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Changes on grape volatile composition after elicitors and nitrogen compounds foliar applications to 'Garnacha', 'Tempranillo' and 'Graciano' vines

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Summary

Grape volatile compounds determine the wine quality and typicity. The aim of this work was to study the effects of several foliar applications in 'Garnacha', 'Tempranillo', and 'Graciano' grapevines in order to enhance their grape volatile composition. The field trial involved the application of two nitrogen compounds, urea (Ur) and phenylalanine (Phe), and two elicitors, methyl jasmonate (MeJ) and a yeast extract (YE). The analysis of grape volatile compounds was carried out by HS-SPME-GC-MS. For 'Garnacha', most terpenes, and C_{13} norisoprenoids increased their grape content by applying Ur and Phe, and especially MeJ. For 'Tempranillo', treatments with Ur and MeJ improved the synthesis of most terpenoids. For 'Graciano', a trend to decrease the terpenoids content in grapes with the treatments was observed; however, Phe application increased C_{13} norisoprenoids content. In conclusion, foliar application of, Phe or Ur, and an elicitor, MeJ, can be a useful tool to improve grape quality.

K e y w o r d s : volatile compounds; grape; must; HS-SPME-GC-MS; aroma; foliar application; elicitors; nitrogen compounds.

Introduction

Grape aroma composition is one of the most important parameters to determine the must and wine quality (Marín-San Román *et al.* 2020b), and it is the result of a multitude of interactions between a large number of chemical compounds belonging to the following families: terpenoids, C_{13} norisoprenoids, benzenoid compounds, esters, thiols, methoxypyrazines, C6 and C9 aldehydes, and alcohols (D'Onofrio *et al.* 2018, Cataldo *et al.* 2021, Garde-Cerdán *et al.* 2021). These compounds are part of the secondary metabolites of plants and form the so-called varietal aroma. Their synthesis depends on several factors such as grape variety, season, terroir, grape maturity, and viticultural practices (Robinson *et al.* 2014, Cataldo *et al.* 2022, Copper *et al.* 2022).

Terpenoids and C_{13} norisoprenoids greatly influence the aroma, because they have low perception thresholds. The most important terpenoids are linalool, α-terpineol, nerol, geraniol, and citronellol; and the most important C_{13} norisoprenoids are β-damascenone and β-ionone. These compounds strongly contribute to floral fragrances (Ribéreau-Gayon *et al*. 2006). C6 compounds are formed from the harvest until the beginning of the alcoholic fermentation and are characterized by herbaceous aromas (Wang *et al.* 2018). These compounds in high concentrations can provide unpleasant aromas (Pedroza *et al*. 2010).

On the other hand, it is known that nitrogen is an essential macronutrient for the correct growth and development of the vine. In turn, nitrogen deficiencies are the main cause of slow or incomplete fermentations (Bisson and Butzke 2000). Several factors affect grapevine nitrogen status, including climate and soil conditions, viticultural practices, and nitrogen form, timing and rate of application (Bell and Henschke 2005). The most common form of nitrogen fertilization is through the soil, so that it is absorbed by the plant's roots. However, contamination problems have led to the development of new fertilization techniques. One of these techniques is the foliar application. This application allows a quick and efficient assimilation, reducing soil contamination. Foliar fertilization is not a substitute for traditional fertilization but allows precise applications at crucial periods of plant development. For these reasons, in recent years, the number of works on foliar applications has increased. The effect of foliar application of nitrogen compounds on the content of phenolic compounds (Portu *et al*. 2015a, b and 2017), in stilbenes content (Garde-Cerdán *et al.* 2015a), in amino acids content (Garde-Cerdán *et al.* 2014 and 2018b, Pérez-Álvarez *et al.* 2017, Jiménez-Moreno *et al.* 2020), in volatile compounds content (Ancín-Azpilicueta *et al.* 2013, Barbosa *et al.* 2009, Garde-Cerdán *et al.* 2015b, Gutiérrez-Gamboa *et al.* 2018a, Rubio-Bretón *et al.* 2018), and chlorophyll and carotenoids content (Gutiérrez-Gamboa *et al.* 2018b) has been studied.

Another of the strategies developed in recent years is the application of elicitors, molecules which are able to trigger plant defense systems (Delaunois *et al.* 2014). Therefore, elicitors could be an alternative for plants to have greater resistance to pathogen attacks, thus limiting the use of pesticides in vineyards and improving the quality of grapes and wines (Bektas and Eulgem 2015). The application of elicitors has been observed to produce higher levels of secondary metabolites such as phenolic compounds (Gómez-Plaza *et al.* 2017, Portu *et al.* 2015c, 2016 and 2018), stilbenes (Gil-Muñoz *et al.* 2017), amino acids (Garde-Cerdán

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et al. 2016), volatile compounds (Garde-Cerdán et al. 2018a), and chlorophyll and carotenoids (Gutiérrez-Gamboa et al. 2018b).

Although foliar application is an increasingly used technique, more trials are necessary to obtain conclusive results. Therefore, the aim of this work was to study the effect of the foliar application of two nitrogen compounds (phenylalanine and urea) and two elicitors (methyl jasmonate and a yeast extract) on the volatile composition of 'Garnacha', 'Tempranillo', and 'Graciano' grapes.

Material and Methods

Study site, grapevine treatments and harvest: The field work was carried out during the 2015 season in commercial vineyards located in Alfaro (warmest and driest area of La Rioja, Spain). 'Garnacha' (*Vitis vinifera* L.) grapevines were grafted in 2009 onto a R-110 rootstock, with a north-south row orientation. The space between rows and within the row was 2.60 m x 1.10 m for a resulting density of 3500 plants·ha^{-1}. The vineyard was located at an altitude of 355 meters above sea level (m.a.s.l.). 'Tempranillo' (*Vitis vinifera* L.) grapevines were grafted in 1999 onto a 1103-Paulsen rootstock, with an east-west row orientation. The space between rows and within the row was 2.80 m x 1.20 m for a resulting density of 3000 plants·ha^{-1}. The vineyard was located at an altitude of 335 m.a.s.l. 'Graciano' (*Vitis vinifera* L.) grapevines were grafted in 1997 onto a 1103-Paulsen rootstock, with an east-west row orientation. The space between rows and within the row was 3.00 m x 1.30 m for a resulting density of 2600 plants·ha^{-1}. The vineyard was located at an altitude of 345 m.a.s.l. All the vineyards were drip irrigated and trained to a vertical shoot position (VSP) trellis system. They were managed using the standard viticultural practices of the region. According to the Agroclimatic Information Service of La Rioja (SIAR), located about 5 km from the vineyards, the annual accumulated rainfall was 301.2 mm, and the annual average temperature was 14.1 °C.

The trials consisted in the application of five treatments, two based on the nitrogen compounds phenylalanine (Phe) and urea (Ur), two based on the elicitors methyl jasmonate (MeJ) and a yeast extract (YE), and a control treatment. All solutions were dissolved in water. The Phe and Ur (Sigma-Aldrich, Madrid, Spain) solutions were prepared at a concentration of 0.75 g N/L (Garde-Cerdán et al. 2014). The MeJ (Sigma-Aldrich) solution was prepared at a concentration of 2.24 g·L^{-1} (Garde-Cerdán et al. 2016), and the YE solution at the concentration of 1.69 g·L^{-1} specified by the manufacturer (Lallemand). The wetting agent Tween 80 (1 mL·L^{-1}) was added to all solutions (Sigma-Aldrich). Control treatment consisted of an aqueous solution containing only Tween 80. 200 mL of each treatment was applied per plant. The treatments were performed in triplicate, with 10 vines per replicate, and were arranged in a complete randomized block design. Each treatment was applied twice, at veraison and one week later. The harvest date was: September 1, 2015 for 'Garnacha'; September 9, 2015 for 'Graciano'; and September 16, 2015 for 'Tempranillo'. Harvest was made when grapes reached their optimum technological maturity, *i.e.* when the probable alcohol reached close to 13 % (v/v). Grapes were manually harvested and then, they were destemmed and crushed into the experimental wine cellar of the ICVV center, situated in Logroño, near to the vineyard. An aliquot (50 mL Falcon) of each must of the three replicates of each treatment and of the three varieties was collected and frozen for subsequent analysis of volatile compounds.

Analysis of grape volatile compounds by HS-SPME-GC-MS: The method used for the extraction of volatile compounds present in the must samples was described by Garde-Cerdán et al. (2018a) The SPME fiber used was DVB/CAR/PDMS (50/30 mm) (Supelco, Bellefonte, PA, USA). The extraction of volatile compounds was carried out by the addition of 2 g of NaCl to 12 mL of must sample, conditioning for 15 min at 60 °C with stirring and introducing the fiber in the headspace for 105 min with stirring.

Once the extraction process was completed, the SPME fiber was manually introduced into the GC injection port at 250 °C and kept during 15 min for desorption. The desorbed volatile compounds were separated in an Agilent 7890A gas chromatograph system (GC) coupled to a quadrupole Agilent 5975C electron ionization mass spectrometric detector (Agilent Technologies, Palo Alto, CA, USA) equipped with a fused silica capillary column SPBTM-20 (30 m x 0.25 mm I.D. x 0.25 µm of film thickness) (Supelco). The carrier gas was helium at a flow rate of 1.2 mL·min^{-1}. For the separation of the compounds, a temperature program was used starting from an initial temperature of 40 °C for 5 min, followed by a gradient of 2 °C·min^{-1} up to a final temperature of 220 °C and a final time of 20 min. Acquisitions were made in Full Scan (35-300 m/z). For the identification of the compounds, the NIST library and the mass spectrum and retention times of the chromatographic standards (Sigma-Aldrich) were used. Since the treatments were performed in triplicate, the results of volatile compounds correspond to the average of the three analyzes (n = 3).

Statistical analysis: The SPSS Version 21.0 for Windows (SPSS, Chicago, USA) statistical package was used for the statistical analysis of the data. The data of the volatile compounds were processed using the analysis of variance (ANOVA). The Duncan multiple comparison test was used to study differences between means, at a probability level of 0.05.

Results and Discussion

Volatile composition of grapes from foliar vine treatments with nitrogen compounds (Phe and Ur): Fig. 1 shows the terpenoids content in the control grapes and in the samples to which nitrogen compounds (Phe and Ur) were applied in vineyards for the varieties 'Garnacha', 'Tempranillo' and 'Graciano'. It can be seen that each treatment affects differently depending on the variety and the compound.

Geranyl acetone was the most abundant terpenoid in 'Garnacha' and 'Tempranillo'. In 'Garnacha', both treatments, Phe (-41 %) and Ur (-18 %), decreased the amount of this

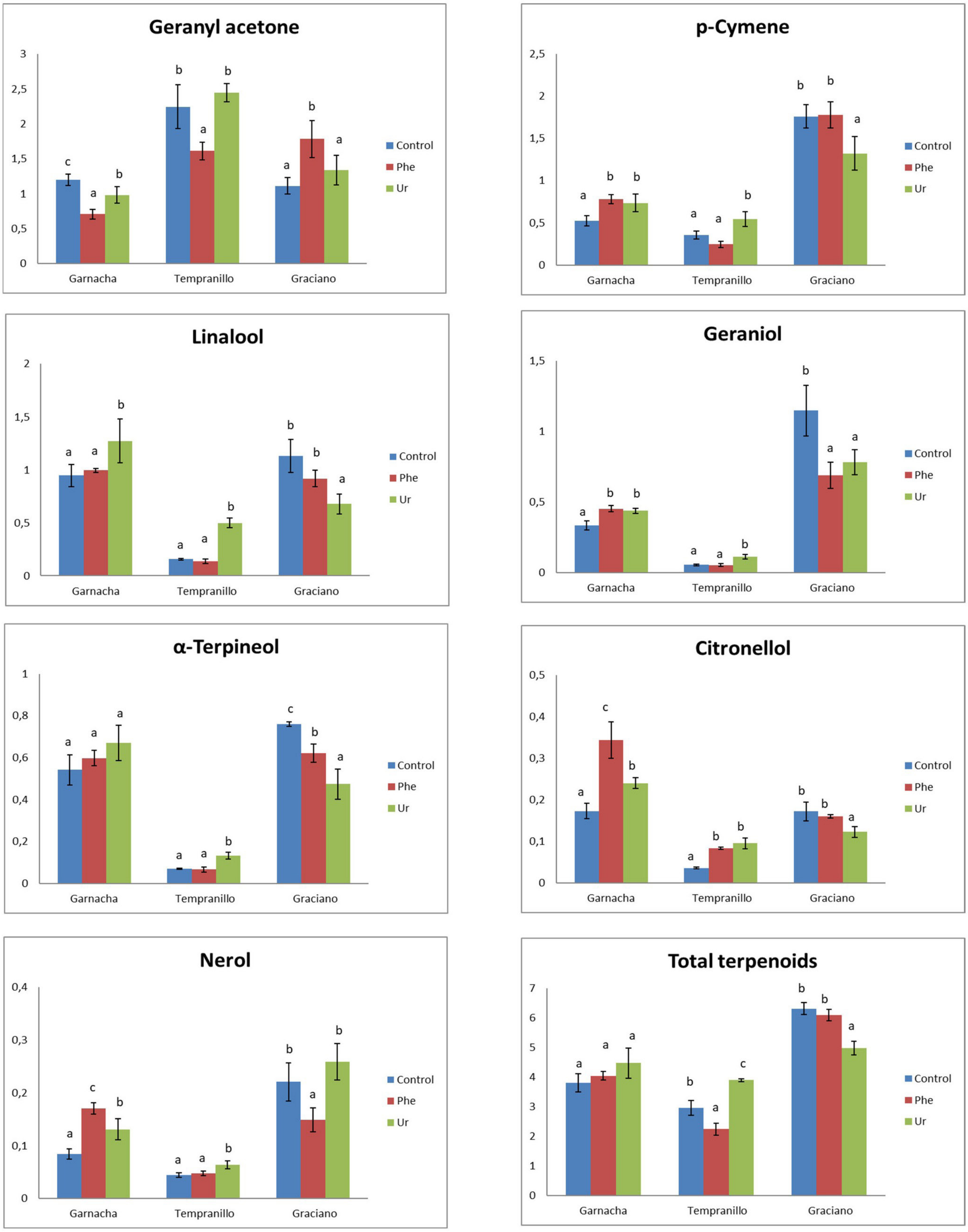

Fig 1: Terpenoids average relative area (x 10^6) in must from grapevines untreated (control) and treated with two different nitrogen compounds, phenylalanine (Phe) and urea (Ur), in 'Garnacha', 'Tempranillo', and 'Graciano'. All parameters are shown with their standard deviation (n = 3). For each variety different letters indicate significant differences between treatments ($p \leq 0.05$).

compound in the grapes. In 'Tempranillo' variety, the foliar application of Phe decreased the amount of geranyl acetone with respect to the control grapes (-28 %). For 'Graciano', Phe treatment increased the geranyl acetone content by 60 % compared to the control grapes.

p-Cymene was the most abundant terpenoid in 'Graciano' variety. For this variety, Ur treatment decreased its quantity by -25 % with respect control grapes. In 'Garnacha' variety, Phe and Ur treatments increased the p-cymene content by 48 % and 40 %, respectively. In 'Tempranillo' variety, only urea increased the amount of this compound by 53 % compared to the control grapes.

Linalool is a very important terpenoid for grape aroma (Ribéreau-Gayon *et al.* 2006). For 'Garnacha' and 'Tempranillo', urea-treated grapes increased the amount of this compound by 34 % and 221 %, respectively, compared to the control. However, in 'Graciano', Ur treatment decreased the linalool content by -40 % compared to the control grapes.

For geraniol in 'Garnacha', both treatments (Phe and Ur) increased its content in grapes by 35 % and 31 %, respectively. In 'Tempranillo', the urea-treated grapes increased the amount of geraniol by 104 %. In 'Graciano', a decrease in the amount of geraniol can be observed, by -40 % in Phe treated grapes and by -32 % in Ur treated grapes.

Regarding α-terpineol, which has a great influence on grape aroma (Ribéreau-Gayon *et al.* 2006), in 'Garnacha' variety, any treatment had a significant effect on the content of this compound. In 'Tempranillo', Ur treated grapes increased the amount of α-terpineol by 90 % compared to the control. In 'Graciano', both treatments, Phe (-18 %) and Ur (-38 %), decreased the α-terpineol content compared to the control grapes.

For citronellol, in 'Garnacha', both Phe (99 %) and Ur (39 %) significantly increased the content of this compound. In 'Tempranillo' grapes, both treatments (Phe and Ur) increased the citronellol content by 133 % and 167 %, respectively. For 'Graciano' grapes, Ur treatment decreased its content by -29 % compared to the control.

Nerol content increased significantly in 'Garnacha' grapes treated with Phe (102 %) and Ur (55 %). In 'Tempranillo' grapes, Ur treatment increased the amount of nerol by 45 % with respect to the control. For 'Graciano', Phe treatment significantly decreased (-33 %) the content of this compound in the grapes.

It is known that terpenoids play an important role in varietal aroma (Ribéreau-Gayon *et al.* 2006). Terpenoids are high volatile compounds, and have low perception threshold, and therefore represent one of the most important group of volatile compounds (Alem *et al.* 2018). For total terpenoids, in 'Garnacha', any treatment had a significant effect on the content of these compounds in grapes. In 'Tempranillo', the Phe treatment decreased (-24 %) the amount of terpenoids in grapes, while Ur treatment increased it (32 %). These results are consistent with those obtained by Garde-Cerdán *et al.* (2015b), where Phe decreased the total terpenoid content in 'Tempranillo'. In 'Graciano', Ur treatment had a negative effect on the total terpenoid content (-21 %).

Fig. 2 shows the content of C_{13} norisoprenoids in the control grapes and in the samples to which nitrogen compounds (Phe and Ur) were applied, for 'Garnacha', 'Tempranillo', and 'Graciano'.

C_{13} norisoprenoids are one of the most important aromatic molecules, and provide fruity and floral notes (Alem *et al.* 2018). (E)-β-Damascenone was the most abundant C_{13} norisoprenoid in control grapes of 'Garnacha', 'Tempranillo', and 'Graciano'. This fact is expected because this compound is one of the most abundant norisoprenoids in the grapes (Sefton *et al.* 2011, Garde-Cerdán *et al*. 2013). In 'Garnacha', both treatments increased the content of β-damascenone in the grapes, Phe by 36 %, and Ur by 29 % in (E) isomer, and Phe by 45 %, and Ur by 35 % in (Z) isomer. In 'Tempranillo', neither of the two treatments showed a significant effect. For 'Graciano', only Phe treatment increased the amount of β-damascenone 25 % for (E) isomer, and 24 % for (Z) isomer. The (E) isomer of β-damascenone is the most abundant isomer in grapes (Sefton *et al.* 2011), which is consistent with the results obtained.

β-Ionone, which provides violet notes (Styger *et al.* 2011), increased in Phe (32 %) and Ur (98 %) treated 'Garnacha' grapes. In 'Tempranillo', only the Phe treatment had an effect, reducing the amount of this compound by -26 % compared to the control. For 'Graciano', the Phe treatment increased β-ionone content by 14 % compared to the control, while Ur decreased it by -23 %. β-Damascenone and β-ionone are the most important C_{13} norisoprenoids in grapes; they contribute to the taste and smell of wine (Sefton *et al*. 2011), providing a honey and violet aroma, respectively (Tomasino 2021).

TDN is one of the most polarising, and maybe the less studied C_{13} norisoprenoid. Its typical aroma descriptor is petrol or kerosene (Tarasov *et al.* 2020). Only Phe treatment increased the amount of this compound in 'Garnacha' grapes (57 %). In 'Tempranillo', both treatments decreased the TDN content in the grapes with respect to the control, -55 % Phe and -46 % Ur. In 'Graciano', no significant differences were found in TDN content between treated grapes and control.

β-Cyclocitral content increased in Phe (34 %) and Ur (56 %) treated grapes compared to control grapes in 'Garnacha'. In 'Tempranillo', Phe decreased the content of this compound by -17 % compared to the control grapes, while Ur increased it by 13 %. In 'Graciano', only Ur treatment showed differences with the control, decreasing the amount of β-cyclocitral by -25 %.

Methyl jasmonate is the least abundant C_{13} norisoprenoid in the control grapes of the three varieties. 'Garnacha' grapes treated with Ur showed a -45 % of this compound compared to the control. In 'Tempranillo', grapes treated with Phe decreased the methyl jasmonate content by -30 % compared to the control, while grapes treated with Ur increased the content of this compound by 68 % compared to the control grapes.

Since (E)-β-damascenone is the most abundant C_{13} norisoprenoid in all three varieties by far, the total amount of total C_{13} norisoporenoids follows a similar trend. In 'Garnacha', both treatments (Phe and Ur) increased the amount of total C_{13} norisoprenoids in treated grapes compared to control grapes, by 37 % and 30 %, respectively. In 'Tempranillo', Phe treatment decreased total C_{13} norisoprenoids content in grapes (-26 %). Finally, 'Graciano' grapes increased their total C_{13} norisoprenoids content when treated with Phe compared to the control grapes (24 %).

Tab. 1 shows the content of benzenoids, esters, and C6 compounds in the musts of grapes treated with phenylalanine and urea, and control grapes of 'Garnacha', 'Tempranillo', and 'Graciano'.

Benzyl alcohol content increased significantly in Phe (65 %) and Ur (76 %) treated grapes of the 'Garnacha'. In 'Tempranillo', only Ur treatment had a significant effect on the amount of this compound, which increased by 77 % compared to the control grapes. In 'Graciano' grapes, Ur

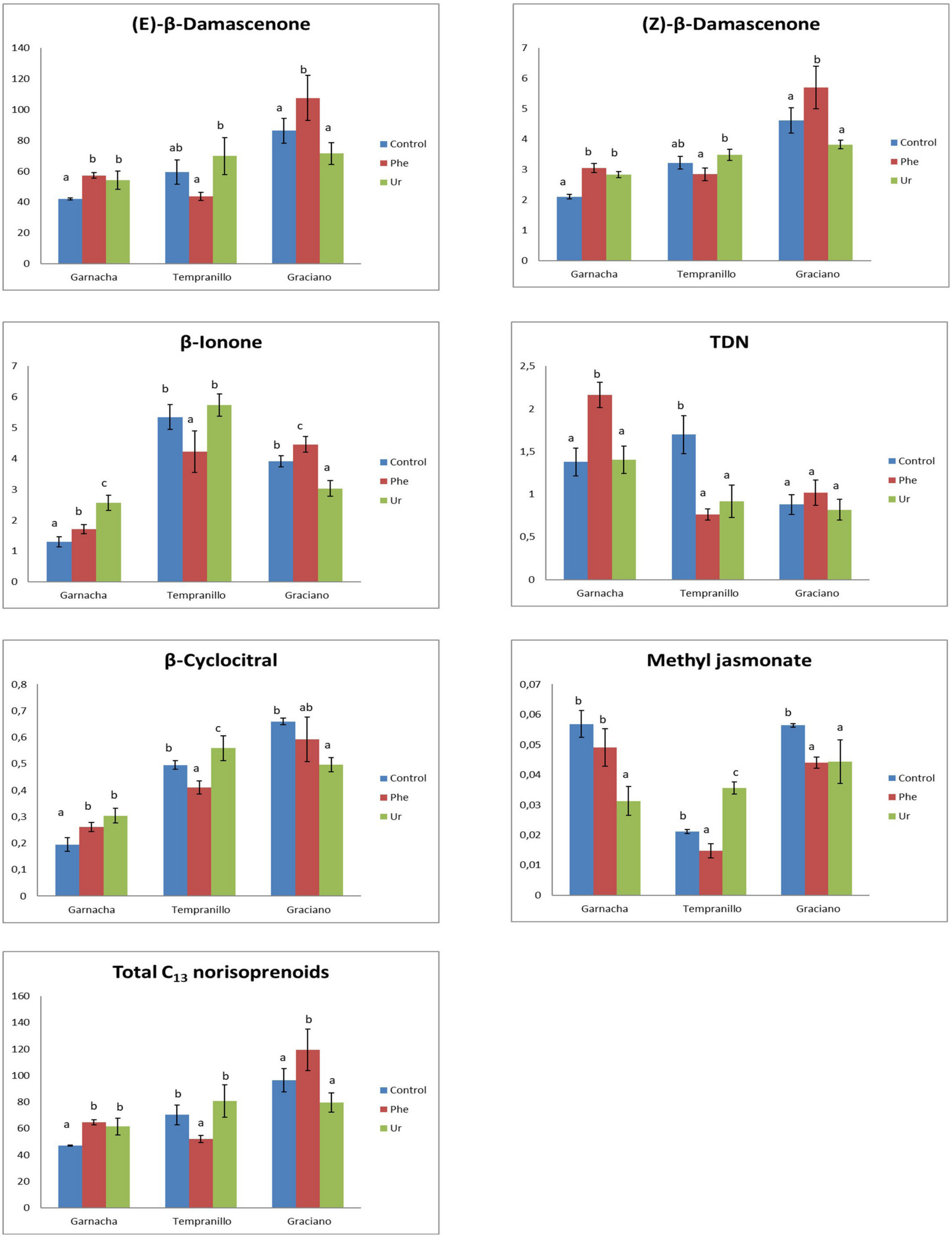

Fig 2: C_{13} norisoprenoids average relative area (x 10^6) in must from grapevines untreated (control) and treated with two different nitrogen compounds, phenylalanine (Phe) and urea (Ur), in 'Garnacha', 'Tempranillo', and 'Graciano'. All parameters are shown with their standard deviation (n = 3). For each variety different letters indicate significant differences between treatments ($p \leq 0.05$). TDN: 1,1,6-Trimethyl-1,2-dihydronaphthalene.

decreased the benzyl alcohol content by -31 % compared to the control grapes. 2-Phenylethanol increased its content by 222 % in Phe treated 'Garnacha' grapes. Ur treatment decreased the amount of this compound by -70 % in this variety with respect to the control. In 'Tempranillo' and 'Graciano', both treatments (Phe and Ur) increased the 2-phenylethanol content in the grapes with respect to the control, 551 % and 559 % respectively for 'Tempranillo', and 95 % and 124 % re-

Table 1

Benzenoids, esters, and C6 compounds average area (x 10^6) in musts from untreated (control) and treated with two nitrogen compounds: phenylalanine (Phe) and urea (Ur) in Garnacha, Tempranillo, and Graciano grape varieties

	Garnacha			Tempranillo			Graciano		
	Control	Phe	Ur	Control	Phe	Ur	Control	Phe	Ur
Benzenoids									
Benzyl alcohol	0.8 ± 0.1a	1.39 ± 0.07b	1.5 ± 0.2b	0.33 ± 0.03a	0.34 ± 0.07a	0.58 ± 0.09b	2.4 ± 0.4b	2 ± 0.3ab	1.7 ± 0.3a
2-Phenylethanol	10 ± 1b	31 ± 4c	2.9 ± 0.4a	0.659 ± 0.006a	4.3 ± 0.7b	4.3 ± 0.7b	2.8 ± 0.1a	5.4 ± 0.3b	6.2 ± 0.7b
2-Phenylethanal	4.8 ± 0.5b	11 ± 1c	2.9 ± 0.1a	1.9 ± 0.1a	2.7 ± 0.4b	2.83 ± 0.09b	2.5 ± 0.3a	4.7 ± 0.3b	2.1 ± 0.3a
Total benzenoids	15 ± 1b	44 ± 5c	7.3 ± 0.2a	2.9 ± 0.1a	7.4 ± 0.3b	7.7 ± 0.6b	7.7 ± 0.5a	12.1 ± 0.5c	10 ± 1b
Esters									
Hexyl acetate	1.9 ± 0.2b	2.3 ± 0.2b	0.9 ± 0.1a	1.24 ± 0.03a	5.0 ± 0.6b	4.6 ± 0.9b	0.64 ± 0.05a	0.54 ± 0.02a	21 ± 3b
2-Hexen-1-ol acetate	5.9 ± 0.3b	0.45 ± 0.05a	0.39 ± 0.06a	0.54 ± 0.06a	1.5 ± 0.3b	1.6 ± 0.2b	0.73 ± 0.08a	0.66 ± 0.03a	5.9 ± 0.5b
Total esters	7.8 ± 0.5c	2.7 ± 0.3b	1.3 ± 0.2a	1.78 ± 0.09a	6.5 ± 0.9b	6 ± 1b	1.4 ± 0.1a	1.21 ± 0.01a	27 ± 4b
Total positive compounds	74.0 ± 0.5a	115 ± 7b	74 ± 7a	78 ± 8a	68 ± 4a	99 ± 11b	112 ± 8a	139 ± 16b	122 ± 5a
C6 compounds									
1-Hexanol	532 ± 75a	462 ± 15a	500 ± 54a	254 ± 9a	399 ± 74b	565 ± 21c	367 ± 10a	457 ± 68b	294 ± 29a
Hexanal	23 ± 3b	49 ± 5c	14 ± 2a	44 ± 2b	6.4 ± 0.3a	8 ± 1a	16 ± 2a	27 ± 4b	12 ± 2a
(E)-2-Hexenal	41 ± 5b	26 ± 3a	50 ± 6c	32 ± 2a	44± 2b	66 ± 8c	56 ± 5a	96 ± 9b	44 ± 2a
Total C6 compounds	596 ± 81a	537 ± 20a	564 ± 49a	330 ± 11a	450 ± 73b	639 ± 15c	439 ± 5a	580 ± 74b	350 ± 30a
Global total	669 ± 81a	650 ± 22a	637 ± 56a	406 ± 19a	517 ± 74b	737 ± 10c	550 ± 11a	717 ± 89b	471 ± 31a

All parameters are given with their standard deviation (n = 3). For each parameter and variety, different letters indicate significant differences between treatments ($p \leq 0.05$). Total positive compounds: sum of terpenoids, C_{13} norisoprenoids, benzenoids, and esters. Global total: sum of terpenoids, C_{13} norisoprenoids, benzenoids, esters, and C6 compounds.

spectively for 'Graciano'. In the work of Ancín-Azpilicueta *et al*. (2013), the Ur treatment also increases the amount of 2-phenylethanol in 'Tempranillo' wine. 2-Phenylethanol was the most abundant benzenoid in 'Garnacha' control samples, which is positive, since it provides a more pleasant aroma to wine (Garde-Cerdán *et al.* 2015b).

For 2-phenylethanal, in 'Garnacha', Phe treatment increased its content by 140 % and Ur treatment decreased it by -40 % with respect to the control grapes. In 'Tempranillo', both treatments (Phe and Ur) increased the content of this compound with respect to the control by 45 % and 51 % respectively. In 'Graciano', treatment with Phe increased the amount of 2-phenyethanal by 89 % compared to the control grapes. 2-Phenylethanal was the most abundant benzenoid in 'Tempranillo' control samples as observed in the works of Garde-Cerdán *et al.* (2015b and 2018a). It can be seen that Phe increased the content of 2-phenylethanol and 2-phenylethanal in all varieties. This was probably due to that the plant transformed the Phe into both aromatic compounds.

The effect of the Phe and Ur treatments on total benzenoids content of the three varieties can also be observed in Tab. 1. In 'Garnacha', it was observed that Phe treatment increased the amount of total benzenoids by 188 % with respect to the control grapes. This is due to the fact that, in this variety, Phe greatly increased the amount of 2-phenylethanol and 2-phenylethanal. Ur treatment decreased the amount of these compounds by -58 %. In 'Tempranillo' and 'Graciano' grapes, both treatments (Phe and Ur) had a positive effect on the total amount of benzenoids, increasing by 157 % and 171 %, respectively, for 'Tempranillo', and by 58 % and 30 %, respectively, for 'Graciano'. Due to the mechanism of transformation of phenylalanine, the amount of total benzenoids was significantly higher with Phe treatments. The same effect can be seen in the work of Garde-Cerdán *et al.* (2015b) for 'Tempranillo' grapes.

Esters play an important role in wine aroma, but are mainly formed during the alcoholic fermentation (Garde-Cerdán *et al.* 2015b, Robinson *et al.* 2014). 2-Hexen-1-ol acetate was the most abundant in 'Garnacha' and 'Graciano' (Tab. 1). In 'Garnacha', both treatments decreased the content of this compound, -92 % Phe and -93 % Ur. In 'Tempranillo', both treatments increased the amount of 2-hexen-1-ol-acetate with respect to the control grapes, 177 % Phe and 190 % Ur. In the 'Graciano', urea was the only treatment that showed significant differences with the control, increasing the 2-hexen-1-ol acetate content by 702 %.

Hexyl acetate was the most abundant ester in 'Tempranillo' control grapes. This is consistent with the findings in Garde-Cerdán *et al.* (2015b). For this variety, both treatments had a significant positive effect, increasing the amount of this compound by 303 % for Phe and 27 % for Ur. For the 'Garnacha' and 'Graciano', Ur was the only treatment that showed significant differences with the control. In 'Garnacha' grapes the hexyl acetate content decreased by -53 % and in 'Graciano' grapes it increased by 3259 %.

Regarding total esters content in 'Garnacha', both treatments (Phe and Ur) had a negative effect, decreasing the amount of total esters compared to the control grapes by -65 % and -84 %, respectively. In 'Tempranillo', grapes treated with Phe (265 %) and Ur (248 %) showed an increase in total ester content. In 'Graciano', grapes treated with the Ur treatment showed an increase in total esters of 1888 % compared to untreated grapes.

Tab. 1 also shows total positive compounds present in Phe and Ur treated grapes and in control grapes. Total positive compounds are the sum of all compounds except C6 compounds. For 'Garnacha' and 'Graciano' varieties, the only treatment effective in increasing these compounds in the grapes compared to the control grapes was Phe (56 % and 24 %, respectively). By contrast, in 'Tempranillo', the only treatment that increased the amount of total positive compounds in the treated grapes with respect to the control grapes was Ur (26 %). C6 compounds, which can provide negative notes to the wine, are derived from the fatty acids and are responsible for green aromas (Alem *et al.* 2018, Robinson *et al.* 2014). 1-Hexanol was the most abundant compound in the control of the three grape varieties. Neither treatment significantly affected the amount of 1-hexanol in 'Garnacha' grapes. In 'Tempranillo', both treatments increased the content of 1-hexanol, Phe by 57 % and Ur by 123 % with respect to the control grapes. In 'Graciano', only Phe had a significant effect, increasing the amount of 1-hexanol by 25 % with respect to the control.

Hexanal content significantly increased in 'Garnacha' grapes with Phe treatment (108 %), and decreased with Ur treatment (-41 %). In 'Tempranillo', it decreased with both treatments, -85 % with Phe and -83 % with Ur. In 'Graciano', the only treatment that showed significant differences with the control with respect to the amount of hexanal was Phe, which increased the hexanal content by 65 %. The (E)-2-hexenal content decreased by -36 % with Phe treatment and increased by 22 % with Ur treatment compared to control grapes. In 'Tempranillo' grapes, the amount of this compound increased by 36 % with Phe treatment and by 103 % with Ur treatment. In 'Graciano', Phe treatment increased the (E)-2-hexenal content by 71 %.

For total C6 compounds, in 'Garnacha', neither of the two nitrogen treatments (Phe and Ur) had a significant effect on the amount of volatile compounds on the grapes. This is positive for the aroma, since these compounds, at high levels, can provide undesirable aromas (Zalacain *et al.* 2007). In 'Tempranillo', the amount of C6 compounds increased more in Ur treated grapes (94 %) than in Phe treated grapes (36 %), although both treatments increased the total amount of C6 compounds compared to the control grapes. For 'Graciano', only Phe treatment increased the amount of these compounds in the treated grapes (32 %) with respect to control grapes.

Volatile composition of grapes from foliar vine treatments with elicitors (MeJ and YE): Fig. 3 shows the terpenoids content in the control grapes and in the samples to which the elicitors (MeJ and YE) were applied in the vineyard, for 'Garnacha', 'Tempranillo', and 'Graciano'. When elicitors are sprayed at different stages of plan development, volatile molecules in vines may be absorbed by leaves and transferred to berries, thus changing the aroma of berries (Li *et al.* 2020).

The control samples are the same as in the previous section, so geranyl acetone remains the major compound in the 'Garnacha' and 'Tempranillo' control grapes. In 'Garnacha', neither of the two treatments (MeJ and YE) showed significant differences in the amount of volatile compounds with the control. In 'Tempranillo', MeJ had no significant effect on the content of this compound. The YE decreased the amount of geranyl acetone by -37 % compared to the control. In 'Graciano', the MeJ treatment increased the amount of this compound by 36 % compared to the control.

p-Cymene was the most abundant compound in 'Graciano' control grapes. For 'Graciano' and 'Tempranillo', YE treatment decreased p-cymene content in both grape varieties with respect to the control by -61 % and -47 %, respectively. In 'Garnacha', MeJ treatment increased the amount of this compound by 143 %.

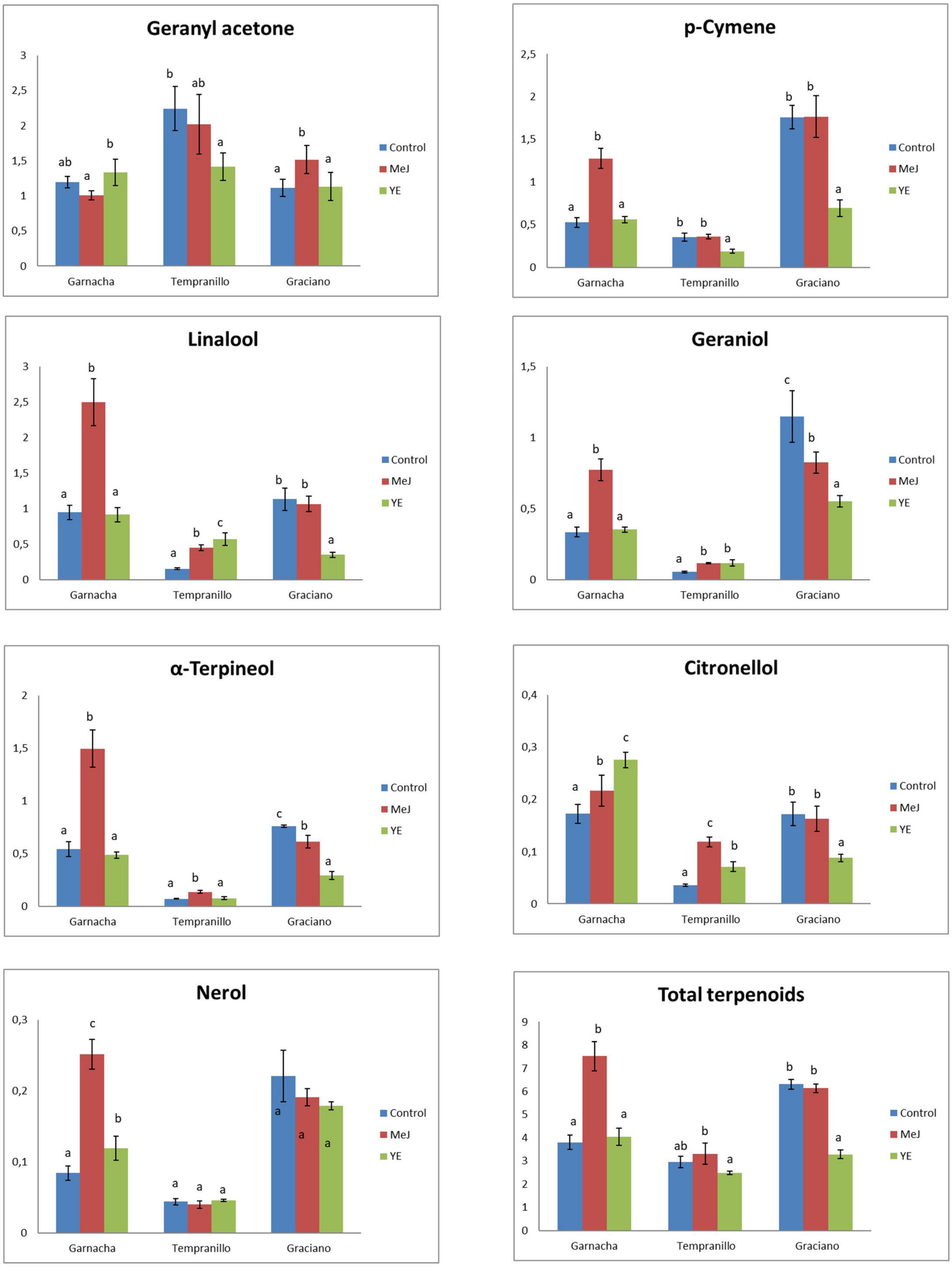

Fig 3: Terpenoids average relative area (x 10^6) in must from grapevines untreated (control) and treated with two different elicitors, methyl jasmonate (MeJ) and a yeast extract (YE), in 'Garnacha', 'Tempranillo', and 'Graciano'. All parameters are shown with their standard deviation (n = 3). For each variety different letters indicate significant differences between treatments ($p \leq 0.05$).

Linalool content increased by 164 % in 'Garnacha' with the MeJ treatment. In 'Tempranillo', both treatments increased the linalool content, MeJ by 189 % and YE by 266 %. In 'Graciano', the only treatment that had an effect was YE, which decreased the amount of linalool by -69 % compared to the control. Geraniol content increased significantly in 'Garnacha' grapes after the application of MeJ. In 'Tempranillo', both treatments increased the geraniol content with respect to the control, 109 % for MeJ and 110 % for YE. These results are in consistent with those obtained by Gómez-Plaza *et al.* (2012) where MeJ increased the amount of geraniol in 'Monastrell' grapes. In 'Graciano', MeJ decreased the amount of geraniol by -28 % and YE by -52 % compared to the control.

Concerning the α-terpineol content, in 'Garnacha' and 'Tempranillo', it only increased with MeJ treatment, by 176 % and 95 %, respectively. The same as in the work of Gómez-Plaza *et al.* (2012), where MeJ increased the amount of this compound in 'Monastrell' grapes. In 'Graciano' the treatments with MeJ and YE decreased the amount of α-terpineol in grapes by -19 % and -61 % compared to the control.

Citronellol content increased significantly with the MeJ and YE treatments in 'Garnacha' (26 % and 60 % respectively) and 'Tempranillo' (234 % and 100 % respectively) grapes compared to the control. YE was more effective in 'Garnacha' and MeJ was more effective in 'Tempranillo'. In 'Graciano', the only treatment that showed significant differences was YE, which reduced the amount of citronellol by -49 % compared to the control.

Nerol content increased significantly in 'Garnacha' grapes with MeJ treatment (198 %) and with the YE treatment (42 %). In 'Tempranillo' and 'Graciano', any treatment had effect on the amount of nerol.

Total terpenoid content of the treated and control grapes of 'Garnacha', 'Tempranillo' and 'Graciano' can be seen at the end of Fig. 3. For 'Garnacha', the only treatment that significantly increased the amount of total terpenoids with respect to the control was MeJ (98 %). These results are in agreement with those obtained by Li *et al*. (2020), where the exogenous MeJ application had a significant promoting effect on the synthesis of free monoterpenes in Italian 'Riesling' grapes. For 'Tempranillo', any treatments showed significant differences with the control. For 'Graciano', YE treatment decreased the content of total terpenoids in the treated grapes compared to control grapes (-48 %). In the work of Gutiérrez-Gamboa *et al.* (2019) both MeJ and YE decreased the total terpenoid content in 'Tempranillo' grapes.

Fig. 4 shows the content of C_{13} norisoprenoids in grapes treated with the elicitors MeJ and YE in 'Garnacha', 'Tempranillo' and 'Graciano'. The most abundant compound in control samples of the three varieties was (E)-β-damascenone. In 'Garnacha', the two elicitors increased the amount of this compound in the grapes compared to the control. MeJ was more effective (67 %) than YE (23 %). In 'Tempranillo', any treatment (MeJ and YE) had effect on the content of (E)-β-damascenone. In 'Graciano', only YE had a significant effect, reducing the amount of this compound by -32 % compared to the control. For (Z)-β-damascenone, MeJ increased the (Z)-β-damascenone content in 'Garnacha' grapes by 57 % compared to the control. In 'Tempranillo', MeJ decreased the amount of (Z)-β-damascenone by -27 % compared to the control. In 'Graciano', YE decreased the amount of (Z)-β-damascenone by -41 %.

The amount of β-ionone in 'Garnacha' grapes was only affected when treated the vines with MeJ, which increased the amount of β-ionone by 79 % compared with control. In 'Tempranillo', YE decreased the amount of β-ionone by -52% compared to the control. In 'Graciano', MeJ increased the content of this compound by 38 % and YE decreased it by -57 % compared to the control. The increase in the content of β-ionone with MeJ application may be due to the fact that MeJ accelerates the degradation of β-carotene (Peña-Cortés *et al.* 2004) which is a β-ionone precursor (Black *et al.* 2015)

The amount of TDN increased with MeJ by 55 % in 'Garnacha' grapes. In 'Tempranillo', both treatments decreased TDN content with respect to the control, MeJ by -67 % and YE by -59 %. In 'Graciano', neither of the two treatments showed significant differences with the control.

The β-cyclocitral content was not affected by any treatment in 'Garnacha'. In 'Tempranillo' and 'Graciano', YE decreased the amount of this compound by -34 % and -59 %, respectively, with respect to the control.

The least abundant C_{13} norisoprenoid in the control grapes was methyl jasmonate. In 'Garnacha', YE treatment decreased the methyl jasmonate content by -57 % compared to the control. In 'Tempranillo', neither of the two treatments showed significant differences in the amount of methyl jasmonate with respect to the control. In 'Graciano', MeJ increased the content of this compound by 77 %, while YE decreased it by -38 %.

Fig. 4 shows the total C_{13} norisoprenoids content in treated and control grapes of the three varieties studied. In 'Garnacha', both treatments increased the total amount of C_{13} norisoprenoids compared to the control grapes. MeJ treatment increased the content of these compounds to a greater extent (67 %) than YE treatment (22 %). The addition of MeJ had a significant effect on the amount of total C_{13} norisoprenoids in 'Garnacha' grapes, this was probably due to the fact that MeJ increases the activity of the enzymes involved in the synthesis of these compounds (Dubery *et al.* 2000, Marín-San Román *et al.* 2020a), which derive from biodegradation of carotenoids (Alem *et al.* 2018, Mendes-Pinto 2009). In 'Tempranillo', neither of the two treatments showed significant differences with the control. For 'Graciano', YE treatment significantly decreased the amount of C_{13} norisoprenoids in treated grapes with respect control grapes (-33 %).

Tab. 2 shows the content of benzenoids, esters, and C6 compounds in control grapes, and grapes treated with the elicitors MeJ and YE in 'Garnacha', 'Tempranillo', and 'Graciano'. For 'Garnacha' and 'Tempranillo', MeJ was the only treatment that increased the amount of benzyl alcohol compound with respect control grapes, 297 % and 70 %, respectively. For 'Graciano', YE treatment decreased the amount of benzyl alcohol with respect to the control grapes (-68 %).

2-Phenylethanol was the most abundant compound in 'Garnacha' and 'Graciano'. In 'Garnacha', both treatments (MeJ and YE) had a negative effect on its contents in the grapes, by -76 % and -53 % respectively. By contrast, for 'Tempranillo', whose control grapes were the least rich in this compound, both treatments increased the amount of 2-phenylethanol by 466 % for the MeJ treatment and by 237 % for the YE treatment with respect to the control. For 'Graciano', YE treatment greatly increased the 2-phenylethanol content in the grapes compared to the control grapes (651 %).

In 'Garnacha', the MeJ treatment increased the content of 2-phenylethanal in the grapes compared to the control grapes

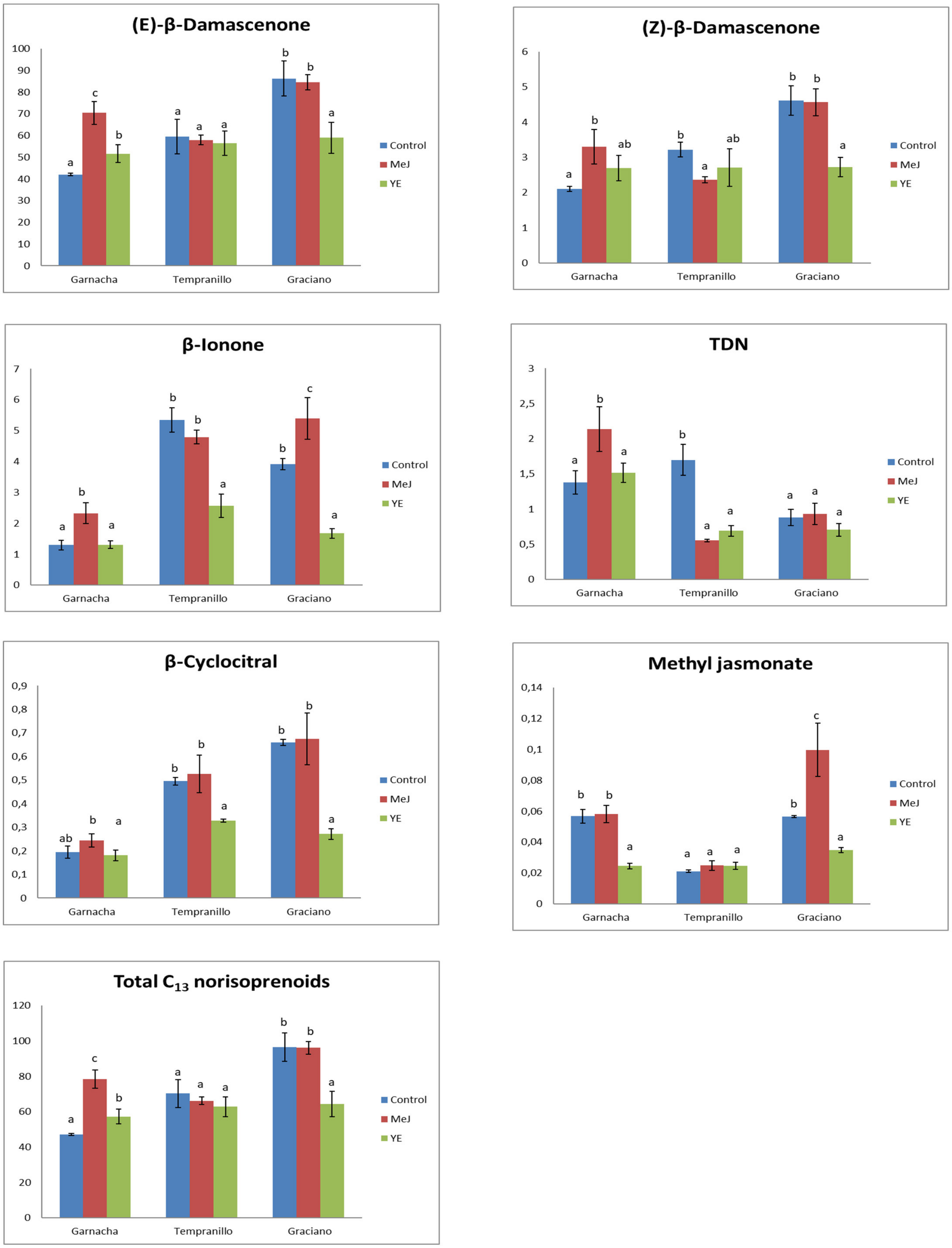

Fig 4. C_{13} norisoprenoids average relative area (x 10^6) in must from grapevines untreated (control) and treated with two different elicitors, methyl jasmonate (MeJ) and a yeast extract (YE), in 'Garnacha', 'Tempranillo', and 'Graciano'. All parameters are shown with their standard deviation (n = 3). For each variety different letters indicate significant differences between treatments ($p \leq 0.05$). TDN: 1,1,6-Trimethyl-1,2-dihydronaphthalene.

(46 %). By contrast, YE treatment had a negative effect on the treated grapes, decreasing the amount of 2-phenylethanal compared to the control grapes (-33 %). For 'Tempranillo', any treatments showed significant differences in 2-phenylethanal content with the control grapes. For 'Graciano', MeJ treatment decreased the amount of 2-phenylethanal in treated grapes compared to control grapes (-23 %). As the most abundant benzenoid in 'Garnacha' and 'Graciano' grapes

Table 2

Benzenoids, esters, and C6 compounds average area (x 10^6) in musts from untreated (control) and treated with two elicitors: methyl jasmonate (MeJ) and a yeast extract (YE) in "Garnacha", "Tempranillo", and "Graciano" grape varieties

	"Garnacha"			"Tempranillo"			"Graciano"		
	Control	MeJ	YE	Control	MeJ	YE	Control	MeJ	YE
Benzenoids									
Benzyl alcohol	0.8 ± 0.1a	3.3 ± 0.5b	1.1 ± 0.2a	0.33 ± 0.03a	0.55 ± 0.08b	0.26 ± 0.04a	2.4 ± 0.4b	2.1 ± 0.4b	0.8 ± 0.1a
2-Phenylethanol	10 ± 1c	2.3 ± 0.3a	4.5 ± 0.8b	0.659 ± 0.006a	3.7 ± 0.7c	2.2 ± 0.4b	2.8 ± 0.1a	2.1 ± 0.4a	21 ± 3b
2-Phenylethanal	4.8 ± 0.5b	7 ± 1c	3.2 ± 0.4a	1.9 ± 0.1ab	2.4 ± 0.3b	1.5 ± 0.3a	2.5 ± 0.3b	1.9 ± 0.2a	2.7 ± 0.2b
Total benzenoids	15 ± 1c	13 ± 2b	8.9 ± 0.8a	2.9 ± 0.1a	6.7 ± 0.5c	4.0 ± 0.7b	7.7 ± 0.5a	6.1 ± 0.7a	24 ± 3b
Esters									
Hexyl acetate	1.9 ± 0.2b	1.6 ± 0.2b	0.36 ± 0.03a	1.24 ± 0.03a	8 ± 1b	42 ± 2c	0.64 ± 0.05a	1.3 ± 0.2a	53 ± 9b
2-Hexen-1-ol acetate	5.9 ± 0.3b	0.48± 0.07a	0.27 ± 0.02a	0.54 ± 0.06a	2.6 ± 0.2c	1.17 ± 0.07b	0.73 ± 0.08a	1.6 ± 0.2b	0.57 ± 0.09a
Total esters	7.8 ± 0.5c	2.0 ± 0.2b	0.629 ± 0.008a	1.78 ± 0.09a	10 ± 1b	43 ± 2c	1.4 ± 0.1a	2.9 ± 0.5a	53 ± 9b
Total positive compounds	74.0 ± 0.5a	101 ± 5b	71 ± 4a	78 ± 8a	86 ± 3a	112 ± 5b	112 ± 8a	111 ± 3a	145 ± 11b
C6 compounds									
1-Hexanol	532 ± 75a	593 ± 73a	477 ± 11a	254 ± 9a	641 ± 21b	652 ± 22b	367 ± 10b	308 ± 39a	263 ± 6a
Hexanal	23 ± 3a	23 ± 3a	20 ± 1a	44 ± 2c	15 ± 1a	31 ± 1b	16 ± 2a	15 ± 3a	25 ± 5b
(E)-2-Hexenal	41 ± 5b	43 ± 5b	26 ± 3a	32 ± 2a	51 ± 9b	67 ± 8c	56 ± 5b	44 ± 4ab	44 ± 8a
Total C6 compounds	596 ± 81ab	659 ± 76b	523 ± 13a	330 ± 11a	708 ± 30b	750 ± 15c	439 ± 5b	368 ± 41a	331 ± 18a
Global total	669 ± 81ab	758 ± 75b	593 ± 9a	406 ± 19a	794 ± 31b	862 ± 15c	550 ± 11b	478 ± 43a	476 ± 22a

All parameters are given with their standard deviation (n = 3). For each parameter and variety, different letters indicate significant differences between treatments ($p \leq 0.05$). Total positive compounds: sum of terpenoids, C_{13} norisoprenoids, benzenoids, and esters. Global total: sum of terpenoids, C_{13} norisoprenoids, benzenoids, esters, and C6 compounds.

was 2-phenylethanol, the total amount of benzenoids in these varieties was strongly influenced by the amount of this compound. Thus, although in 'Garnacha' the MeJ treatment was the most effective in increasing the benzyl alcohol and 2-phenylethanal content in the grapes, this effect was not observed in the amount of total benzenoids. In this case, the two treatments show a decrease in the amount of total benzenoids by -17 % and -42 %, respectively, compared to the control grapes. In 'Tempranillo', both treatments increased the amount of total benzenoids in the grapes by 133 % in the MeJ treatment and 40 % in the YE treatment, with respect to the control. In 'Graciano', the grapes to which

YE was applied showed an increase in the total amount of benzenoids (215 %).

Regarding the esters, YE treatment decreased the amount of hexyl acetate by -81 % compared to control grapes. For 'Tempranillo', both treatments increased the hexyl acetate content in treated grapes with respect to control grapes, the increase being much greater when YE was applied (3257 %) than with MeJ (508 %). In 'Graciano', YE treatment greatly increased the hexyl acetate content in the grapes with respect to the control (8188 %). It can be observed that 'Garnacha' is very rich in 2-hexen-1-ol acetate in control grapes. The application of both treatments greatly reduces the 2-hexen-1-ol acetate content in the treated grapes compared to control grapes (-92 % MeJ and -95 % YE). In 'Tempranillo', both treatments have a positive effect on the amount of 2-hexen-1-ol acetate in the grapes by 387 % in MeJ treatment and 117 % in YE treatment. In 'Graciano', the MeJ treatment was the only one that increased the amount of 2-hexen-1-ol acetate in treated grapes compared to control grapes (123 %). For total esters, in 'Garnacha', the two elicitors reduced the amount of total esters in treated grapes compared to control grapes, by -74 % MeJ and -92 % YE. In 'Tempranillo' and 'Graciano', the effect of YE treatment on the amount of hexyl acetate was very pronounced, so it had a great influence on the amount of total esters. Thus, it can be observed that, for these two grape varieties, the amount of total esters follows the same trend as hexyl acetate.

Tab. 2 shows the amount of total positive compounds. For 'Garnacha', the only treatment that increased the amount of these compounds with respect to the control was MeJ (36 %). For 'Tempranillo' and 'Graciano', the only treatment effective in increasing the content of Total positive compounds was YE (44 % and 30 %, respectively).

The last family of compounds that can be observed in Tab. 2 are the C6 compounds. 1-Hexanol was the most abundant compound in the control grapes of the three varieties. In 'Garnacha', neither of two treatments had a significant effect on the amount of this compound. In 'Tempranillo', both treatments increased the 1-hexanol content with respect to the control, 152 % MeJ and 157 % YE. By contrast, in 'Graciano', both treatments decreased the amount of 1-hexanol with respect control grapes, by -16 % MeJ and -28 % YE.

The content of hexanal present in 'Garnacha' was not affected by the application of the elicitors. In 'Tempranillo', both treatments decreased the amount of this compound in treated grapes with respect control grapes (MeJ by -65 % and YE by -29 %). In 'Graciano', YE increased the content of this compound in treated grapes by 56 % with respect to the control.

The amount of (E)-2-hexenal decreased in 'Garnacha' grapes when treated with YE (-36 %). In 'Tempranillo', both treatments increased the content of this compound in the grapes with respect to the control (58 % for MeJ and 106 % for YE). In 'Graciano', the only treatment that showed significant differences with the control was YE, which decreased the amount of (E)-2-hexenal by -22 %.

Total amount of C6 compounds shown by 'Garnacha' grapes was not affected by any treatment. In 'Tempranillo', both treatments increased the amount of C6 compounds compared to the control grapes. The MeJ treatment increased by 115 % and the YE by 127 %. For 'Graciano', both elicitors decreased the C6 content in grapes compared to control grapes (-16 % MeJ and -25 % YE).

Conclusions

The application of nitrogen compounds (Phe and Ur) and elicitors (MeJ and YE) improved the synthesis of some volatile compounds in 'Garnacha', 'Tempranillo' and 'Graciano' grapes. The effect of foliar applications on volatile composition was dependent on the grape variety. For 'Garnacha', the treatments that most increased the amount of volatile compounds were Phe and MeJ; for 'Tempranillo', Ur and MeJ; and for 'Graciano', the most effective treatment was Phe. Consequently, depending on the variety, foliar application of MeJ and both nitrogen compounds could be a useful tool to improve grape quality.

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