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Could foliar applications of methyl jasmonate and methyl jasmonate + urea improve must grape aroma composition?

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Abstract

BACKGROUND: Grape aromas are formed by a great number of volatile compounds. Methyl jasmonate (MeJ) and urea (Ur) foliar applications have been studied to improve grape quality, but their combined application has never been studied.

RESULTS: In both seasons, MeJ application enhanced terpenoids and C6 compounds synthesis, though decreased alcohols content. Moreover, MeJ + Ur treatment reduced benzenoids and alcohols and did not affect C_{13}-norisoprenoids content. However, there was no clear effect of these treatments on the rest of the volatile compounds. Multifactorial analysis showed a season effect on all volatile compounds, except terpenoids. Discriminant analysis showed a good separation among samples under treatment criterion. The great effect of MeJ treatment on terpenoids was probably due to this elicitor influencing their biosynthesis.

CONCLUSION: Season has a strong influence on grapes aromatic composition since it affects all volatile compound families except terpenoids. MeJ foliar application enhanced terpenoids, C_{13}-norisoprenoids and C6 compounds synthesis, whereas decreased alcohols content; however, MeJ + Ur foliar treatment did not affect C_{13}-norisoprenoids and C6 compounds, and decreased benzenoids and alcohols grape compounds. Therefore, no synergistic effect was observed between Ur and MeJ on grape volatile compounds biosynthesis. Foliar application of MeJ seems to be sufficient to improve the aromatic quality of grapes.

Keywords: methyl jasmonate; urea; Tempranillo; foliar application; volatile compounds

INTRODUCTION

Grape aromas are comprising of several hundreds of volatile compounds which belong to different chemical groups. The compounds responsible for the called varietal aroma or primary aroma are mainly, terpenoids, C_{13}-norisoprenoids, esters, and benzenoid compounds.[1-3] Terpenoids contribute to the floral and citrus character, within these compounds are α-terpineol, nerol, geraniol, linalool, and citronellol, among others.[4] The C_{13}-norisoprenoids are important for the floral aroma of grapes, among which, β-damascenone and β-ionone are the most important, providing rose and violet aromas.[5] Both, terpenoids and C_{13}-norisoprenoids are closely related in variety and have the lowest perception thresholds among the volatile compounds that are studied in this work.[6,7] The C6 compounds are related to the herbaceous aroma of grapes, being predominantly (*Z*)-3-hexen-1-ol and hexanol.[6] In grapes, few esters are present, but their aroma is described as fruity. Benzenoid compounds come from phenolic acids and are related to fruity aromas.[3,8]

Numerous approaches have been studied for improving the grape aroma, such as agronomic practices, clonal selection, and the foliar application to grapevines of elicitors.[3,7] Elicitors are compounds that do not show any antimicrobial activity, but are able to trigger the defense mechanism in plants, increasing the secondary metabolites production, such as phenolic and volatile compounds.[6,8,9] Methyl jasmonate (MeJ) is an elicitor studied as foliar application to grapevines to enhance grape quality, and its effect on aromatic profile of grapes has been also studied.

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D'Onofrio *et al.*[8] showed an increase in the content of several volatile compounds in grapes. Terpenoids have undergone the highest increase, but also isoamyl alcohol, methyl vanillate, acetovanillate, and *cis*-3-hexenyl hexanoate increased their concentration. Previously, Gómez-Plaza *et al.*[6] described an increase on the content of hexanal, *E*-2-hexenal, (*E*)-2-hexenol, and also, a higher content of terpenoids when compared with control grapes, after MeJ application, while in another study it was observed that MeJ application increased the content of secondary metabolites from shikimate pathway and induced the lipid peroxidation and lipoxygenase (LOX) activity.[10,11] Furthermore, Wang *et al.*[12] studied the effect of jasmonates in grapes and showed an increase in terpene content and the expression of terpene genes in grapes. It is noteworthy that Garde-Cerdán *et al.*[3] investigated the effect of MeJ foliar application to Tempranillo grapevines on grape aroma composition over three vintages. These authors suggested that its application to vineyard can lead to an activation of the terpene metabolism which cause the accumulation of some of these compounds in grapes. Although, they also concluded that the influence of MeJ on grape volatile composition was season dependent. Moreover, Gutiérrez-Gamboa *et al.*[13] described a decrease on the content of several volatile compounds (terpenoids, C_{13}-norisoprenoids, benzenoids, and esters) in grapes after foliar application of MeJ to vineyard.

Furthermore, nitrogen foliar application has been studied to improve certain grape components related with its quality, such as phenolic and volatile compounds.[14] Nitrogen is an important compound in grapes since nitrogen participates in the processes from grapevine growth to wine fermentation and its use in vineyard can improve grape and wine quality. In fact, urea (Ur) has been thoroughly studied due to its physico-chemical characteristics, owing its high water solubility and small molecular size.[15,16] The effect of foliar application of Ur to vineyard with respect to volatile compounds has been studied. Garde-Cerdán *et al.*[17] described that the highest dose of Ur produced an increase on geraniol, MeJ, benzyl alcohol and total positive compounds (sum of terpenoids, C_{13}-norisoprenoids, esters, and benzenoid compounds), while 2-phenylethanal, (*E*)-2-hexenal and C6 compounds underwent a decrease in comparison with control grapes; whereas the grapes from grapevines foliar sprayed with the lowest dose of Ur increased the geraniol, *β*-ionone, and benzyl alcohol content, while (*Z*)-*β*-damascenone, 2-phenylethanal and total benzenoids were decreased compared to control. Furthermore, Garde-Cerdán *et al.*[5] described a decrease of the content of terpenoids, C_{13}-norisoprenoids, esters, benzenoids and C6 compounds in grapes after the foliar treatment with Ur. In addition, the effectiveness of foliar application has been associated with climate, variety, timing of the application and season dependent and seems to be more effective when the requirement of nitrogen is bigger in vines.[15,18,19]

Here we are concerned that the combined foliar application of these two compounds, MeJ and Ur have not been studied previously on must volatile composition. Considering the earlier mentioned, the foliar application of MeJ + Ur could be a good practice to improve grape quality. Therefore, the aim of this work was to study the influence of MeJ and MeJ + Ur on Tempranillo grape volatile composition over two consecutive seasons.

MATERIALS AND METHODS

Vineyard site, grapevine treatments and samples

Red grapes from Tempranillo (*Vitis vinifera* L.) variety grown in the experimental vineyard of Finca La Grajera, located in Logroño, La Rioja, Spain (latitude: 42° 26′ 25.36″ N; longitude: 2° 30′ 56.41″ W; 456 m above sea level) during 2019 and 2020 seasons were used. Vines grafted onto a R-110 rootstock were planted in 1997 and were trained to a VSP (vertical shoot positioned) trellis system with a vine spacing of 2.80 m × 1.25 m. In this study, three treatments were carried out by foliar application to vineyard of: (i) control, (ii) methyl jasmonate (MeJ), and (iii) methyl jasmonate plus urea (MeJ + Ur). To apply the treatments, aqueous solutions were prepared with a concentration of 10 mmol L^{-1} of MeJ (following previous studies[3,20]) and the same concentration of MeJ + Ur in a total dose of 6 kg N ha^{-1} (according to Pérez-Álvarez *et al.*[16]); Tween 80 (1 mL L^{-1}) was used as wetting agent. Control plants were sprayed with a water solution of Tween 80 alone. The treatments were applied to vineyard twice, at veraison and 1 week later, and, for each application, 200 mL per plant was sprayed over leaves. The treatments were performed in triplicate and were arranged in a complete randomized block design along the vineyard, with 10 vines for each replication and treatment.

Grapes were harvested at their optimum technological maturity, i.e., when the probable alcohol reached 13°, and the weight of 100 berries remained constant. A set of 100 berries was collected haphazard, these berries were separated and weighed to obtain the average berry weight. Grape berries were then crushed to obtain the must and general parameters and volatile composition were determined.

Determination of general parameters in the musts

The °Brix, probable alcohol, pH, and total acidity in must were analyzed according to the official methods of OIV (International Organization of Vine and Wine).[21] Analysis of glucose, glucose + fructose, malic acid, total phenols, amino nitrogen, ammonium nitrogen and yeast assimilable nitrogen (YAN) were carried out using a Miura One enzymatic analyser (TDI, Barcelona, Spain). As the treatments were performed in triplicate, the results of these parameters are shown as the average of three analyses ($n = 3$).

Analysis of must volatile compounds by HS-SPME-GC–MS

The determination of volatile compounds in must was carried out by headspace-solid-phase microextraction (HS-SPME) and its later analysis was performed by gas chromatography (GC) coupled to mass spectrometry (MS), according to the methodology described by Garde-Cerdán *et al.*[3] Briefly, in a 20 mL glass vial (Supelco, Bellenfonte, PA, USA), 9 mL of must sample, 2.5 g of sodium chloride (NaCl) and 10 μL of internal standard (2-octanol) were added. Then, the vial was placed in the GC–MS (Agilent, Palo Alto, CA, USA). The sample conditioning was at a controlled temperature of 60 °C during 15 min with stirring (500 rpm). Afterwards, the SPME fiber [DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane), 50/30 μm, Supelco] was inserted automatically in the head space to extract the volatile compounds, during 105 min at 60 °C with stirring (500 rpm). After concluding the extraction process, the SPME fiber, which contains the volatile compounds of the samples, was immediately introduced into the GC injection port at 250 °C and kept for 15 min during desorption of volatile compounds. The chromatograph system (GC) and the column used was the same described in Garde-Cerdán *et al.*[3] Identification was performed using the NIST library and comparing with the mass spectrum and retention index of chromatographic standards (Sigma-Aldrich, Madrid, Spain), when these are available, but also by comparison with data found in bibliography. A semi-quantification was carried out, relating the areas of each compound to the area and known concentration of the internal standard. Since treatments were performed in triplicate, the results of grape volatile compounds are expressed as the average of the three replicates ($n = 3$).

Statistical analyses

SPSS version 21.0 statistical package for Windows (SPSS, Chicago, IL, USA) was used for the statistical elaboration of the data. General parameters and volatile compounds data were processed using the analysis of variance (ANOVA) ($P \leq 0.05$). Differences among means were compared using the Duncan test. A multifactor analysis was carried out to study the effect of treatment, season, and their interaction. Indeed, a discriminant analysis was performed with the volatile compounds data in order to classify the different samples.

RESULTS AND DISCUSSION

General parameters in the musts

Table 1 shows the enological parameters in the grapes from control and vines treated with MeJ and with MeJ + Ur, in 2019 and 2020 seasons. In 2019, MeJ foliar application produced a significant decrease in °Brix, and therefore, a reduction in the probable alcohol compared to the control, whereas MeJ + Ur treatment did not produce differences, in these parameters, with control and MeJ grapes. The decrease in the probable alcohol undergone by MeJ grapes is interesting to palliate the effect of climate change, that are producing wines with a high alcohol level,[22] and has been previously described.[8,11] Moreover, MeJ and MeJ + Ur foliar applications decreased glucose and fructose content with respect to control grapes. Wang *et al.*[12] recently described this effect of MeJ treatment in a study about the influence of hormone applications to *Gewürztraimer* berries. In addition, MeJ treatment can accelerate or delay the ripening of grapes when applied at low or high concentration.[8,23] However, the foliar application of both treatments increased total phenols, ammonium nitrogen and amino nitrogen content, as well as YAN, with respect to control grapes. García-Pastor *et al.*[23] and Portu *et al.*[24] reported that MeJ increases grapes total phenolic concentration because of the improvement on phenylalanine ammonia lyase (PAL) activity. In 2020, grapes from grapevines treated with MeJ showed a higher content of malic acid than control grapes but did not show differences on this parameter with respect to MeJ + Ur foliar treatment. The rest of the general parameters did not show differences among control grapes and grapes from grapevines with foliar applications.

Previous studies showed slight differences or no differences in general parameters after MeJ treatment in agreement with the results of 2020 season.[3,20,25] With respect to Ur foliar application, some authors have described that application did not affect the general parameters, except for nitrogen content, which underwent an increase after Ur foliar application.[26]

YAN must concentrations of control grapes in both seasons were quite different, they varied from 196.51 to 273.69 mg N L^{-1} in control grapes in 2019 and 2020 seasons, respectively. This could be explained by meteorological conditions, August 2020 was more rainy than August 2019 season (32.9 L m^{-2} in 2020 *versus* 11.5 L m^{-2} in 2019) which could explain the higher weight of 100 berries in 2020 season than in 2019 (Table 1). The recording of greater preharvest rainfalls favors the absorption of more nitrogen and nutrients from the soil through the roots[27] which could explain the differences among seasons on must nitrogen content.

Table 2 shows the multifactor ANOVA of must general parameters, with the two factors studied, treatment and season. The treatment factor affected total phenols content, amino nitrogen and YAN content. Both treatments produced a significant increase in total phenols content, whereas only MeJ + Ur foliar application produced a significant increase in amino nitrogen and YAN content when compared with control grapes. Season factor affected the weight of 100 berries, which was higher in 2020 season as earlier mentioned, this effect can be explained by the higher rains recorded in 2020. However, total acidity, fructose, malic acid, total phenols and amino nitrogen content were higher in 2019 season, also according with the differences on climatic conditions, since higher preharvest rainfall recorded in 2020 season can produced a dilution effect on some general parameters.[11] Treatment and season interaction affected the content of nitrogen parameters: ammonium nitrogen, amino nitrogen and YAN.

Table 1. General parameters in grapes from control, methyl jasmonate (MeJ) and MeJ + urea (MeJ + Ur) treatments, in 2019 and 2020 seasons

	2019			2020		
	Control	MeJ	MeJ + Ur	Control	MeJ	MeJ + Ur
Weight of 100 berries (g)	113.68 ± 11.07a	141.81 ± 27.18a	131.52 ± 25.19a	199.57 ± 7.27a	207.67 ± 40.39a	222.83 ± 25.25a
°Brix	24.70 ± 0.72b	22.23 ± 1.17a	23.03 ± 0.60ab	22.30 ± 0.92a	22.17 ± 2.31a	22.77 ± 0.74a
Probable alcohol (% *v/v*)	14.63 ± 0.49b	12.92 ± 0.80a	13.48 ± 0.42ab	12.97 ± 0.63a	12.89 ± 1.58a	13.29 ± 0.51a
pH	3.83 ± 0.05a	3.78 ± 0.10a	3.80 ± 0.04a	3.76 ± 0.01a	3.70 ± 0.07a	3.71 ± 0.03a
Total acidity (g L^{-1})[†]	4.61 ± 0.11a	5.20 ± 0.36a	5.11 ± 0.36a	4.12 ± 0.33a	4.54 ± 1.08a	3.83 ± 0.13a
Glu + Fru (g L^{-1})	249.86 ± 9.97b	215.50 ± 12.29a	226.67 ± 5.67a	216.42 ± 10.70a	218.62 ± 26.56a	228.85 ± 9.85a
Glu (g L^{-1})	120.18 ± 5.13b	102.88 ± 6.89a	107.43 ± 3.65a	107.31 ± 4.54a	106.08 ± 12.84a	113.11 ± 6.85a
Fru (g L^{-1})	129.68 ± 4.84b	112.62 ± 5.43a	119.25 ± 2.52a	109.11 ± 6.53a	112.54 ± 13.76a	115.75 ± 3.49a
Malic acid (g L^{-1})	2.24 ± 0.24a	2.54 ± 0.32a	2.45 ± 0.46a	1.21 ± 0.08a	1.54 ± 0.22b	1.42 ± 0.05ab
Total phenols (mg L^{-1})	1185.33 ± 72.31a	1306.57 ± 61.35b	1351.83 ± 29.05b	541.60 ± 64.02a	603.07 ± 73.82a	578.17 ± 82.64a
Ammonium nitrogen (mg N L^{-1})	78.00 ± 8.22a	106.34 ± 15.68b	118.30 ± 6.54b	121.16 ± 3.52a	101.66 ± 19.58a	109.72 ± 8.59a
Amino nitrogen (mg N L^{-1})	118.51 ± 14.33a	202.11 ± 50.59b	237.60 ± 30.51b	152.53 ± 14.33a	139.63 ± 35.64a	149.89 ± 7.06a
YAN (mg N L^{-1})	196.51 ± 21.18a	308.45 ± 64.76b	355.90 ± 31.59b	273.69 ± 17.69a	241.29 ± 55.05a	259.61 ± 13.65a

Abbreviations: Fru, fructose; Glu, glucose; YAN, yeast assimilable nitrogen.

[†] As g L^{-1} of tartaric acid. All parameters are listed with their standard deviation ($n = 3$). For each season and parameter, different letters indicate significant differences between the samples ($P \leq 0.05$).

Table 2. 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) treatments) and season (2019 and 2020) and their interaction (treatment × season)

	Weight of 100 berries (g)	°Brix	Probable alcohol (% *v/v*)	pH	Total acidity ($g\ L^{-1}$)[†]	Glu + Fru ($g\ L^{-1}$)	Glu ($g\ L^{-1}$)	Fru ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	Total phenols ($mg\ L^{-1}$)	Ammonium nitrogen ($mg\ N\ L^{-1}$)	Amino nitrogen ($mg\ N\ L^{-1}$)	YAN ($mg\ N\ L^{-1}$)
Treatment (T)													
Control	156.63a	23.50a	13.80a	3.79a	4.37a	233.14a	113.74a	119.39a	1.73a	863.47a	99.58a	135.52a	235.10a
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	104.00 a	170.87 ab	274.87 ab
MeJ + Ur	177.18a	22.90a	13.39a	3.76a	4.47a	227.76a	110.27a	117.50a	1.94a	965.00b	114.01a	193.75b	307.76b
Season (S)													
2019	129.00a	23.32a	13.68a	3.80a	4.98b	230.68a	110.16a	120.52b	2.41b	1281.24b	100.88a	186.07b	286.95a
2020	210.02 b	22.41a	13.51a	3.72a	4.17a	221.30a	108.83a	112.46a	1.39a	574.28a	110.85a	147.35a	258.20a
Interaction													
T × S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	**	**

Abbreviations: Fru, fructose; Glu, glucose; YAN, yeast assimilable nitrogen.

[†] As $g\ L^{-1}$ of tartaric acid. For each parameter and factor, different letters indicate significant differences between samples ($P \leq 0.05$). Interaction: n.s., not significant ($P > 0.05$); **, $P \leq 0.01$.

Influence of the foliar MeJ and MeJ + Ur treatments on must volatile compounds

Figures 1–3 and Table 3 show the results of must volatile primary aroma content in control and treated grapevines with MeJ and with MeJ + Ur, in 2019 and 2020 seasons. Tempranillo variety is included into the 'neutral' varieties, in which most of the volatile compounds in grapes are in the bound glycoside form, which is odorless.[28]

In 2019 season, MeJ treatment sprayed to grapevines increased the must concentration of *p*-cymene (97%), linalool (142%), α-terpinol (167%) and total terpenoids (45%) content with respect to the control treatment (Fig. 1(b–d,h), respectively). These results agree with the effect of MeJ foliar application observed by Marín-San Román *et al.*[7] Geranic acid was the only terpenoid that underwent a significant decrease in its content (34%) in MeJ grapes when compared with control grapes (Fig. 1(f)). The content of geraniol and geranyl acetone was not affected by the MeJ foliar application to the grapevines (Fig. 1(e,g), respectively). Furthermore, Wang *et al.*[12] described an increase on terpenoid concentrations and into the expression of terpene genes in grapes, which support the increase of terpenoids observed in MeJ grapes (Fig. 1). However, MeJ + Ur foliar application produced a different effect, that is, a decrease on limonene (41%), geranic acid (51%), geranyl acetone (69%) and total terpenoids (29%) content respect to the control berries (Fig. 1(a,f,g,h), respectively). The rest of the terpenoids was not affected by the MeJ + Ur foliar application to the grapevines. Moreover, some differences among treatments were found; in this sense, MeJ grapes showed a higher content of limonene, *p*-cymene, linalool, α-terpineol, geranyl acetone, and total terpenoids when compared with MeJ + Ur grapes. The increase on the synthesis of *p*-cymene observed in MeJ grapes was previously described by Garde-Cerdán *et al.*,[3] although Gutiérrez-Gamboa *et al.*[13] observed a decreased on the content of all terpenoids in berries from grapevines treated with MeJ. However, Garde-Cerdán *et al.*[17] described an increase on geraniol after Ur foliar application to grapevine, at the two doses studied, whereas Garde-Cerdán *et al.*[5] observed a decrease on the synthesis of terpenoids in grapes from grapevines sprayed with Ur, as we observed in MeJ + Ur grapes. In this season, geranic acid was the most abundant terpenoid in control grapes followed by *p*-cymene and limonene. These three compounds represented a 67% of total terpenoids in the control samples.

In 2020, the effect of both treatments over terpenoids was different to the effect observed in 2019 season, this agrees with the season or climatical dependance of the effect of foliar treatments.[3,19] MeJ and MeJ + Ur grapes showed a higher content of *p*-cymene (114% and 97%, respectively), linalool (141% and 73%, respectively), α-terpineol (91% and 156%, respectively), geranic acid (175% and 63%, respectively), geranyl acetone (135% and 51%, respectively) and total terpenoids (102% and 77%, respectively) than control grapes (Fig. 1(b–d,f–h), respectively). These results are consistent with previous studies carried out with different varieties, Monastrell and Sangiovese, where an increase of total terpenoids was described in MeJ grapes.[6,8] and with the study of Wang *et al.*,[12] where an increase on the expression of terpene genes in grapes was described after MeJ treatment. Furthermore, MeJ + Ur grapes presented a higher content of geraniol (77%) when compared with control grapes (Fig. 1(e)). Differences among treatments also were found: MeJ + Ur grapes were characterized by a lower content of linaool, geranic acid, and geranyl acetone and, a higher content of α-terpineol when compared with

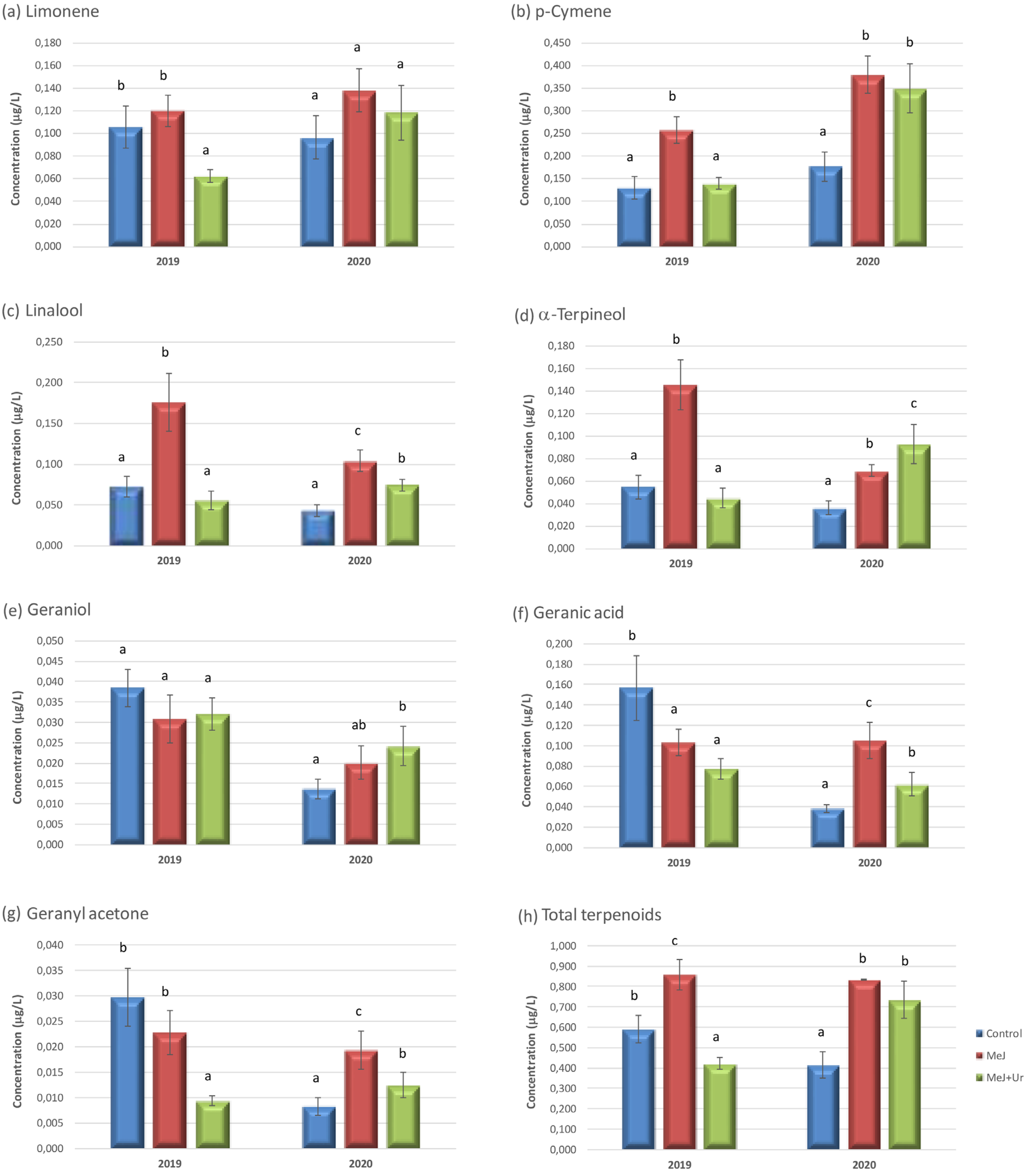

Figure 1. Terpenoids concentration (μg L^{-1}) in grapes from control, methyl jasmonate (MeJ) and MeJ + urea (MeJ + Ur) treatments in 2019 and 2020 seasons. All parameters listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between samples ($P \leq 0.05$).

MeJ grapes (Fig. 1). *p*-Cymene was the most abundant terpenoid in control grapes in this season, followed by limonene and linalool, representing a 77% of total terpenoids in the control samples.

Multifactor analysis showed MeJ foliar treatment increased all terpenoids, except for geraniol, geranic acid and geranyl acetone, when compared with control grapes (Table 4). Therefore, MeJ treatments seem not to improve the synthesis of derivates of

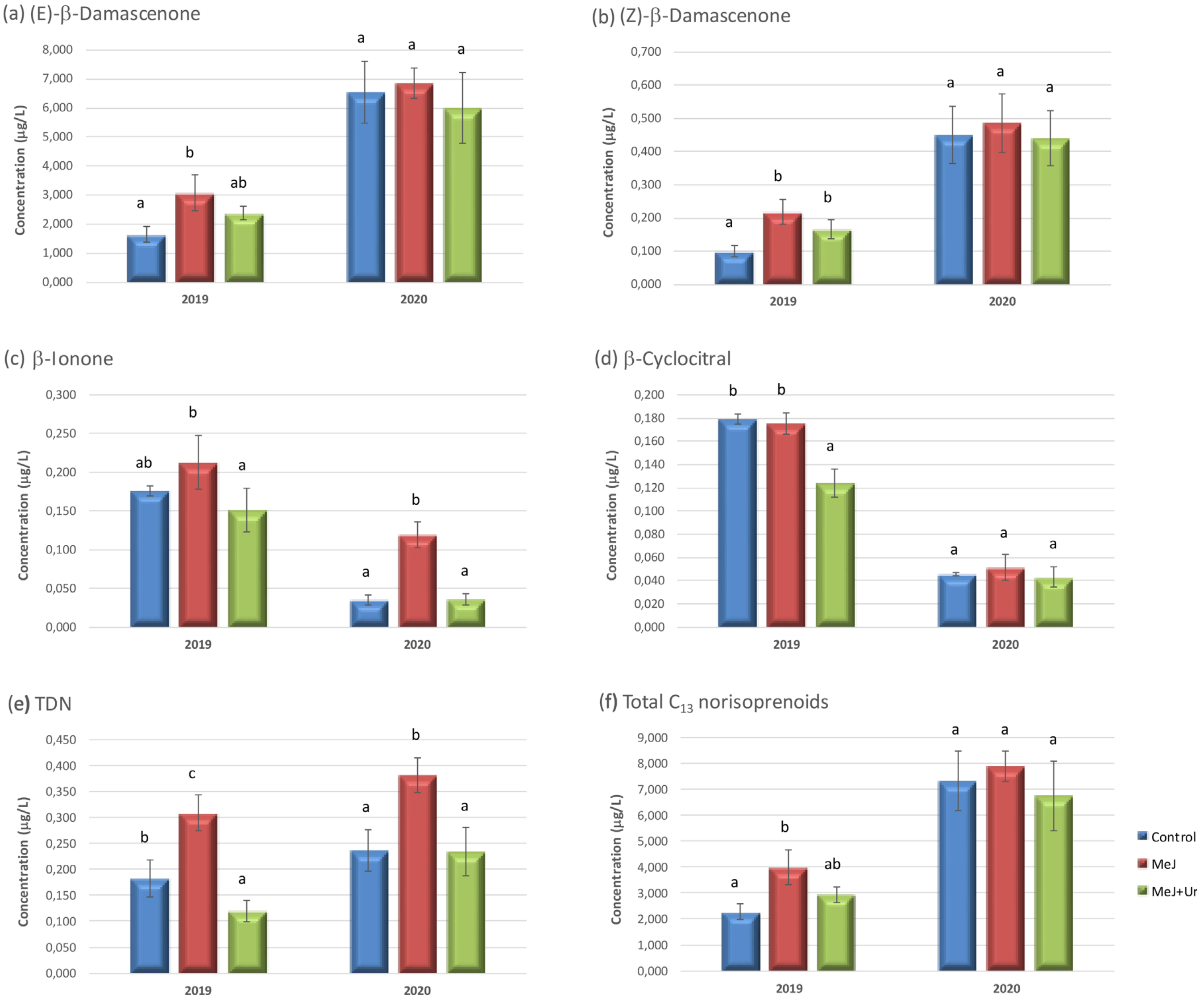

Figure 2. The C_{13} norisoprenoids concentration (μg L^{-1}) in grapes from control, methyl jasmonate (MeJ) and MeJ + urea (MeJ + Ur) treatments in 2019 and 2020 seasons. All parameters listed with their standard deviation ($n = 3$). For each season and compound, different letters indicate significant differences between samples ($P \leq 0.05$). TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene.

geraniol but, MeJ foliar treatment increases the synthesis of terpenoids as earlier mentioned. MeJ + Ur foliar application increased *p*-cymene and *α*-terpineol content when compared with control grapes. Season affected the individual content of terpenoids, but not the total content. Limonene and *p*-cymene content was higher in 2020 season, whereas the rest of individual terpenoids showed a higher content in 2019 season. Treatment and season interaction was significant for the content of all terpenoids.

With respect to C_{13}-norisoprenoids, in 2019 season, MeJ grapes showed a higher content of (*E*)-*β*-damascenone (88%), (*Z*)-*β*-damascenone (119%), TDN (69%) and total C_{13}-norisoprenoids (75%) when compared to control grapes (Fig. 2(a,b,e,f), respectively); whereas MeJ + Ur grapes showed a higher content of (*Z*)-*β*-damascenone (65%) and a lower content of *β*-cyclocitral (31%) and TDN (35%) respect to control grapes (Fig. 2(b,d,e), respectively). MeJ grapes presented a higher content of *β*-ionone, *β*-cyclocitral and TND with respect to MeJ + Ur grapes (Fig. 2(c,d, e)). This significant effect of MeJ foliar treatment on C_{13}-norisoprenoids content can be explained because MeJ treatment increases the biosynthesis of these compounds, that are derivates from the biodegradation of carotenoids.[29] Garde-Cerdán *et al.*[17] described an increase in *β*-ionone and a decrease on (*Z*)-*β*-damascenone when compared with control grapes, with the lowest dose of Ur applied. Cheng *et al.*[14] described in their work that foliar nitrogen application during veraison did not have a negative effect on volatile content in grapes. However, Garde-Cerdán *et al.*[3] observed that MeJ foliar application produced grapes with lower *β*-damascenone and did not affect the *β*-ionone content, when compared with control grapes, but these effects changed with the season, since, in the other year of the study, MeJ treatment did not affect total C_{13}-norisoprenoids content.

In 2020, MeJ grapes showed a higher content of *β*-ionone (24%) and TDN (62%) than control grapes (Fig. 2(c,e), respectively). MeJ + Ur did not showed differences with control grapes in any C_{13}-norisoprenoids (Fig. 2). MeJ treatment increased, in both seasons, the *β*-ionone content in grapes, probably due to the fact that MeJ can accelerate the degradation of *β*-carotene, which is a *β*-ionone precursor.[7]

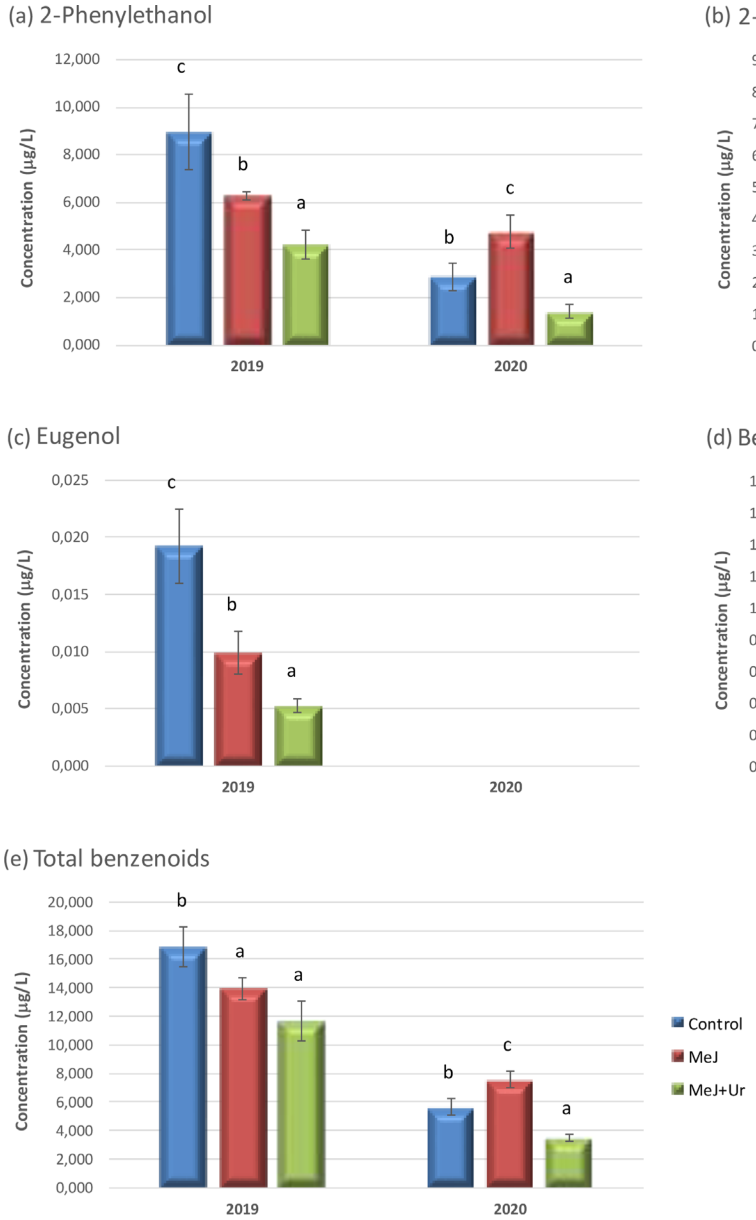

Figure 3. Benzenoid compounds concentration (µg L^{-1}) in grapes from control, methyl jasmonate (MeJ) and MeJ + urea (MeJ + Ur) treatments in 2019 and 2020 seasons. All parameters listed with their standard deviation ($n = 3$). For each season and compound, different letters indicate significant differences between samples ($P \leq 0.05$).

(*E*)-*β*-Damascenone was the most abundant C_{13}-norisoprenoid in all samples and seasons (Fig. 2), as was expected, since this compound is one of the most abundant C_{13}-norisoprenoids in grapes.[7] This compound is very important because it contributes to the odor of wines due to its low odor threshold and it is related to a baked apple aroma.[30]

The multifactor analysis showed MeJ foliar application increased the content of *β*-ionone, TDN and total terpenoids when compared with control grapes. MeJ + Ur foliar treatment did not affect C_{13}-norisoprenoids, except for *β*-cyclocitral, which underwent a decrease in comparison with control grapes. Season affected C_{13}-norisoprenoids, showing a higher content of (*E*)-*β*-damascenone, (*Z*)-*β*-damascenone, TDN and total C_{13}-norisoprenoids in 2020 season, and the content of *β*-ionone and *β*-cyclocitral were higher in 2019 season. Treatment and season interaction only was significant for *β*-cyclocitral. Therefore, MeJ + Ur foliar treatment did not improve the C_{13}-norisoprenoids content in grapes.

With respect to benzenoid compounds, in 2019 season, MeJ and MeJ + Ur grapes showed a lower content of 2-phenylethanol (30% and 53%, respectively), eugenol (49% and 73%, respectively), benzyl alcohol (44% and 59%, respectively) and total benzenoids (17% and 31%, respectively) when compared with control grapes (Fig. 3(a,c,d,e), respectively). As can be observed, both treatments produced a generalized decrease of benzenoids, with the decrease being stronger for MeJ + Ur grapes. Therefore, MeJ and MeJ + Ur treatments did not favor the synthesis of these compounds. In 2020, MeJ grapes showed an increase of 2-phenylethanol (66%) and total benzenoids (34%) with respect to control grapes (Fig. 3(a,e), respectively); whereas MeJ + Ur grapes were characterized

Table 3. Alcohols, carbonyl compounds, C6 compounds and other compounds concentration (μg L^{-1}) in grapes from control, methyl jasmonate (MeJ) and MeJ + urea (MeJ + Ur) treatments, in 2019 and 2020 seasons

	2019			2020		
	Control	MeJ	MeJ + Ur	Control	MeJ	MeJ + Ur
Alcohols						
n-Heptanol	0.062 ± 0.010b	0.046 ± 0.008ab	0.034 ± 0.007a	0.047 ± 0.002b	0.044 ± 0.009b	0.028 ± 0.002a
n-Octanol	0.191 ± 0.014b	0.174 ± 0.017b	0.126 ± 0.022a	0.326 ± 0.018b	0.234 ± 0.042a	0.183 ± 0.032a
n-Nonanol	0.064 ± 0.006b	0.059 ± 0.010b	0.036 ± 0.000a	0.197 ± 0.036b	0.245 ± 0.048b	0.076 ± 0.015a
1-Octen-3-ol	0.595 ± 0.043b	0.296 ± 0.063a	0.259 ± 0.057a	0.174 ± 0.036b	0.074 ± 0.006a	0.101 ± 0.020a
2-Ethyl-1-hexanol	3.088 ± 0.060b	1.798 ± 0.309a	2.083 ± 0.432a	1.870 ± 0.131c	0.863 ± 0.132a	1.362 ± 0.102b
Total	4.001 ± 0.108b	2.373 ± 0.387a	2.539 ± 0.505a	2.613 ± 0.048c	1.460 ± 0.156a	1.750 ± 0.142b
Carbonyl compounds						
Heptanal	0.055 ± 0.009b	0.034 ± 0.007a	0.037 ± 0.005a	0.014 ± 0.002c	0.007 ± 0.001a	0.010 ± 0.001b
(*E*)-2-Octenal	0.059 ± 0.005a	0.051 ± 0.009a	0.049 ± 0.005a	0.043 ± 0.009b	0.024 ± 0.004a	0.036 ± 0.005ab
Nonanal	0.204 ± 0.039b	0.115 ± 0.028a	0.129 ± 0.016a	0.381 ± 0.074b	0.143 ± 0.025a	0.239 ± 0.043a
(*E*)-2-Nonenal	0.065 ± 0.007a	0.068 ± 0.007a	0.107 ± 0.018b	0.047 ± 0.008b	0.031 ± 0.001a	0.028 ± 0.005a
Decanal	0.076 ± 0.013b	0.070 ± 0.011b	0.041 ± 0.008a	0.112 ± 0.023c	0.068 ± 0.014b	0.032 ± 0.007a
(*E*,*E*)-2,4-Hexadienal	1.177 ± 0.245ab	1.567 ± 0.261b	0.767 ± 0.136a	0.711 ± 0.133b	0.208 ± 0.015a	0.995 ± 0.202c
(*E*,*E*)-2,4-Nonadienal	0.097 ± 0.011a	0.112 ± 0.026a	0.082 ± 0.009a	0.040 ± 0.005b	0.026 ± 0.005a	0.049 ± 0.003b
γ-Decalactone	0.125 ± 0.024b	0.157 ± 0.030b	0.070 ± 0.003a	0.146 ± 0.029a	0.141 ± 0.021a	0.178 ± 0.028a
6-Methyl-3,5-heptadien-2-one	0.086 ± 0.017b	0.079 ± 0.015b	0.037 ± 0.006a	0.022 ± 0.005ab	0.029 ± 0.004b	0.018 ± 0.003a
Total	1.942 ± 0.278b	2.254 ± 0.286b	1.318 ± 0.194a	1.515 ± 0.258b	0.676 ± 0.049a	1.587 ± 0.165b
C6 compounds						
n-Hexanol	5.904 ± 1.031a	7.018 ± 1.447a	7.380 ± 1.475a	22.311 ± 3.544a	42.324 ± 4.178b	25.531 ± 4.995a
Hexanal	22.040 ± 2.145b	28.064 ± 5.929b	13.214 ± 0.911a	11.784 ± 1.942a	16.831 ± 2.431b	14.308 ± 1.256ab
(*Z*)-3-Hexen-1-ol + (*E*)-2-Hexen-1-ol	1.027 ± 0.187b	0.340 ± 0.065a	0.419 ± 0.051a	0.669 ± 0.115a	1.080 ± 0.206b	0.414 ± 0.076a
(*E*)-2-Hexenal	5.474 ± 1.044b	10.305 ± 2.251c	2.493 ± 0.207a	9.629 ± 0.776a	19.002 ± 3.906b	12.783 ± 0.678a
Total	34.445 ± 3.815a	45.727 ± 8.718b	23.506 ± 0.687a	44.393 ± 4.949a	79.237 ± 5.398b	53.036 ± 3.747a
Other compounds						
Hexyl acetate	n.d.	n.d.	n.d.	0.206 ± 0.043a	0.721 ± 0.159b	0.096 ± 0.020a
Methyl jasmonate	0.064 ± 0.006ab	0.077 ± 0.009b	0.059 ± 0.010a	1.738 ± 0.381b	0.222 ± 0.038a	0.664 ± 0.133a

Note: All parameters are listed with their standard deviation ($n = 3$). For each season and compound, different letters indicate significant differences between the samples ($P \leq 0.05$). n.d.: not detected.

by a lower content of 2-phenylethanol (51%) and total benzenoids (38%) when compared with control grapes (Fig. 3(a,e), respectively). Eugenol was not found in grapes from 2020 season. Overall, 2-phenylethanol was the most abundant benzenoid compound in both seasons, as previously described by other authors.[7,17] 2-Phenylethanol is related to rose aromatic descriptor.[5,30]

There are contradictory results in previous studies about the effect of MeJ foliar application in benzenoid compounds. Garde-Cerdán *et al.*[3] showed different effects of MeJ foliar application in each season studied: no effect, a decrease, and an increase of total benzenoids. This can be explained by the meteorological dependence of MeJ treatment described by Paladines-Quezada *et al.*[11] These authors also described that meteorological conditions influenced most of the components of the cell wall and can affect the content of various components in grapes. Furthermore, Marín-San Román *et al.*[7] did not observe an effect of MeJ treatment on these compounds. With regard to the effect of Ur foliar application on benzenoid compounds, only the benzyl alcohol content was affected, which underwent a decrease.[5] This effect of reduction of the content of benzyl alcohol also was observed in MeJ + Ur grapes in 2019 season, however, in 2020, differences on the content of benzyl alcohol were not found.

With regard to the multifactor analysis, MeJ foliar application produced a decrease in eugenol and benzyl alcohol when compared with control grapes; whereas MeJ + Ur treatment decreased all benzenoid compounds, except for 2-phenylethanal, and total benzenoids when compared with control grapes (Table 4). MeJ grapes showed a higher content of 2-phenylethanol, eugenol and total benzenoids than MeJ + Ur grapes. Season affected all benzenoids compounds, where content was higher in 2019 than in 2020. Treatment and season interaction was significant for all benzenoids, except for 2-phenylethanal.

Table 3 shows the content of alcohols, carbonyl compounds, C6 compounds and other compounds for the control and treated grapes in both seasons. In 2019, MeJ grapes presented a lower content of 1-octen-3-ol (50%), 2-ethyl-1-hexanol (42%) and total alcohols (41%) respect to control grapes. MeJ + Ur grapes showed a lower content of all individual alcohols and, therefore, a lower total alcohols content (37%) when compared with control grapes. In 2020, a similar trend was observed with respect to alcohols. MeJ grapes showed a lower content of *n*-octanol (28%), 1-octen-3-ol (57%), 2-ethyl-1-hexanol (54%) and total alcohols (44%) with respect to control grapes (Table 3). MeJ + Ur sprayed on grapevines produced a decrease on all alcohols (33%) when compared with control grapes, although the content of 2-ethyl-1-hexanol was higher in MeJ + Ur than MeJ grapes.

Table 4. Multifactor analysis of variance of grape volatile compounds (expressed as μg L^{-1})

	Treatment (T)			Season (S)		
	Control	MeJ	MeJ + Ur	2019	2020	Interaction (T × S)
Terpenoids						
Limonene	0.101a	0.129b	0.091a	0.096a	0.118b	*
p-Cymene	0.154a	0.319c	0.244b	0.176a	0.302b	**
Linalool	0.058a	0.140b	0.065a	0.101b	0.074a	**
α-Terpineol	0.045a	0.107c	0.069b	0.082b	0.066a	***
Geraniol	0.026a	0.025a	0.028a	0.034b	0.019a	**
Geranic acid	0.098b	0.104b	0.070a	0.112b	0.069a	***
Geranyl acetone	0.019b	0.021b	0.011a	0.021b	0.013a	***
Total	0.501a	0.846b	0.577a	0.622a	0.661a	***
C_{13}_norisoprenoids						
(*E*)-*β*-Damascenone	4.093a	4.964a	4.190a	2.361a	6.471b	n.s.
(*Z*)-*β*-Damascenone	0.275a	0.353a	0.302a	0.161a	0.459b	n.s.
β-Ionone	0.105a	0.186b	0.094a	0.180b	0.063a	n.s.
β-Cyclocitral	0.112b	0.113b	0.083a	0.159b	0.047a	***
TDN	0.210a	0.346b	0.177a	0.204b	0.284b	n.s.
Total	4.795a	5.942b	4.846a	3.065a	7.324b	n.s.
Benzenoid compounds						
2-Phenylethanol	5.917b	5.528b	2.828a	6.500b	3.016a	***
2-Phenylethanal	4.492a	4.722a	4.331a	6.717b	2.313a	n.s.
Eugenol	0.010c	0.005b	0.003a	0.011b	n.d.a	***
Benzyl alcohol	0.826b	0.511a	0.411a	0.933b	0.232a	***
Total	11.245b	10.766b	7.573a	14.161b	5.561a	**
Alcohols						
n-Heptanol	0.055c	0.045b	0.031a	0.048b	0.040a	n.s.
n-Octanol	0.258c	0.204b	0.155a	0.164a	0.248b	*
n-Nonanol	0.130b	0.152b	0.056a	0.053a	0.173b	***
1-Octen-3-ol	0.384b	0.185a	0.180a	0.384b	0.116a	***
2-Ethyl-1-hexanol	2.479c	1.330a	1.723b	2.323b	1.365a	n.s.
Total	3.307b	1.916a	2.144a	2.971b	1.941a	n.s.
Carbonyl compounds						
Heptanal	0.034b	0.020a	0.024a	0.042b	0.010a	n.s.
(*E*)-2-Octenal	0.051b	0.038a	0.043ab	0.053b	0.034a	n.s.
Nonanal	0.292c	0.129a	0.184b	0.149a	0.254b	*
(*E*)-2-Nonenal	0.056a	0.049a	0.068b	0.080b	0.035a	***
Decanal	0.094c	0.069b	0.036a	0.062a	0.071a	*
(*E,E*)-2,4-Hexadienal	0.944a	0.888a	0.881a	1.170b	0.638a	***
(*E,E*)-2,4-Nonadienal	0.069a	0.069a	0.065a	0.097b	0.065a	**
γ-Decalactone	0.135a	0.149a	0.124a	0.117a	0.155b	**
6-Methyl-3,5-heptadien-2-one	0.054b	0.054b	0.027a	0.067b	0.023a	**
Total	1.729a	1.465a	1.452a	1.838b	1.259a	***
C6 compounds						
n-Hexanol	14.107a	24.671b	16.456a	6.767a	30.055b	***
Hexanal	16.912a	22.448b	13.761a	21.106b	14.308a	**
(*Z*)-3-Hexen-1-ol + (*E*)-2-Hexen-1-ol	0.848b	0.710b	0.416a	0.595a	0.721a	***
(*E*)-2-Hexenal	7.552a	14.653b	7.638a	6.091a	13.805b	*
Total	39.419a	62.482b	38.271a	34.559a	58.889b	**
Other compounds						
Hexyl acetate	0.103a	0.361b	0.048a	n.d.a	0.341b	***
Methyl jasmonate	0.901c	0.149a	0.362b	0.066a	0.875b	***

Note: For each parameter and factor, different letters indicate significant differences between samples ($P \leq 0.05$). Interaction: *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$, and n.s., not significant ($P > 0.05$).
Abbreviations: MeJ, methyl jasmonate; MeJ + Ur, MeJ + urea; TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene.

As far as we are concerned, there is few previous works that have studied the effect of MeJ and Ur foliar applications on alcohols. D'Onofrio *et al.*[8] described the effect of MeJ treatment on Sangiovese grapes in 1-octen-3-ol and 1-octanol. 1-Octen-3-ol was not affected by treatment whereas 1-octanol underwent an increase after MeJ treatment. Gómez-Plaza *et al.*[6] showed an absence of effect on 2-ethyl-1-hexanol content after MeJ treatment. Therefore, in view of the results for both seasons, MeJ and MeJ + Ur foliar application did not improve the biosynthesis of alcohols in grapes. Furthermore, a decrease on grapes alcohol content was observed after foliar treatments. 2-Ethyl-1-hexanol was the most abundant alcohol within this family of compounds in all samples, and in both seasons.

Multifactor analysis showed that MeJ grapes presented lower content of all individual alcohols and total alcohols, with the exception of *n*-nonanol, in comparison with control grapes, whereas MeJ + Ur grapes underwent a decrease on all alcohols and total alcohols (Table 4). Season affected alcohols, showing higher content of *n*-heptanol, 1-octen-3-ol, 2-ethyl-1-hexanol and total alcohols in 2019 season than in 2020. Treatment and season interaction was significant for *n*-octanol, *n*-nonanol and 1-octen-3-ol.

Carbonyl compounds are substances that have one or more aldehyde and ketone functions, and mainly are formed in alcoholic fermentation.[31] However, they are also present in small quantities in grapes. In 2019, MeJ foliar application produced grapes with a lower content of heptanal (38%) and nonanal (44%) than control grapes. Grapes from grapevines treated with MeJ + Ur showed a lower content of heptanal (33%), nonanal (37%), decanal (46%), γ-decalactone (44%), 6-methyl-3,5-heptadien-2-one (57%), and total carbonyl compounds (32%) and a higher content of (*E*)-2-nonenal (64%) when compared with control grapes. Some differences were found among treatments, MeJ grapes showed lower content of (*E*)-2-nonenal, but higher content of decanal, (*E*,*E*)-2,4-hexadienal, γ-decalactone, 6-methyl-3,5-heptadien-2-one, and total carbonyl compounds when compared with MeJ + Ur grapes. Therefore, MeJ + Ur treatment showed a greater effect on carbonyl compounds, producing grapes with the lowest carbonyl compounds content, whereas MeJ grapes did not show differences in total carbonyl compounds content with control grapes. In 2020, MeJ treatment sprayed to grapevines decreased the content of all carbonyl compounds and the total content (55%), except for γ-decalactone and 6-methyl-3,5-heptadien-2-one in comparison with control grapes. Foliar application of MeJ + Ur to grapevines decreased the content of heptanal (25%), nonanal (37%), (*E*)-2-nonenal (39%), and decanal (71%), and increased the content of (*E*,*E*)-2,4-hexadienal (40%) when compared with control grapes. The foliar treatments also showed differences, MeJ grapes were characterized by a lower content of heptanal, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-nonadienal, and total carbonyl compounds and a higher content of decanal and 6-methyl-3,5-heptadien-2-one than MeJ + Ur grapes. The most abundant carbonyl compound was (*E*,*E*)-2,4-hexadienal in all samples studied. Therefore, these foliar applications did not enhance the biosynthesis of carbonyl compounds. Gómez-Plaza *et al.*[6] described that heptanal and the sum of carbonyl compounds were not affected by MeJ treatment, whereas nonanal and (*E*)-2-octenal underwent a decrease after MeJ treatment, and (*E*,*E*)-2,4-hexadienal increased its content in MeJ grapes when compared with control grapes. These results partially agree with the MeJ effect observed.

Regarding the multifactor analysis, MeJ foliar treatment produced a decrease in some carbonyl compounds, i.e., heptanal, (*E*)-2-octenal, nonanal, and decanal, but total carbonyl compounds were not affected; while MeJ + Ur grapes showed a decrease on heptanal, nonanal, and decanal, and an increase on (*E*)-2-nonenal content when compared with control grapes (Table 4). Season affected all carbonyl compounds, except for decanal, that did not show differences among seasons. In 2019, the content of heptanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-nonadienal, 6-methyl-3,5-heptadien-2-one, and total carbonyl compounds was higher than in 2020 season. This season was characterized by a higher content of nonanal and γ-decalactone. Treatment and season interaction was significant for all carbonyl compounds and total carbonyl content, except for heptanal and (*E*)-2-octenal.

The C6 compounds can derived from fatty acids and are associated with green aromas in grapes.[29] These compounds are formed in the LOX pathway and are related with the 'green' fresh notes in wine,[32] although in high concentrations they give negative notes to the wine. In 2019 vintage, grapes from grapevines treated with MeJ showed lower content of (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol (67%) and higher content of (*E*)-2-hexenal (88%) and total C6 compounds (33%) when compared with control grapes. Whereas MeJ + Ur grapes showed a lower content of hexanal (40%), (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol (59%) and (*E*)-2-hexenal (54%) when compared with control grapes. Differences among treatments were found, MeJ grapes showed higher content of hexanal, (*E*)-2-hexenal and total C6 compounds when compared with MeJ + Ur grapes. Ju *et al.*[32] also reported an improvement on the content of C6 compounds after MeJ treatment, which can be related with the increase of LOX activity. In 2020 season, MeJ grapes showed a higher content of all C6 compounds and therefore a higher total C6 content than control grapes, whereas MeJ + Ur grapes did not present differences on C6 compounds in comparison with the content of control grapes. MeJ grapes showed a higher content of all C6 compounds than MeJ + Ur grapes except for the hexanal content. Garde-Cerdán *et al.*[3] described that the effect of MeJ foliar treatment on C6 compounds depended on the vintage. In the first season studied, these authors observed an increase in the hexanal content, as observed in MeJ grapes from 2020 season, and a decrease in (*Z*)-3-hexen-1-ol and *n*-hexanol content but, total C6 content was not affected. The decrease in (*Z*)-3-hexen-1-ol was observed in MeJ grapes from 2019. However, in the second vintage of their study, MeJ treatment increased *n*-hexanol content, as found in MeJ grapes from 2020, and once again a decrease on (*Z*)-3-hexen-1-ol content. These authors showed an improvement on C6 compounds after MeJ foliar application in one of the seasons studied, as we found in both vintages. However, Marín-San Román *et al.*[7] described an absence of effect on C6 compounds in grapes after MeJ foliar application, whereas Garde-Cerdán *et al.*[5] showed no effect for total C6 compounds after foliar treatment with Ur, which agrees with the results of MeJ + Ur grapes in both vintages, since MeJ + Ur grapes did not show differences in total C6 compounds when compared with control grapes. In 2019 season, hexanal was the most abundant C6 compound, whereas in 2020 *n*-hexanol was the predominant.

Multifactor analysis showed that MeJ grapes underwent an increase on all C6 compounds, except for (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol, and total C6 in comparison with control grapes (Table 4). However, MeJ + Ur grapes did not show differences from control grapes, with the exception of (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol, which underwent a decrease. The season affected all C6 compounds, except for (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol. The content of *n*-hexanol, (*E*)-2-hexenal and total C6 compounds was higher in 2020 than in 2019. Treatment and season interaction affected all C6 compounds, and the total content.

Finally, in 2019, hexyl acetate was not detected but MeJ was (Table 3). Differences among control and treated grapes were not found, but treatments showed differences. MeJ grapes

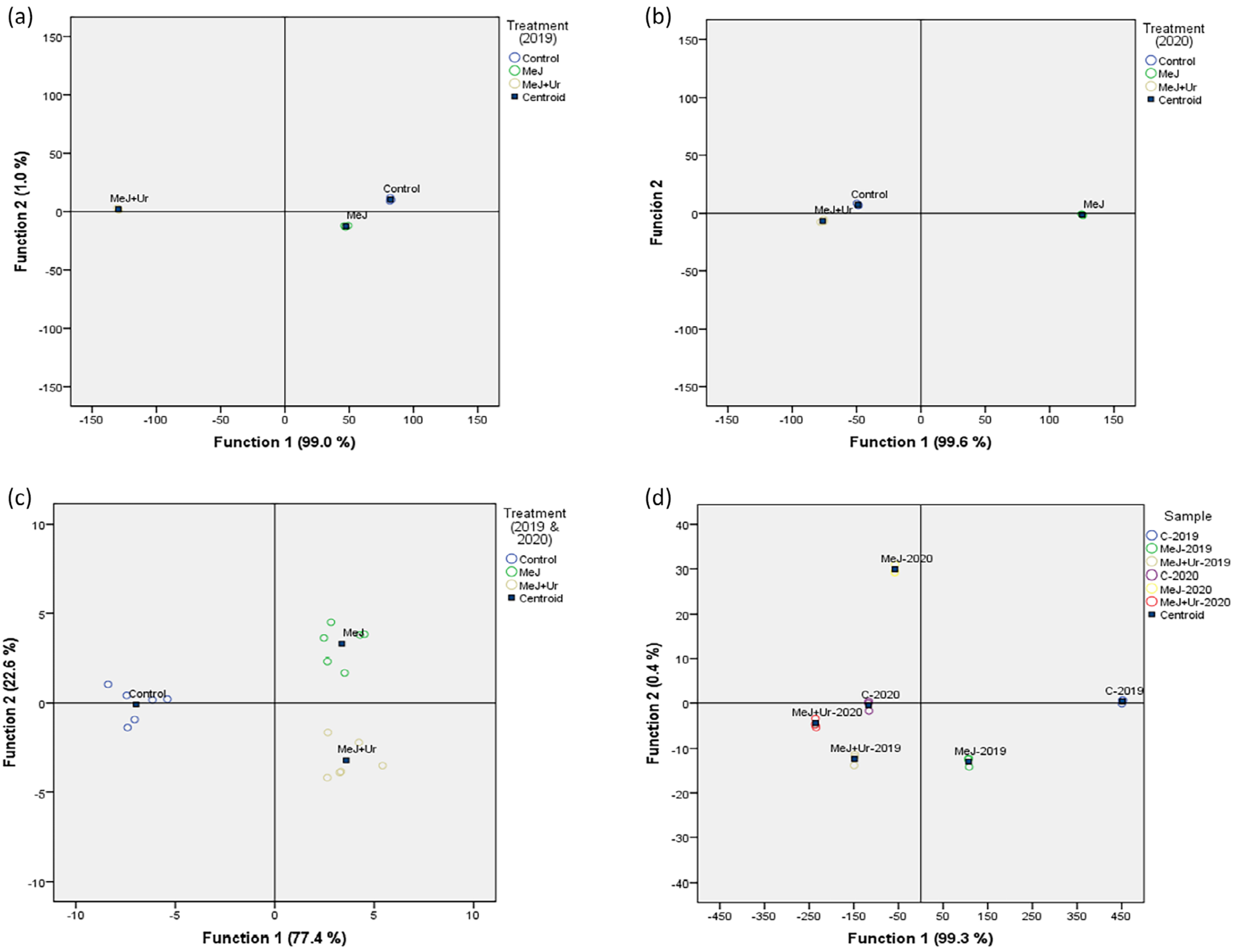

Figure 4. Discriminant analysis of volatile compounds content (μg L^{-1}) in grapes from control, methyl jasmonate (MeJ) and MeJ + urea (MeJ + Ur) treatments, in (a) 2019 season, (b) 2020 season, (c) 2019 and 2020 seasons with treatment as discriminant factor and (d) 2019 and 2020 seasons with sample as discriminant factor.

presented higher MeJ content than MeJ + Ur grapes. In 2020, MeJ grapes showed the highest hexyl acetate content, whereas control grapes and MeJ + Ur grapes did not show differences between them. Marín-San Román *et al.*[7] did not observe an effect on the content of hexyl acetate in grapes after foliar spraying of MeJ, whereas Garde-Cerdán *et al.*,[3] in their studies on the effect of MeJ on three vintages, described a different effect on these compounds, hexyl acetate and MeJ, in each season, so it is possible that the effect of season on these compounds was higher than the effect of the treatment. For the influence of Ur foliar application, Garde-Cerdán *et al.*[17] observed an increase on MeJ content after applying the highest dose of Ur when compared with control grapes, where the MeJ was not detected. Also, these authors described an increase on hexyl acetate content in grapes after Ur foliar application, in contrast with that observed in MeJ + Ur grapes from 2020 season. However, in Garde-Cerdán *et al.*,[5] Ur foliar treatment did not produce an increase of hexyl acetate content, as described in MeJ + Ur grapes from 2020.

Multifactor analysis showed that MeJ foliar treatment increased hexyl acetate content when compared with control grapes, whereas MeJ + Ur treatment did not affect this compound (Table 4). Season strongly affected the content of hexyl acetate, since in 2019 it was not detected. Treatment and season interactions significantly affected the content of hexyl acetate. MeJ and MeJ + Ur treatments affected the MeJ content, which underwent a decrease. Its content was affected by season, being higher in 2020 season. Treatment and season interactions affected the MeJ content.

Discriminant analysis

Figure 4 shows the discriminant analysis carried out with the grape volatile compounds of control and treated samples from: (Fig. 4(a)) 2019 season, (Fig. 4(b)) 2020 season, (Fig. 4(c)) both seasons with treatment as discriminant factor, and (Fig. 4(d)) both seasons with sample as discriminant factor.

Considering samples from 2019 season (Fig. 4(a)), Function 1 explained 99% and Function 2 explained 1%, so total of variance explained was 100%. The variables that contributed the most to the discriminant model were (*E*)-2-octenal, eugenol, nonanal, and α-terpineol (Function 1) and α-terpineol and (*E*)-2-octenal (Function 2). The discriminant model showed a clear separation among samples (Fig. 4(a)). Therefore, samples were separated under treatment criteria in this first vintage. MeJ + Ur samples were located farther from MeJ and control samples in agreement with its higher differences in grape volatile composition, since MeJ + Ur grapes showed a lower content of terpenoids, eugenol and carbonyl compounds than control and MeJ grapes. Considering samples from 2020 season (Fig. 4(b)), Function 1 explained 99.6% and Function 2 explained 0.4%, so total of variance explained was 100%. The variables that contributed the most to the discriminant model were (*E*)-2-hexenal, heptanol, ß-ionone and hexyl acetate (in both functions). Samples showed a good separation under treatment criteria, but the trend observed was the opposite than in 2019 season. In 2020, MeJ grapes were located farther from MeJ + Ur and control grapes, which were located nearer between them (Fig. 4(b)). This result can be explained because MeJ grapes showed, in 2020, the highest (*E*)-2-hexenal and ß-ionone content.

Taking into account the samples from both seasons and using treatment as discriminant factor (Fig. 4(c)), Function 1 explained 77.4% and Function 2 explained 22.6%, thus total of variance explained was 100%. The variables that contributed the most to the discriminant model were (*E*)-2-nonenal, 2-ethyl-1-hexanol, decanal, and γ-decalactone (Function 1) and ß-ionone, TDN, decanal, and (*E*)-2-nonenal (Function 2). Samples showed a good separation, control grapes were located in the negative side of Function 1 due to its highest content of 2-ethyl-1-hexanol and decanal, whereas treatments were separated in agreement with the highest content of ß-ionone and TDN of MeJ grapes and the higher content of (*E*)-2-nonenal that MeJ + Ur grapes showed (Fig. 4(c)).

Finally, Fig. 4(d) shows the discriminant analysis of samples from both seasons using sample as discriminant factor. Function 1 explained 99.3% and Function 2 explained 0.4%, so total of variance explained was 99.7%. The variables that contributed the most to the discriminant model were eugenol, heptanol, (*E*)-2-nonenal, and (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol (Function 1) and hexyl acetate, (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol, γ-decalactone, heptanol, and (*E*)-ß-damascenone (Function 2). A diagonal line can separate the samples under season criterion, locating samples from 2019 in the right side and samples from 2020 in the left side (Fig. 4(d)). Control sample from 2019 was the most different sample on this season, was located in the right side of the Function 1 in agreement with its high content of heptanol, 2-phenylethanol, and eugenol. MeJ + Ur sample from 2019 was located in the left side of Function 1 due to its highest content of (*E*)-2-nonenal. MeJ sample from 2019 showed intermediate contents of 2-phenylethanol and eugenol. MeJ sample from 2020 was the most different sample in this year, was located in the positive side of Function 2 and characterized by the highest content of (*Z*)-3-hexen-1-ol + (*E*)-2-hexen-1-ol. Control and MeJ + Ur samples were located nearer, indicating less differences among them. These samples were located in the left side of Function 1 due to its similar content on (*E*)-2-nonenal (Fig. 4(d)). Thus, season has an influence on grape volatile composition, but treatment also influenced the volatile composition of the different samples showing their separation under one unique criterion.

CONCLUSIONS

To the authors' knowledge, this is the first work that studies the effect of foliar application of MeJ plus Ur combined on grape volatile composition. In the multifactorial analysis of grape general parameters, it was observed that both treatments increased the total phenol content in musts, and MeJ + Ur treatment increased the amino nitrogen and YAN must content. Higher preharvest rainfall recorded in 2020 produced grapes with a higher weight and, a dilution effect on some general parameters, such as total acidity, fructose or total phenols content. Furthermore, foliar application of MeJ increased the synthesis of terpenoids and C6 compounds, whereas produced a decrease in alcohols in both seasons. MeJ + Ur foliar treatment did not affect C_{13}-norisoprenoids and C6 compounds, while decreased benzenoids and alcohols. With respect to the other families of volatile compounds, foliar treatments did not exert a consistent effect, due to different climatic conditions among seasons. Multifactorial analysis confirmed the enhancement of volatile composition after MeJ foliar application. MeJ foliar application seems to be a good tool to enhance grape volatile composition. Season affected all families of volatile compounds except for terpenoids, due to the great effect of MeJ treatment on the synthesis of these compounds. In addition, MeJ treatment improved the production of volatile aromas from fatty acids, C6 compounds. This article contributes to the understanding of the biochemical response of grapevines to exogenous applications of MeJ and MeJ + Ur on grape volatile composition. A synergetic effect among MeJ and Ur has not been observed in the biosynthesis of volatile compounds, therefore it seems that foliar application of MeJ is enough to enhance grape volatile composition.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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