	MeJ
M-3-glcU+M-3-gal	11.79±3.03	14.02±2.47	12.65±1.83	14.26±1.47	4.24±0.89	4.58±0.33	6.72±0.78A	8.41±0.84B
M-3-glc	44.65±9.14	48.18±3.53	44.21±6.17	49.67±5.99	27.03±8.21	28.07±4.78	28.53±2.27	30.92±3.61
Q-3-glcU	16.39±0.82	18.82±7.14	23.21±1.60a	33.52±4.92b	15.84±0.29	17.70±3.20	31.11±4.70a	42.75±5.33b
Q-3-glc	19.18±4.57	18.19±7.89	20.74±2.06a	31.15±5.93b	17.15±4.14	17.99±2.26	23.17±2.28a	31.84±3.66b
L-3-glc	5.87±1.13	6.19±0.61	6.91±1.15	7.33±1.22	6.01±0.86	6.43±0.51	8.19±0.35	9.06±0.86
K-3-gal	0.63±0.11	0.76±0.48	0.68±0.07a	1.37±0.23b	0.94±0.08	1.00±0.08	0.51±0.03a	0.71±0.08b
K-3-glc	6.04±1.40	6.20±3.35	5.74±0.39a	10.64±2.54b	2.93±1.13	3.38±0.27	3.32±0.28a	5.26±0.71b
I-3-glc	1.43±0.33	1.21±0.45	1.74±0.45	2.15±0.61	5.87±1.38	6.40±0.65	6.56±0.59a	8.48±0.95b
S-3-glc	2.80±0.39A	3.53±0.28B	4.04±0.69	4.35±0.77	8.99±0.92	9.53±1.16	11.50±0.53	12.72±0.90
∑ flavonols	108.8±20.9	115.1±24.8	119.9±13.9A	154.4±22.1B	88.98±17.12	95.09±11.05	120.0±10.0A	150.5±16.4B

688 Nomenclature abbreviations: M, myricetin, Q, quercetin; L, laricitrin; K, kaempferol; I, isorhamnetin; S, syringetin; glcU, glucuronide; gal, galactoside; glc, glucoside.
689 All parameters are listed with their standard deviation (n = 3). For each parameter, growing season and variety, different letters indicate significant differences between the
690 samples at $p \leq 0.05$ (lower case) and $p \leq 0.10$ (upper case).

Table 5. Flavanols, phenolic acids and stilbenes content (mg kg^{-1}) in control grapes and grapes from vines treated with methyl jasmonate (MeJ) over two growing seasons (2015 and 2016) in two different grape varieties (Tempranillo and Graciano).

	Tempranillo				Graciano			
	2015		2016		2015		2016	
	Control	MeJ	Control	MeJ	Control	MeJ	Control	MeJ
Flavanols								
Catechin	74.12±0.41	86.77±9.60	59.09±4.79	57.07±7.48	96.71±16.85	114.0±15.7	97.92±7.77	104.12±7.57
Epicatechin	72.73±7.20	76.94±12.44	31.15±2.99	31.48±3.58	183.1±12.6	211.4±19.9	128.9±11.7	132.2±5.5
Epicatechin-3-gallate	23.55±5.35	28.09±3.34	21.01±2.80	23.46±2.18	76.23±8.55	77.51±3.02	37.86±5.30	37.80±1.63
Epigallocatechin	0.86±0.46	1.39±0.38	0.23±0.35	0.37±0.33	3.20±0.53	3.76±0.38	2.18±0.71	2.25±0.27
Procyanidin B1	45.01±4.10	49.98±4.21	16.10±1.18	15.43±0.36	70.04±3.50	72.74±4.86	35.42±4.11	37.81±2.04
Procyanidin B2	28.96±2.79	31.79±4.48	n.d.	n.d.	59.33±7.15	62.52±4.76	54.30±2.15	54.86±2.68
Total	245.2±11.3	275.0±27.4	127.6±7.8	127.8±12.8	488.7±31.8	541.9±35.2	356.6±29.2	369.1±12.4
Hydroxybenzoic acid								
Gallic acid	27.76±1.35	28.18±3.75	11.98±0.51	13.15±0.89	12.07±0.44	12.97±0.70	7.85±0.94	8.62±0.33
Hydroxycinnamic acids								
trans-Caftaric acid	34.70±2.14	39.23±5.45	13.69±3.16	19.44±3.53	11.36±1.78	13.62±1.04	22.57±10.06	26.71±3.98
trans+*cis*-Coutaric acids	36.45±2.43	37.71±4.33	16.36±2.54	20.16±3.35	7.73±0.96	8.56±1.11	15.48±5.83	17.62±1.95
trans-Fertaric acid	4.06±0.15	4.25±0.46	1.73±0.01	1.87±0.16	2.99±0.08	2.94±0.26	2.48±0.26a	3.02±0.05b
Total	75.20±4.71	81.19±9.84	31.77±5.62	41.47±6.93	22.09±2.70	25.12±2.26	40.53±16.12	47.35±5.96
Stilbenes								
trans-Piceid	0.74±0.16a	1.84±0.03b	0.63±0.06a	1.30±0.33b	11.52±1.43	14.36±1.83	16.68±2.23a	21.24±1.53b
cis-Piceid	0.14±0.03a	0.65±0.12b	1.37±0.25	1.58±0.34	2.82±0.48A	3.74±0.12B	12.65±1.11	14.98±1.60
trans-Resveratrol	0.09±0.02a	0.23±0.06b	n.d.	n.d.	0.29±0.03	0.41±0.18	1.17±0.34a	2.16±0.37b
Viniferin	0.02±0.03a	0.12±0.03b	0.16±0.01	0.16±0.00	0.27±0.02a	0.38±0.02b	0.28±0.02A	0.37±0.06B
Total	0.98±0.21a	2.83±0.18b	2.16±0.31A	3.04±0.60B	14.90±1.91A	18.88±1.79B	30.77±3.49a	38.75±2.96b

All parameters are listed with their standard deviation (n = 3). For each parameter, growing season and variety, different letters indicate significant differences between the samples at $p \leq 0.05$ (lower case) and $p \leq 0.10$ (upper case). n.d. = not detected.

Table 6. Percentage of variability attributable to each factor (treatment, growing season and variety) and the interaction between them on the total amount of the main group of phenolic compounds in grape.

	Treatment	Growing season	Variety	Treatment X Growing season	Treatment X Variety	Growing season X Variety	Treatment X Growing season X Variety	Residual
Anthocyanins	5.79*	49.00***	27.11***	0.24 NS	2.03 NS	0.50 NS	0.47 NS	14.85
Acylated anthocyanins	8.80**	76.11***	3.75*	0.37 NS	0.55 NS	0.26 NS	0.03 NS	10.14
Non-acylated anthocyanins	5.11*	42.93***	32.01***	0.45 NS	2.29 NS	0.53 NS	0.58 NS	16.10
Flavonols	14.00**	43.58***	4.42 NS	6.42 NS	0.04 NS	2.99 NS	0.03 NS	28.52
Flavanols	0.69*	24.47***	72.51***	0.37 NS	0.10 NS	0.12 NS	0.01 NS	1.73
Hydroxycinnamic acids	2.21	6.12**	30.35***	0.19 NS	0.12 NS	52.04***	0.00 NS	8.97
Stilbenes	1.80***	11.56***	74.55***	0.08 NS	0.71**	9.90***	0.21 NS	1.19

Statistically significant at: *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$, respectively. NS, not significant.