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Effects of foliar application of methyl jasmonate and/or urea, conventional or via nanoparticles, on grape volatile composition

Lesly L. Torres-Díaz,[a] Eva P Pérez-Álvarez,[a*] Belén Parra-Torrejón,[b] Sandra Marín-San Román,[a] Itziar de Sáenz de Urturi,[a] Gloria B Ramírez-Rodríguez,[b] Rebeca Murillo-Peña,[a] Miriam González-Lázaro,[a] José M Delgado-López[b] and Teresa Garde-Cerdán[a*]

Abstract

BACKGROUND: Viticulture has adapted foliar applications of biostimulants as a tool to improve crop quality. Recently, nanotechnology has been incorporated as a strategy to reduce the loss of biostimulants and treat nutrient deficiencies. Therefore, the present study aimed to investigate the effect of foliar applications of amorphous calcium phosphate nanoparticles (ACP) doped with methyl jasmonate (ACP-MeJA) and urea (ACP-Ur), individually or together (ACP-MeJA+Ur), on the content of volatile compounds in 'Tempranillo' grapes, compared to the conventional application of MeJA and Ur, individually or in combination (MeJA+Ur).

RESULTS: The results showed that nanoparticle treatments reduced the total C6 compounds and some carbonyl compounds in the grape musts. This is of novel interest because their presence at high levels is undesirable to quality. In addition, some aroma-positive compounds such as nerol, neral, geranyl acetone, β-cyclocitral, β-ionone, 2-phenylethanal and 2-phenylethanol increased, despite applying MeJA and Ur at a lower dose.

CONCLUSION: Consequently, although few differences in grape volatile composition were detected, nanotechnology could be an option for improving the aromatic quality of grapes, at the same time as reducing the required doses of biostimulants and generating more sustainable agricultural practices.

Keywords: grape; methyl jasmonate; nanoparticles; 'Tempranillo'; urea; volatile compounds

INTRODUCTION

Both viticulture and winemaking are essential practices representing a critical economic activity in numerous regions worldwide. In 2020, the global vineyard area exceeded 7.3 million hectares, with Spain covering the largest vineyard acreage.[1] 'Tempranillo', a widely cultivated red grape variety in Spain, stands out as the quintessential variety in the Rioja region. It is characterised by its versatility because it adapts to different soils and climatic conditions.[2] The wines elaborated from this grape variety are characterised by aromas evoking forest fruits, plum, strawberry and wildflowers. Aroma plays a pivotal role in the character and quality of wine and is primarily influenced by the grape's composition.[3] In the present study, primary aromas were studied, which in turn are classified as varietal and pre-fermentative aromas, encompassing various compounds families, such as terpenoids, C_{13} norisoprenoids, C6 compounds, alcohols, esters, benzenoids and carbonyl compounds.[3-5]

Grape quality is predominantly conditioned by climate, agronomic practices, soil type and grape variety.[6-8] The climate is undoubtedly a critical factor in all agricultural systems; its influence is much more notable in viticulture and, thus, in winemaking. Although grapevines exhibit adaptability to different climatic conditions, and are resistant to moderate heat and water

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* Correspondence to: E P Pérez-Álvarez or T Garde-Cerdán, Grupo VIENAP, Instituto de Ciencias de la Vid y del Vino (CSIC, Gobierno de La Rioja, Universidad de La Rioja). Ctra. de Burgos, km. 6. 26007 Logroño, Spain. E-mail: evapilar.perez@icvv.es (Pérez-Álvarez); teresa.garde.cerdan@csic.es (Garde-Cerdán)

a Grupo VIENAP, Instituto de Ciencias de la Vid y del Vino (CSIC, Gobierno de La Rioja, Universidad de La Rioja), Logroño, Spain

b Facultad de Ciencias, Departamento de Química Inorgánica, Universidad de Granada, Granada, Spain

stress, they are susceptible to severe stress by extreme weather events.[9] The climate change causes a mismatch between technological and phenolic maturities, which affects grape and wine quality. Moreover, agronomic practices, such as leaf removal, cluster thinning and soil fertilisation, can also influence the synthesis of primary and secondary metabolites, and therefore modify the grape aromatic composition. To mitigate environmental effects and prevent nutritional wastage and deficiencies, alternative techniques have been proposed.[10] These include the foliar application of biostimulants to the vineyard because of the rapid and efficient use by plants of the applied products. Some of the advantages obtained from this technique are the reduction of costs and the contribution to sustainable and environmentally friendly ecological agriculture.[4,11-13]

The biostimulants used in the present study were methyl jasmonate (MeJA) and urea (Ur). MeJA has been used in recent years as a defence agent against pathogens and as a strategy to stimulate the synthesis of secondary metabolites in plants, as well as to extend the shelf life and improve the quality of food during harvest and storage.[14-21] Currently, urea fertilisers account for the majority of the world's nitrogen fertiliser applications because of their high nitrogen concentration and low costs.[22] However, some disadvantages have been reported in the use of biostimulants, especially MeJA, which is expensive, has low water solubility and is highly volatile[21,23,24] and, in addition, several applications are necessary to achieve an effect.[21,24] Urea traditionally has been economic. However, the international association of fertiliser manufacturers announced the rise in prices of this product as a result of the record prices that were to be reached in natural gas in Europe.[25] Hence, nanotechnology is opening up as an alternative to improve plant nutrition, reduce the loss of nutrients in crops and limit unwanted environmental effects,[26] in addition to striving to maintain the balance between doses/benefits.[21,24,27] Although the use of this type of materials in agribusiness has been suggested, their application in viticulture is relatively recent compared to other agri-food sectors.[28]

In this context, Garde-Cerdán *et al.*[21] reported that the application of MeJA and MeJA-doped nanoparticles in 'Tempranillo' variety increased the content of some anthocyanins, flavonols, flavanols and non-flavonoid compounds compared to the control treatment, highlighting the potential of employing MeJA-doped nanoparticles to enhance the phenolic composition of grapes, as well as reducing maturity decoupling and the environmental impact. Furthermore, Gimenez-Bañón *et al.*[29] investigated the impact of MeJA-doped nanoparticles on the volatile composition of 'Monastrell' wines over three seasons, reporting that nano-MeJA treatment generally increased the volatile composition to a similar degree to that obtained with conventionally used MeJA, but at a dose that was 10 times lower. Marín-San Román *et al.*[30] found that MeJA application increased the concentration of terpenoids, C_{13} norisoprenoids and total C6 compounds, whereas amorphous calcium phosphate nanoparticles (ACP)-MeJA enhanced the amount of terpenoids, and benzenoid compounds.

Pérez-Álvarez *et al.*[31] managed to increase the amino acids content in 'Tempranillo' grapes after foliar application of nanoparticles doped with urea and underscored that the use of nanoparticles could reduce the dose of fertiliser. In the same way, Gil-Muñoz *et al.*[23] reported that the conventional application of MeJA and nanoparticles doped with MeJA increased the concentration of amino acids in 'Monastrell' grapes, although better results were found when applying the biostimulants doped to the nanoparticles. Parra-Torrejón *et al.*[24] designed amorphous calcium phosphate nanoparticles doped with MeJA aiming to increase the content of stilbenes in wine. In addition, these lower cytotoxicity was reported when using MeJA on nanoparticles, characteristics that are important for the use of biostimulants more safely and efficiently in agribusiness.

ACP are materials analogous to the materials that make up the bone structure of mammals.[32,33] This type of material has gained significant interest in agribusiness because of its composition, mainly calcium and phosphorus, two essential nutrients for plants; its high surface reactivity, which gives it the ability to be doped with different ions and biomolecules; its biodegradability and biocompatibility; and its shape and size, which are also an advantage because they allow easier penetration into plants.[33-35] Considering the notable benefits that have been achieved with the use of urea and MeJA to improve the quality of grapes, there is the question of whether applying MeJA- and Ur-doped nanoparticles (ACP-MeJA: 1 mM; ACP-Ur: 0.4 kg N ha^{-1}) has the same effect as applying them conventionally. Furthermore, is it possible to improve the grape volatile composition by exogenously applying nanoparticles doped with MeJA and/or Ur? What are the effects on the aroma composition of grapes of applying MeJA and/or Ur individually and in combination? It is worth noting that there are no studies about the use of these two biostimulants together, either freely or in combination, in the composition of grapes, and no research has investigated the effect of urea supported in nanoparticles on the aromatic composition of grapes of the 'Tempranillo' variety. Considering the above, it is possible that nanoparticles doped with one of the biostimulants improve the volatile composition of 'Tempranillo' grapes, or, failing that, give similar results as when MeJA and/or Ur are applied in a conventional way. Therefore, the present study aimed to compare the effect of foliar application of MeJA and/or urea, conventionally and in apatite nanoparticles, applied both individually and together, on the volatile composition of 'Tempranillo' grapes.

MATERIALS AND METHODS

Vineyard site and experimental design

'Tempranillo' (*Vitis vinifera* L.) variety grown in a commercial vineyard in Monte Cantabria, Logroño, La Rioja (North of Spain) was employed for this trial, in the 2021 vintage. The vineyard (latitude 42°28′48.77″N; longitude 2°26′0.4″W) is 492 m above sea level, with a planting density of 2922 plants ha^{-1}, and with distances of 3 m between rows and 1.2 m between vines. The vines were trained on a single vertical trellis system, were pruned leaving four to five thumbs (two buds per thumb) per plant and grown in rainfed conditions using traditional cultural practices in D.O.Ca. Rioja. The site has a semi-arid continental Mediterranean climate, with warm and dry summers and the rainfall period concentrated mainly at spring.

The work involved the foliar application of the following seven treatments: control (water), conventional methyl jasmonate solution (MeJA, 10 mM), ACP loaded with MeJA (ACP-MeJA, 1 mM), urea solution (Ur, 6 kg N ha^{-1}), ACP loaded with urea (ACP-Ur, 0.4 kg N ha^{-1}), MeJA and urea (MeJA+Ur, 10 mM + 6 kg N ha^{-1}) and ACP loaded with MeJA and Ur (ACP-MeJA+Ur, 1 mM + 0.4 kg N ha^{-1}). Urea and MeJA are commercial products (Sigma-Aldrich, Madrid, Spain). ACP-Ur and ACP-MeJA were prepared according to the methodology described elsewhere.[21,24,31,35] For preparation of nanoparticles loaded with the two molecules, ACP-MeJA+Ur, first, ACP nanoparticles were synthesised by mixing two solutions of equal volume (2 L): (i) an aqueous solution containing 0.2 mol L^{-1} calcium chloride and 0.2 mol L^{-1} sodium citrate and (ii) an aqueous solution containing 0.12 mol L^{-1} dipotassium phosphate and 0.1 mol L^{-1} sodium carbonate. After 5 min, the ACP precipitate was collected and repeatedly washed with ultrapure water by centrifugation (3428 × *g* for 15 min). Subsequently, ACP nanoparticles were dispersed in 1.5 L of a 4.3% m/v urea solution and then 4 mL of methyl jasmonate was added to the solution. The mixture was left under agitation for 24 h and then ACP-MeJA+Ur nanoparticles were collected by centrifugation as described before and stored at room temperature. MeJA and urea loading was quantified by UV-visible spectroscopy and elemental analysis, respectively, as previously described.[24,35]

The experimental design was carried out in randomised blocks with seven treatments, in triplicate ($7 \times 3 = 21$ trials), with five vines per replicate (105 plants in total). For each plant, 200 mL of solution (this volume is sufficient to cover a medium-sized vine, and thus avoid wasting biostimulant through dripping) were applied twice, at veraison and 1 week later on. To perform the treatments, aqueous solutions were prepared using Tween 80 (Sigma-Aldrich) as wetting agent (1 mL L^{-1}).

Measures taken to prevent any cross-media effects arising from the application of the foliar treatments under investigation, applications of the treatments were made by alternating the rows, suggesting that the treatments were applied in one row and then not in the next, rather than continuously.

According to the methodology of Garde-Cerdán *et al.*,[36] grapes were hand-harvested at their optimum point of technological maturity, such that the weight of 100 berries was constant, and the probable alcohol was around 13% (v/v). A set of 250 grapes was collected haphazard per treatment and replicated (a set of 50 grape berries was frozen and stored at −20 °C until must volatile composition analysis was performed). Then, the remaining grapes were destemmed and crushed to obtain the musts, and the general parameters were analysed.

Determination of general parameters in musts

The grape must was analysed for enological parameters, such as °Brix, probable alcohol, pH and total acidity, according to the official methods established by the OIV.[37] Glucose + fructose, glucose (and fructose indirectly, via subtraction of glucose + fructose − glucose), tartaric and malic acids, total phenols and nitrogen fractions were determined using a Miura One enzymatic equipment (TDI, Barcelona, Spain). The yeast assimilable nitrogen (YAN) was calculated as the sum of amino and ammonium nitrogen content.

Because the treatments were performed in triplicate, the results of these parameters are shown as the average of three analyses ($n = 3$).

Analysis of volatile compounds in the musts by headspace solid-phase microextraction (HS-SPME)-gas chromatography (GC)-mass spectrometry (MS)

Determination of volatile compounds in the musts was carried out by HS-SPME, and their subsequent analysis by GC-MS, according to the method described by Garde-Cerdán *et al.*[36] The SPME fibre used was divinylbenzene/carboxen/polydimethylsiloxane (50/30 μm) (Supelco, Bellenfonte, PA, USA). In a 20 mL vial (Supelco), 9 mL of sample, 2.5 g NaCl and 10 μL of internal standard (2-octanol; Sigma-Aldrich) were added. After adding a stir bar, the vial was closed and placed in the GC-MS (Agilent, Palo Alto, CA, USA). Sample conditioning was performed at 60 °C, for 15 min and with stirring. After this step, the fibre was automatically inserted into the headspace to extract the volatile compounds, for 105 min, with agitation.

After the extraction process, the fibre was immediately introduced into the GC injection port at 250 °C and held for 15 min for desorption of the compounds of interest. The capillary column used for analyte separation is a SPB-20 (30 m × 0.25 mm inner diameter × 0.25 μm film thickness) (Supelco). Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The chromatographic conditions used were initial temperature, 40 °C for 5 min, a temperature gradient of 2 °C min^{-1}, up to a final temperature of 220 °C, which was maintained for 20 min (total time = 115 min). The ionisation of the volatile compounds was performed at 70 eV. The detector worked at full scan mode (*m/z* 35–300). Identification was carried out using the NIST library and compared with the chromatographic standards' mass spectra and retention time, when available, and with data found in the literature. A semi-quantification was performed by relating the areas of each compound to the area and known concentration of the internal standard.

Because the treatments were performed in triplicate, the results of volatile compounds are expressed as the mean concentration of the three replicates ($n = 3$).

Statistical analysis

The statistical elaboration of the data was performed using SPSS, version 21.0 (IBM Corp. Armonk, NY, USA). General parameters and volatile compounds data were processed using analysis of variance (ANOVA) ($P \leq 0.05$). Differences between samples were compared using the Duncan test at a 95% probability level. Discriminant analysis was performed to classify the different samples according to their volatile composition.

RESULTS AND DISCUSSION

Non-toxic and biodegradable ACP nanoparticles provided slow release of MeJA or urea, as well as demonstrating a protective action against thermal degradation and ensuring a sustained supply of the biostimulants, resulting in a significant efficiency increase. Thus, ACP nanoparticles allow reduction of the biostimulant dosage by 10 or 15 times (MeJA or Ur, respectively), at the same time as maintaining the quality of the grapes.[21,29-31,33,35] In the present study, the effect of the foliar application of ACP nanoparticles functionalised with both molecules, MeJA and urea (ACP-MeJA+Ur), on the volatile composition of 'Tempranillo' grapes was evaluated for the first time. The effect of this novel nanoformulation was compared to the individual effect of each molecule, either free (MeJA, Ur) or loaded on the nanoparticle (ACP-MeJA, ACP-Ur), as well as the combinatorial effect of both free molecules (MeJA+Ur).

Effect of the foliar treatments on the must general parameters

The results of the general parameters in grapes of the control samples and those of the treatments (MeJA, ACP-MeJA, Ur, ACP-Ur, MeJA+Ur and ACP-MeJA+Ur) are shown in Table 1. The physico-chemical parameters, weight of 100 berries, °Brix, probable alcohol, Glu + Fru, Glu, Fru, pH, total acidity and tartaric acid, showed no changes between treated and control samples (Table 1); those results are in agreement with previous studies, where the application of conventional or nanoparticle-doped MeJA and/or Ur does not impact the majority of the general

Table 1. General parameters in grapes from control, methyl jasmonate (MeJA), methyl jasmonate on nanoparticles (ACP-MeJA), urea (Ur), urea on nanoparticles (ACP-Ur), methyl jasmonate + urea (MeJA+Ur) and methyl jasmonate + urea on nanoparticles (ACP-MeJA+Ur) foliar treatments.

	Control	MeJA	ACP-MeJA	Ur	ACP-Ur	MeJA+Ur	ACP-MeJA+Ur
Weight of 100 berries (g)	221.65 ± 8.11 a	230.00 ± 18.18 a	216.56 ± 35.38 a	237.75 ± 36.80 a	235.34 ± 15.92 a	213.31 ± 19.99 a	224.72 ± 5.72 a
°Brix	21.53 ± 0.75 a	20.80 ± 0.87 a	21.27 ± 1.69 a	21.87 ± 2.28 a	21.40 ± 0.52 a	21.27 ± 1.76 a	20.57 ± 0.55 a
Probable alcohol (% v/v)	12.45 ± 0.51 a	11.95 ± 0.59 a	12.27 ± 1.15 a	12.69 ± 1.57 a	12.35 ± 0.35 a	12.27 ± 1.20 a	11.76 ± 0.34 a
Glu + Fru (g L^{-1})	205.57 ± 10.30 a	199.08 ± 9.42 a	215.32 ± 21.32 a	213.05 ± 26.55 a	214.55 ± 4.71 a	203.99 ± 22.61 a	206.36 ± 8.64 a
Glu (g L^{-1})	102.48 ± 6.15 a	96.51 ± 5.96 a	106.11 ± 10.27 a	105.05 ± 16.13 a	104.64 ± 2.49 a	99.39 ± 13.19 a	101.36 ± 6.77 a
Fru (g L^{-1})	103.08 ± 4.16 a	102.57 ± 3.46 a	109.22 ± 11.06 a	108.00 ± 10.52 a	109.92 ± 3.25 a	104.60 ± 9.57 a	105.00 ± 1.98 a
pH	3.67 ± 0.09 a	3.70 ± 0.06 a	3.73 ± 0.21 a	3.73 ± 0.11 a	3.73 ± 0.07 a	3.87 ± 0.05 a	3.86 ± 0.13 a
Total acidity (g L^{-1})[a]	4.89 ± 0.17 a	4.90 ± 0.35 a	4.53 ± 0.09 a	4.89 ± 0.51 a	4.93 ± 0.17 a	4.96 ± 0.45 a	4.88 ± 0.14 a
Tartaric acid (g L^{-1})	5.76 ± 0.21 a	5.74 ± 0.39 a	5.49 ± 0.07 a	5.32 ± 0.31 a	5.51 ± 0.26 a	5.74 ± 0.44 a	5.57 ± 0.26 a
Malic acid (g L^{-1})	2.17 ± 0.05 a	2.09 ± 0.28 a	2.22 ± 0.23 a	2.28 ± 0.11 a	2.38 ± 0.23 ab	2.71 ± 0.20 b	2.05 ± 0.32 a
Total phenols (mg L^{-1})	712.57 ± 50.41 c	611.10 ± 22.26 a	602.17 ± 34.10 a	623.27 ± 20.87 ab	697.03 ± 51.83 bc	673.10 ± 16.67 abc	607.07 ± 13.16 a
Ammonium nitrogen (mg N L^{-1})	68.01 ± 4.62 a	85.97 ± 7.63 b	63.70 ± 9.53 a	65.52 ± 7.44 a	56.42 ± 5.19 a	72.80 ± 12.87 ab	66.56 ± 11.15 a
Amino nitrogen (mg N L^{-1})	164.59 ± 11.00 a	248.68 ± 16.62 c	175.19 ± 8.83 ab	153.44 ± 13.45 a	153.28 ± 11.65 a	260.96 ± 19.24 c	190.74 ± 13.25 b
YAN (mg N L^{-1})	232.60 ± 15.60 ab	334.65 ± 23.35 c	238.89 ± 12.62 ab	218.96 ± 20.34 ab	209.70 ± 16.79 a	333.76 ± 31.44 c	257.30 ± 23.00 b

[a] As g L^{-1} tartaric acid. Glu: glucose; Fru: fructose; YAN: yeast assimilable nitrogen. All parameters are listed with their standard deviation ($n = 3$). For each parameter, different lowercase letters indicate significant differences between the samples ($P \leq 0.05$).

parameters of the must.[20,23,27,33,38,39] On the other hand, the foliar application of some treatments affected the rest of the musts enological parameters. The malic acid content increased by approximately 24.88% in the musts with the foliar application of MeJA +Ur, showing significant differences compared to control samples (Table 1). Garde-Cerdán *et al.*[40] reported, in the 2019 vintage, that the concentration of malic acid in MeJA-treated vines showed no difference compared to the control. However, in the 2020 vintage, the concentration of malic acid was slightly higher with the foliar application of MeJA and MeJA+Ur. From this, it can be inferred that, when conducting the study across different vintages, the concentration of malic acid in the must depends on various factors, such as climatic conditions and the degree of fruit ripeness.[27] The total phenols content decreased by 14.24%, 15.49%, 12.53% and 14.81%, with the application of MeJA, ACP-MeJA, Ur and ACP-MeJA+Ur, respectively, compared to the control samples. Additional research is needed to thoroughly investigate the impact of nano-treatments in total phenols content.

Regarding nitrogen content, significant effects were only observed with the MeJA treatment (Table 1). Ammonium nitrogen increased (26.46%) with the application of MeJA compared to control must. Similar results were observed for amino nitrogen and YAN content because the application of MeJA and MeJA+Ur significantly increased these nitrogen fractions in the must compared to the control. Amino nitrogen increased by about 51.09% and 58.55%, respectively, whereas YAN increased by about 43.87% and 43.49%, respectively (Table 1). Similar results were reported in must from Tempranillo grapes treated with MeJA and MeJA+Ur in the 2019 vintage.[40] MeJA may affect the expression of genes linked to nitrogen metabolism in plants, potentially leading to increased synthesis of proteins and nitrogen compounds, thereby raising nitrogen concentration in grapevine tissues.[41,42] This could also explain the similar nitrogen fraction concentrations observed in Ur-treated grapevines compared to the control. Garde-Cerdán *et al.*[40] noted significant differences in YAN concentrations between the control grapes in 2019 and 2020, with higher levels in 2020. They attributed this variation to weather conditions, particularly the wetter conditions in 2020 promoting nutrient uptake and berry weight. They also emphasised the role of plant nutritional needs in nitrogen uptake.

On the other hand, the results of the statistical analysis concerning the influence of the application method (conventional *versus* nanoparticles; individual *versus* combined) on the general parameters of the musts are shown in Table 2. Considering the effect of the form of application of the treatments (conventional *versus* nanoparticles), statistically significant differences were observed in some parameters with the application of MeJA *versus* ACP-MeJA, obtaining higher contents of ammonium nitrogen, amino nitrogen and YAN, in the samples treated with MeJA compared to ACP-MeJA, whereas no significant effects were observed for any of the general parameters studied when Ur *versus* ACP-Ur treatments were applied (Table 2). Similarly, when applying MeJA+Ur, higher concentrations of malic acid, total phenols, amino nitrogen and YAN were obtained compared to the application of ACP-MeJA+Ur (Table 2). Finally, the combination of these biostimulants showed significant effects on some of the general parameters, i.e. total acidity, malic acid, total phenols, ammonium nitrogen, amimo nitrogen and YAN (Table 2), with their contents being intermediate or similar to that of some of the biostimulants applied individually. However, malic acid and total phenols increased significantly with the MeJA+Ur combination respect to the individual treatments (Table 2). Therefore, general

Table 2. One-factor ANOVA for enological parameters: conventional *versus* nano (MeJA/ACP-MeJA; Ur/ACP-Ur; MeJA+Ur/ACP-MeJA+Ur) and individual *versus* combined (MeJA/Ur/MeJA+Ur; ACP-MeJA/ACP-Ur/ACP-MeJA+Ur).

	MeJA/ ACP-MeJA		Ur/ACP-Ur		MeJA+Ur/ACP-MeJA+Ur		MeJA/Ur/MeJA+Ur		ACP-MeJA/ACP-Ur/ACP-MeJA+Ur	
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Weight of 100 berries (g)	0.343	0.590	0.011	0.922	0.903	0.396	0.674	0.545	0.519	0.619
°Brix	0.181	0.692	0.119	0.747	0.434	0.546	0.284	0.762	0.528	0.615
Probable alcohol (% v/v)	0.187	0.687	0.129	0.738	0.489	0.523	0.291	0.757	0.585	0.586
Glu + Fru (g L^{-1})	1.457	0.294	0.009	0.928	0.029	0.873	0.346	0.721	0.403	0.685
Glu (g L^{-1})	1.963	0.234	0.002	0.967	0.053	0.829	0.362	0.711	0.338	0.726
Fru (g L^{-1})	0.985	0.377	0.091	0.778	0.005	0.946	0.315	0.741	0.465	0.649
pH	0.045	0.842	0.000	1.000	5.741	0.075	4.235	0.071	0.114	0.894
Total acidity (g L^{-1})	3.237	0.146	0.015	0.909	0.103	0.764	0.025	0.976	7.664	0.022 a/b/b
Tartaric acid (g L)	1.206	0.334	0.651	0.465	0.313	0.606	1.169	0.373	0.127	0.883
Malic acid (g L^{-1})	0.407	0.558	0.488	0.523	9.147	0.039	7.044	0.027 a/a/b	1.178	0.370
Total phenols (mg L^{-1})	0.030	0.872	5.229	0.084	29.013	0.006	8.036	0.020 a/a/b	2.464	0.166
Ammonium nitrogen (mg N L^{-1})	9.981	0.034	3.017	0.157	0.403	0.560	3.463	0.050 b/a/ab	1.016	0.417
Amino nitrogen (mg N L^{-1})	45.736	0.002	0.000	0.989	27.092	0.006	37.673	0.000 b/a/b	8.187	0.019 ab/a/b
YAN (mg N L^{-1})	39.049	0.003	0.369	0.576	11.561	0.027	20.465	0.002 b/a/b	5.344	0.046 ab/a/b

For the factor individual *versus* combined, different lowercase letters indicate significant differences among treatments, for each parameter ($P \leq 0.05$).

parameters in grapes were slightly affected by the foliar treatments, with a greater influence on the nitrogen fractions and total phenols content in grapes.

Influence of the foliar treatments on must volatile compounds: comparison with the control samples

The results of the volatile compounds identified in the control musts and in the musts from the foliar treatments studied are presented Figs 1 and 2 and Tables 3 and 4. In total, 38 volatile compounds were identified, belonging to the terpenoids, C_{13} norisoprenoids, benzenoid compounds, alcohols, carbonyl compounds, esters and C6 compounds chemical families.

Terpenoids

Figure 1 shows the terpenoids content in grapes. Terpenoids play a key role in grape varietal aroma because they contribute to the floral and citrus character.[4,5] Some of the most odiferous terpenoids are linalool, geraniol, nerol and α-terpineol.

The linalool content in the musts decreased with the foliar applications of ACP-Ur and ACP-MeJA+Ur (49.32% and 40.67%, respectively) (Fig. 1a). On the other hand, the α-terpineol concentration in the musts increased by approximately 93.97% with Ur treatment (Fig. 1b). The γ-geraniol content in the musts was not affected by any of the treatments carried out in the vineyard (Fig. 1c). However, nerol synthesis decreased with foliar applications of Ur and MeJA+Ur (37.26% and 57.86%, respectively) (Fig. 1d). Geraniol biosynthesis was not favoured with ACP-MeJA+Ur application (Fig. 1e). In relation to neral, a decrease in its concentration was recorded in samples treated with Ur, MeJA+Ur and ACP-MeJA+Ur; this decrease was 46.83%, 62.19% and 38.9%, respectively (Fig. 1f). Finally, the concentration of geranyl acetone decreased in samples treated with ACP-MeJA, Ur and MeJA+Ur; in turn, the ACP-Ur treatment was the only one that significantly increased its content (44.56%) (Fig. 1g). Consequently, the total terpenoids content decreased by 36.33%, 46.25% and 18.83% with the foliar applications of ACP-MeJA, Ur and MeJA+Ur, respectively (Fig. 1h).

Some research has reported the role of MeJA in activating terpenoids metabolism by increasing geranylgeranyl diphosphate synthase,[43] in contrast to the findings of the present study, where MeJA grapes did not show differences in terpenoids content compared to the control ones. However, the foliar application of MeJA on Sangiovese grapes led to an increase in the concentration of volatile compounds in both berries and wines, especially terpenoids, despite the naturally low levels of these volatile compounds in this grape variety.[7] Similarly, Gómez-Plaza *et al.*[43] reported that exogenous application of MeJA in 'Monastrell' berries stimulated terpenoids synthesis, presenting higher levels than in untreated grapes. Nevertheless, Garde-Cerdán *et al.*[4] did not show significant differences among untreated berries and grapes from vines foliar treated with proline, phenylalanine and urea in 'Tempranillo', results that agree with those of the present study when MeJA application was performed.

C_{13} norisoprenoids

Figure 2 presents the C_{13} norisoprenoids identified in the must samples. C_{13} norisoprenoids stem from the degradation of carotenoids.

First, it was demonstrated that β-cyclocitral content decreased (29.18%) in musts with Ur application (Fig. 2a). As for the TDN, β-damascenone and α-ionone, their concentration remained similar to the control samples (Fig. 2b–d). These findings align with those of Garde-Cerdán *et al.*,[4] who found that the concentrations of C_{13} norisoprenoids in 'Tempranillo' grapes treated with phenylalanine, urea and proline showed no significant differences compared to the control. On the other hand, β-ionone content in the grape musts decreased with ACP-MeJA, Ur and MeJA+Ur applications, by 30.25%, 35.80% and 51.62%, respectively (Fig. 2e). Finally, methyl jasmonate concentration decreased with all foliar treatments carried out in the vines (Fig. 2f). Therefore, the content of total C_{13} norisoprenoids also decreased between 36.96% and 55.48% with foliar applications of ACP-Ur, MeJA+Ur and ACP-MeJA+Ur (Fig. 2g).

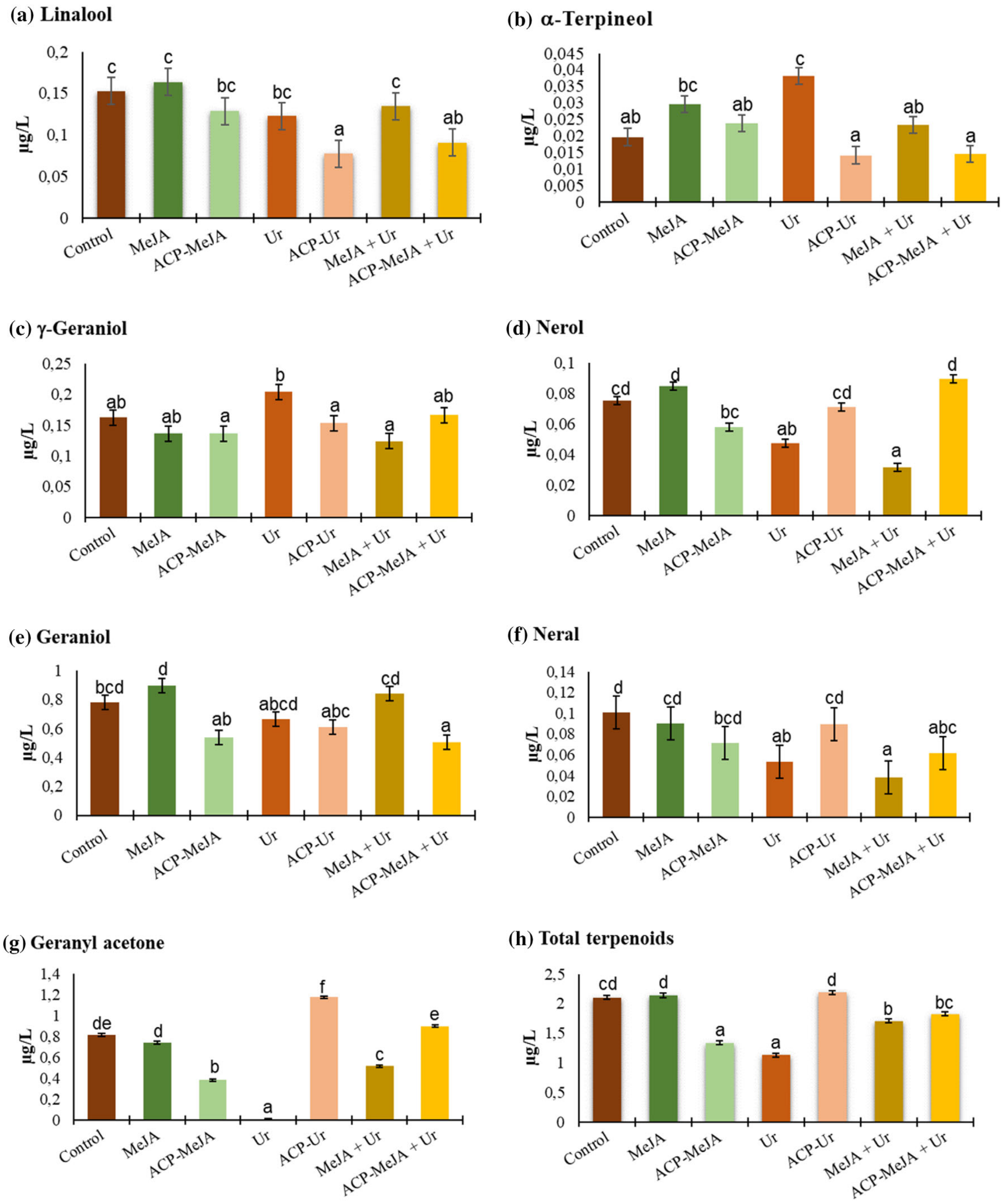

Figure 1. Terpenoids concentration (µg L^{-1}) (a, linalool; b, α-terpineol; c, γ-geraniol; d, nerol; e, geraniol, f, neral; g, geranyl acetone; h, total terpenoids) in grapes from control, methyl jasmonate (MeJA), methyl jasmonate on nanoparticles (ACP-MeJA), urea (Ur), urea on nanoparticles (ACP-Ur), methyl jasmonate + urea (MeJA+Ur) and methyl jasmonate + urea on nanoparticles (ACP-MeJA+Ur) foliar treatments. All parameters are listed with the SD (n = 3). Different lowercase letters indicate significant differences between samples ($P \leq 0.05$) for each season and compound.

After assessing the effect of MeJA on 'Tempranillo' grapes during three vintages, Garde-Cerdán *et al.*[36] observed that the formation of volatile compounds depends on several factors, such as variety, viticultural practices, climatic conditions, soil characteristics and degree of fruit ripening.

Within this family, the most important compounds are β-damascenone and β-ionone, as they are the most important contributors to odour and flavour, providing aromas of roses and violets.[4,36,43] Similar to terpenoids, this family has low perception thresholds, thus enhancing the aroma of many grape varieties.[44]

Benzenoid compounds

Two benzenoid compounds were identified: 2-phenylethanal and 2-phenylethanol (Table 3). The ACP-MeJA, Ur, MeJA+Ur and ACP-MeJA+Ur treatments stimulated the synthesis of 2-phenylethanal in the musts, obtaining a higher content compared to the control samples, at 136.74%, 55.24%, 172.02% and 66.05%, respectively.

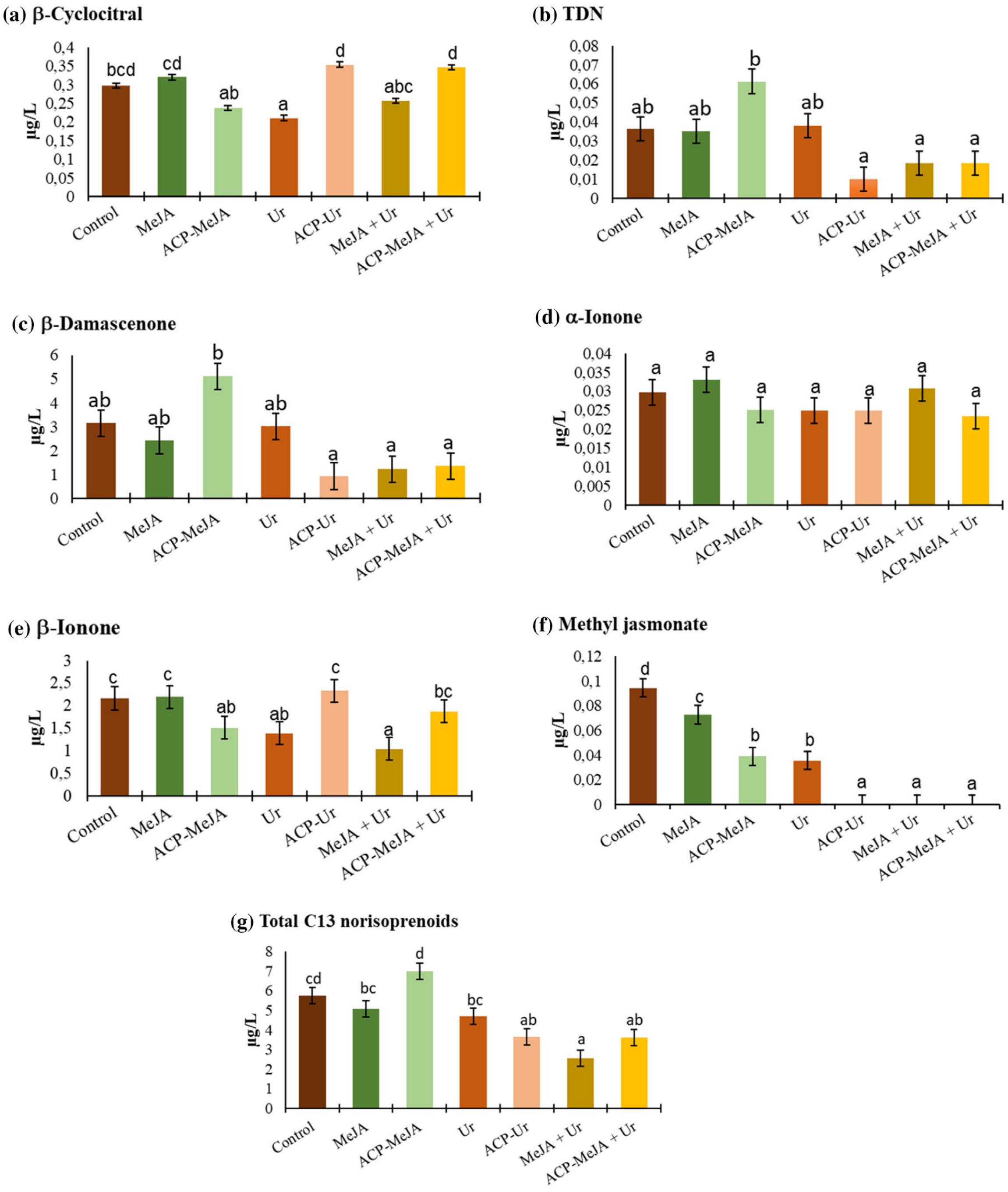

Figure 2. C_{13} norisoprenoids concentration (µg L^{-1}) (a, β-cyclocitral; b, TDN; c, β-damascenone; d, α-ionone; e, β-ionone; f, methyl jasmonate; g, total C_{13} norisoprenoids) in grapes from control, methyl jasmonate (MeJA), methyl jasmonate on nanoparticles (ACP-MeJA), urea (Ur), urea on nanoparticles (ACP-Ur), methyl jasmonate + urea (MeJA+Ur) and methyl jasmonate + urea on nanoparticles (ACP-MeJA+Ur) foliar treatments. All parameters are listed with the SD ($n = 3$). Different lowercase letters indicate significant differences between samples ($P \leq 0.05$) for each season and compound. TDN, 1,1,6-trimethyl-1,2-dihydronaphthalene.

On the other hand, 2-phenylethanol content decreased (61.46%) in the samples from Ur foliar applications. Garde-Cerdán *et al.*[36] reported that benzenoid compounds and their total content in 'Tempranillo' grapes were not affected by foliar application of MeJA, in agreement with the results of the present study, because the only treatment that affected the content of total benzenoid compounds was the foliar application of Ur, reducing its content in the musts (Table 3).

Alcohols

Concerning alcohols, most treatments did not affect heptanol concentration, except for the foliar application of Ur and MeJA +Ur, which resulted in a higher concentration compared to the control sample. Specifically, increases of 114.29% and 214.29%, respectively, were observed (Table 3). Similarly, the concentration of 1-octen-3-ol increased by 75.74% in the samples treated with ACP-Ur, whereas ACP-MeJA and MeJA+Ur decreased their

Table 3. Concentration (μg L^{-1}) of benzenoid compounds, alcohols, carbonyl compounds, esters, and C6 compounds in grapes from control, methyl jasmonate (MeJA), methyl jasmonate on nanoparticles (ACP-MeJA), urea (Ur), urea on nanoparticles (ACP-Ur), methyl jasmonate + urea (MeJA+Ur) and methyl jasmonate + urea on nanoparticles (ACP-MeJA+Ur) foliar treatments.

	Control	MeJA	ACP-MeJA	Ur	ACP-Ur	MeJA+Ur	ACP-MeJA+Ur
Benzenoid compounds							
2-Phenylethanal	1.09 ± 0.01 a	1.45 ± 0.29 ab	2.59 ± 0.14 d	1.70 ± 0.17 bc	1.12 ± 0.1 a	2.98 ± 0.12 e	1.82 ± 0.25 c
2-Phenylethanol	6.37 ± 0.15 b	6.07 ± 1.49 b	4.41 ± 2.02 ab	2.45 ± 0.10 a	5.37 ± 1.03 b	3.76 ± 0.11 ab	5.14 ± 0.25 b
Total benzenoid compounds	7.46 ± 0.10 b	7.52 ± 1.67 b	7.01 ± 2.11 b	4.16 ± 0.19 a	6.49 ± 0.64 b	6.74 ± 0.16 b	6.96 ± 0.51 b
Alcohols							
Heptanol	0.07 ± 0.01 a	0.11 ± 0.01 ab	0.13 ± 0.05 ab	0.15 ± 0.04 b	0.08 ± 0.00 a	0.22 ± 0.03 c	0.12 ± 0.01 ab
1-Octen-3-ol	1.36 ± 0.25 c	1.22 ± 0.08 bc	0.68 ± 0.19 a	1.01 ± 0.14 abc	2.39 ± 0.52 d	0.8 ± 0.08 ab	1.36 ± 0.15 c
2-Ethyl-1-hexanol	2.3 ± 0.09 b	2.18 ± 0.23 b	1.74 ± 0.46 ab	1.78 ± 0.48 ab	1.8 ± 0.1 ab	1.46 ± 0.03 a	1.85 ± 0.33 ab
1-Nonanol	0.49 ± 0.09 a	0.59 ± 0.1 a	1.21 ± 0.35 b	0.51 ± 0.14 a	0.55 ± 0.07 a	1.46 ± 0.09 b	0.8 ± 0.14 a
1-Decanol	0.1 ± 0.01 ab	0.1 ± 0.02 b	0.11 ± 0.03 b	0.1 ± 0.04 ab	0.05 ± 0.01 a	0.1 ± 0.00 ab	0.05 ± 0.01 a
1-Dodecanol	0.17 ± 0.01 b	0.13 ± 0.00 ab	0.11 ± 0.04 ab	0.08 ± 0.01 a	0.15 ± 0.01 ab	0.12 ± 0.00 ab	0.28 ± 0.06 c
Total alcohols	4.49 ± 0.17 a	4.34 ± 0.17 ab	3.98 ± 0.75 ab	3.64 ± 0.58 a	5.03 ± 0.45 b	4.16 ± 0.01 ab	4.45 ± 0.28 ab
Carbonyl compounds							
(*E*)-2-Heptenal	0.21 ± 0.05 b	0.08 ± 0.01 a	0.07 ± 0.01 a	0.09 ± 0.00 a	0.36 ± 0.12 c	0.1 ± 0.01 a	0.16 ± 0.00 ab
Nonanal	3.51 ± 0.37 bc	2.68 ± 0.26 ab	2.18 ± 0.79 a	1.87 ± 0.28 a	5.56 ± 1.04 d	2.88 ± 0.52 ab	4.66 ± 0.44 cd
(*E*)-2-Nonenal	0.48 ± 0.13 bc	0.61 ± 0.11 cd	0.29 ± 0.04 a	0.27 ± 0.06 a	0.69 ± 0.08 d	0.41 ± 0.09 ab	0.53 ± 0.13 bcd
Decanal	0.41 ± 0.07 de	0.33 ± 0.05 cd	0.19 ± 0.06 ab	0.18 ± 0.06 a	0.29 ± 0.03 bc	0.44 ± 0.05 e	0.23 ± 0.01 abc
γ-Decalactone	0.11 ± 0.02 a	0.15 ± 0.02 a	0.1 ± 0.02 a	0.34 ± 0.08 c	0.14 ± 0.05 a	0.25 ± 0.00 b	0.11 ± 0.03 a
(*E*,*E*)-2,4-Hexadienal	1.29 ± 0.27 c	0.78 ± 0.14 b	0.46 ± 0.1 a	0.38 ± 0.04 a	0.82 ± 0.13 b	0.28 ± 0.01 a	0.35 ± 0.01 a
(*E*,*E*)-2,4-Heptadienal	1.19 ± 0.09 d	0.85 ± 0.12 c	0.48 ± 0.24 ab	0.21 ± 0.00 a	0.69 ± 0.21 bc	0.28 ± 0.03 a	0.37 ± 0.06 a
6-Methyl-3,5-heptadien-2-one	0.04 ± 0.00 de	0.03 ± 0.00 cd	0.02 ± 0.00 b	0.03 ± 0.00 bc	0.04 ± 0.01 e	0.00 ± 0.00 a	0.03 ± 0.00 cd
(*E*,*E*)-2,4-Nonadienal	0.38 ± 0.04 c	0.36 ± 0.03 c	0.2 ± 0.02 a	0.25 ± 0.02 ab	0.47 ± 0.08 d	0.23 ± 0.03 a	0.32 ± 0.02 bc
(*E*,*E*)-2,4-Decadienal	0.08 ± 0.01 a	0.06 ± 0.01 a	0.04 ± 0.01 a	0.04 ± 0.01 a	0.46 ± 0.08 b	0.02 ± 0.00 a	0.05 ± 0.01 a
Total carbonyl compounds	7.72 ± 0.66 d	5.93 ± 0.57 bc	4.03 ± 1.138 a	3.64 ± 0.24 a	9.52 ± 1.65 e	4.90 ± 0.59 ab	6.80 ± 0.56 cd
Esters							
Hexyl acetate	0.04 ± 0.00 a	0.03 ± 0.00 a	0.04 ± 0.01 a	0.03 ± 0.01 a	0.05 ± 0.03 a	0.22 ± 0.01 b	0.02 ± 0.00 a
Ethyl 2-hexenoate	0.03 ± 0.00 ab	0.04 ± 0.00 bc	0.02 ± 0.00 a	0.03 ± 0.01 ab	0.04 ± 0.01 bc	0.08 ± 0.00 d	0.05 ± 0.01 c
Total esters	0.07 ± 0.00 ab	0.07 ± 0.00 ab	0.06 ± 0.01 a	0.06 ± 0.01 a	0.09 ± 0.02 b	0.3 ± 0.01 c	0.07 ± 0.01 ab
C6 compounds							
1-Hexanol	15.58 ± 2.4 ab	19.02 ± 0.28 b	13.95 ± 1.87 ab	40.42 ± 2.32 d	14.91 ± 1.41 ab	25.9 ± 5.58 c	11.78 ± 0.67 a
(*Z*)-3-Hexen-1-ol	0.44 ± 0.08 b	0.95 ± 0.17 c	1.54 ± 0.06 d	0.47 ± 0.02 b	0.36 ± 0.01 ab	0.21 ± 0.02 a	0.21 ± 0.03 a
(*E*)-2-Hexen-1-ol	3.49 ± 0.53 ab	3.027 ± 0.21 ab	1.57 ± 0.02 a	5.99 ± 2.15 c	1.83 ± 0.12 a	4.35 ± 0.32 bc	1.91 ± 0.21 a
Hexanal	47.15 ± 5.94 d	45.21 ± 3.74 cd	22.69 ± 5.68 a	28.32 ± 5.09 ab	36.16 ± 2.38 bc	41.02 ± 5.97 cd	30.66 ± 4.18 ab
(*E*)-2-Hexenal	19.91 ± 1.79 cd	21.16 ± 2.31 d	9.89 ± 3.19 a	16.09 ± 0.89 b	12.31 ± 1.41 a	17.24 ± 0.74 bc	10.85 ± 1.86 a
Total C6 compounds	86.57 ± 9.78 c	89.36 ± 5.86 c	49.64 ± 7.06 a	91.27 ± 4.93 c	65.57 ± 5.32 b	88.74 ± 6.96 c	55.41 ± 6.44 ab

All parameters are shown with their standard deviation ($n = 3$). For each compound, different lowercase letters indicate significant differences between treatments ($P \leq 0.05$).

Table 4. One-factor ANOVA for volatile compounds: conventional *versus* nano (MeJA/ACP-MeJA; Ur/ACP-Ur; MeJA+Ur/ACP-MeJA+Ur) and individual *versus* combined (MeJA/Ur/MeJA+Ur; ACP-MeJA/ACP-Ur/ACP-MeJA+Ur).

	MeJA/ACP-MeJA		Ur/ACP-Ur		MeJA+Ur/ACP-MeJA+Ur		MeJA/Ur/MeJA+Ur		ACP-MeJA/ACP-Ur/ACP-MeJA+Ur	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Terpenoids										
Linalool	2.64	0.18	0.012	0.92	11.52	0.027	2.46	0.17	0.07	0.94
α-Terpineol	0.67	0.47	30.01	0.01	25.55	0.015	5.39	0.06	1.97	0.25
γ-Geraniol	0.45	0.57	7.497	0.05	10.14	0.033	6.54	0.04 ab/b/a	0.95	0.45
Nerol	1.77	0.31	14.36	0.03	72.36	0.003	11.64	0.04 b/a/a	4.22	0.08
Geraniol	5.89	0.09	0.28	0.64	10.83	0.046	2.05	0.22	0.47	0.66
Neral	0.86	0.40	48.69	0.01	19.21	0.012	21.24	0.00 b/a/a	1.10	0.40
Geranyl acetone	30.88	0.03	603.50	0.00	46.59	0.002	95.23	0.00 c/a/b	82.62	0.00 a/c/b
Total terpenoids	16.95	0.01	71.99	0.00	1.68	0.264	19.55	0.00 c/a/b	23.34	0.00 a/c/b
C_{13} norisoprenoids										
β-Cyclocitral	2.23	0.21	23.62	0.01	70.19	0.001	6.02	0.04 b/a/ab	5.06	0.05 a/b/b
TDN	0.89	0.44	3615.12	0.00	0.00	0.982	10.40	0.05 b/b/a	4.09	0.11
β-Damascenone	1.54	0.34	127.40	0.01	0.68	0.497	40.62	0.01 b/b/a	3.39	0.17
α-Ionone	1.17	0.34	0.00	0.99	6.81	0.080	2.13	0.21	0.04	0.96
β-Ionone	3.31	0.17	26.59	0.01	21.42	0.010	11.04	0.01 b/a/a	6.24	0.04 a/b/ab
Methyl jasmonate	14.45	0.03	31.22	0.01	–	–	59.71	0.00 c/b/a	56.52	0.00 b/a/a
Total C_{13} norisoprenoids	1.65	0.27	18.82	0.01	72.06	0.001	56.09	0.00 b/b/a	5.15	0.05 b/a/a
Benzenoid compounds										
2-Phenylethanal	1.308	0.32	15.97	0.06	8.41	0.063	7.504	0.04 b/a/ab	0.33	0.73
2-Phenylethanol	25.008	0.04	24.33	0.02	35.15	0.027	31.51	0.01 a/a/b	50.98	0.00 c/a/b
Total benzenoid compounds	0.109	0.76	35.83	0.00	0.50	0.518	9.79	0.01 b/a/b	0.14	0.87
Alcohols										
Heptanol	0.395	0.57	7.41	0.11	13.53	0.067	6.12	0.09	1.66	0.30
1-Octen-3-ol	20.88	0.02	22.38	0.02	22.05	0.018	9.26	0.02 b/ab/a	17.02	0.01 a/b/a
2-Ethyl-1-hexanol	1.51	0.31	0.01	0.94	2.45	0.216	2.08	0.24	0.08	0.92
1-Nonanol	5.55	0.14	0.16	0.72	43.63	0.007	62.32	0.00 a/a/b	6.75	0.05 b/a/ab
1-Decanol	0.00	0.97	2.12	0.24	30.47	0.012	0.04	0.96	6.23	0.04 b/a/a
1-Dodecanol	0.55	0.54	62.68	0.00	10.58	0.047	34.69	0.01 b/a/b	10.03	0.02 a/a/b
Total alcohols	0.67	0.46	10.76	0.03	3.22	0.147	3.34	0.11	2.89	0.13
Carbonyl compounds										
(*E*)-2-Heptenal	1.63	0.33	18.81	0.02	68.58	0.00	3.56	0.11	9.61	0.05 a/b/ab
Nonanal	0.69	0.47	21.96	0.02	20.38	0.01	3.85	0.12	14.48	0.00 a/b/b
(*E*)-2-Nonenal	22.91	0.01	51.46	0.00	1.72	0.26	11.40	0.01 b/a/a	14.99	0.00 a/b/b
Decanal	8.95	0.04	9.26	0.04	27.28	0.04	13.64	0.01 b/a/b	4.62	0.07
γ-Decalactone	7.23	0.07	13.24	0.04	31.21	0.01	12.89	0.02 a/b/ab	0.68	0.55
(*E*,*E*)-2,4-Hexadienal	7.73	0.07	32.38	0.01	46.77	0.02	22.41	0.00 b/a/a	15.06	0.01 a/b/a
(*E*,*E*)-2,4-Heptadienal	5.59	0.09	9.50	0.05	21.78	0.04	42.90	0.00 b/a/a	1.76	0.28
6-Methyl-3,5-heptadien-2-one	19.31	0.02	8.43	0.06	121.52	0.00	86.39	0.00 b/b/a	7.61	0.04 a/b/ab
(*E*,*E*)-2,4-nonadienal	45.73	0.02	23.25	0.01	15.10	0.03	16.381	0.01 b/a/a	23.81	0.00 a/c/b

Table 4. Continued

	MeJA/ACP-MeJA		Ur/ACP-Ur		MeJA+Ur/ACP-MeJA+Ur		MeJA/Ur/MeJA+Ur		ACP-MeJA/ACP-Ur/ACP-MeJA+Ur	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
(*E*,*E*)-2,4-Decadienal	4.68	0.10	87.26	0.00	27.91	0.01	18.51	0.01 c/b/a	83.28	0.00 a/b/a
Total carbonyl compounds	6.84	0.06	37.05	0.00	16.23	0.02	16.21	0.00 c/a/b	15.70	0.00 a/c/b
Esters										
Hexyl acetate	1.61	0.33	1.56	0.34	430.49	0.00	264.30	0.00 a/a/b	1.68	0.32
Ethyl 2-hexenoate	30.91	0.03	2.31	0.23	10.48	0.08	29.17	0.01 a/a/b	9.17	0.03 a/ab/b
Total esters	1.99	0.23	5.04	0.09	533.98	0.00	437.07	0.00 a/a/b	2.25	0.19
C6 compounds										
1-Hexanol	14.39	0.06	250.83	0.00	11.47	0.04	14.27	0.02 a/b/a	3.01	0.16
(*Z*)-3-Hexen-1-ol	19.50	0.02	33.81	0.01	0.05	0.83	39.66	0.00 c/b/a	644.50	0.00 c/b/a
(*E*)-2-Hexen-1-ol	96.19	0.01	13.33	0.03	80.53	0.01	2.76	0.21	3.71	0.12
Hexanal	30.28	0.01	5.84	0.07	6.07	0.07	9.22	0.01 b/a/b	6.92	0.04 a/b/ab
(*E*)-2-Hexenal	21.85	0.02	10.83	0.05	30.54	0.00	7.46	0.03 b/a/a	0.88	0.47
Total C6 compounds	48.69	0.00	7.13	0.06	37.03	0.00	0.05	0.95	5.54	0.04 a/b/ab

content in the musts (50% and 41.18%, respectively). The 2-ethyl-1-hexanol content decreased with the application of MeJA+Ur (36.52%) (Table 3). Conversely, 1-nonanol content in the musts increased by 146.94% and 197.96% with ACP-MeJA and MeJA+Ur foliar applications, respectively. The concentration of 1-decanol did not show any change between the control sample and the foliar treatments (Table 3). Furthermore, the concentration of 1-dodecanol increased in the musts (by 64.71%) with the application of ACP-MeJA+Ur, whereas the application of Ur decreased the level of this compound by 52.94%. The total content of alcohols was solely influenced by the foliar application of ACP-Ur, resulting in an increase in their content (approximately 12.03%) compared to the control sample (Table 3).

Carbonyl compounds

It was observed that the (*E*)-2-heptenal content decreased with the foliar application of all treatments studied except for ACP-Ur, which increased its concentration by 71.43%, and ACP-MeJA+Ur, which did not influence in the content of this compound compared to the control samples (Table 3). Furthermore, foliar application of MeJA also produced a 64.34%, 59.66% and 47.80% reduction in the concentration of (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal and total carbonyl compounds, respectively, with respect to the control must. Overall, the foliar application of ACP-MeJA and Ur decreased in the musts the concentration of most of the compounds belonging to this family, except γ-decalactone and (*E*,*E*)-2,4-decadienal. The decrease with these applications ranged from 37–50% to 25–57%, respectively (Table 3). The opposite effect was observed with the application of ACP-Ur because this treatment increased the content of (*E*)-2-heptenal (71.43%), nonanal (58.40%), (*E*)-2-nonenal (43.75%), (*E*,*E*)-2,4-nonadienal (23.68%), (*E*,*E*)-2,4-decadienal (475%) and total carbonyl compounds (23.32%), whereas it decreased the content of decanal (28.27%), (*E*,*E*)-2,4-hexadienal (36.43%) and (*E*,*E*)-2,4-heptadienal (42.02%) with respect to the control must. Similarly, the application of MeJA+Ur decreased the content of (*E*)-2-heptanal, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal, 6-methyl-3,-5-heptadien-2-one and (*E*,*E*)-2,4-nonadienal, as well as total carbonyl compounds, by 36–100%, and increased the content of γ-decalactone (127.27%) (Table 3). Treatment ACP-MeJA+Ur had a significant effect on some compounds [decanal, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal], reducing their content by 43–72.87% compared to the control must (Table 3).

Within this family, nonanal was the main major compound, increasing significantly after treatment with urea nanoparticles. Similarly, Cheng *et al.*[38] reported a high nonanal content after foliar application of urea in Cabernet Sauvignon wine.

Esters

Ethyl esters have low detection thresholds and therefore play an essential role in the fruity aromas of wines; it should be noted that these compounds are formed mainly during alcoholic fermentation.[13] Hexyl acetate increased by approximately 450% in the samples treated with MeJA+Ur, showing differences between the control sample and the other treatments. The same results were observed for ethyl 2-hexenoate and total esters, which increased by 166.67% and 328.57%, respectively, when MeJA+Ur was applied (Table 3). It should be noted that ethyl 2-hexenoate also increased (66.67%) in the musts from ACP-MeJA+Ur. Previously, Garde-Cerdán *et al.*[4] observed that none of the nitrogen treatments applied to 'Tempranillo' vines affected the formation of esters identified in the grapes. No significant differences were observed between the control and foliar-treated samples, as was observed for Ur and ACP-Ur treatments (Table 3). In turn, when applying MeJA to strawberries during postharvest to improve aromatic and flavour qualities, de la Peña *et al.*[45] found that this elicitor has a significant effect on the biosynthesis of volatile compounds in this fruit because most of the compounds identified were higher than those found in the control sample; for example, ethyl hexanoate was found to be the most affected by the MeJA treatment. Therefore, in the present study, only the combined application of MeJA and Ur affected the content of this family of compounds (Table 3).

C6 compounds

Finally, the concentration of 1-hexanol in the musts increased with the foliar application of Ur and MeJA+Ur compared to the control and treated samples. The increase corresponds to 159% and 66.24%, respectively (Table 3). 1-Hexanol is characterised by herbaceous and fatty odours (related to harmful effects on the wine), and so its concentration at high levels can be undesirable. Similarly, the (*Z*)-3-hexen-1-ol content in the musts increased by 115.91% and 250% with the application of MeJA and ACP-MeJA, respectively, whereas it decreased (approximately 52.27%) in the samples treated with MeJA+Ur and ACP-MeJA+Ur (Table 3). In the case of (*E*)-2-hexen-1-ol, a higher concentration was found in Ur must, compared to the control sample. The concentration of hexanal and (*E*)-2-hexenal in the musts decreased between 19.19% and 51.88% in the samples treated with ACP-MeJA, Ur, ACP-Ur and ACP-MeJA+Ur with respect to the control sample (Table 3). By contrast, Cheng *et al.*[38] reported that urea application significantly increased (*E*)-2-hexenal levels in Cabernet Sauvignon grapes. Similarly, Gómez-Plaza *et al.*[43] obtained the highest relative concentrations of hexanal and (*E*)-2-hexenal when treating grapes with MeJA. In summary, the foliar application of ACP-MeJA, ACP-Ur and ACP-MeJA+Ur decreased the content of total C6 compounds by up to 42.66% (Table 3).

In the present study, the most abundant C6 compound in the control sample was hexanal, whereas the least abundant in this group was (*Z*)-3-hexen-1-ol, which is in agreement with the results reported by López-Tamames *et al.*[46] and Garde-Cerdán *et al.*[4] in 'Tempranillo' grapes.

It was evident that the major compounds were C6 compounds, which, in high concentrations, can contribute negative notes to the wine. These compounds are derived from fatty acids and are responsible for green aromas.[13] These results are in agreement with those reported by Cheng *et al.*[38] who investigated the effects of foliar nitrogen application on the volatile composition of Cabernet Sauvignon grapes from veraison to pre-harvest.

One-factor statistical analysis

Effect of conventional treatments compared to nano treatments

Table 4 shows the results of the one-factor statistical analysis, aiming to assess the impact of the treatment application mode on the volatile composition of 'Tempranillo' grape must.

Terpenoids

In this family, the application of MeJA and/or ACP-MeJA only presented statistically significant effects on geranyl acetone and total terpenoids, obtaining higher contents when MeJA was applied (Table 4). On the other hand, it was found that, when Ur was applied, a higher concentration of α-terpineol and γ-geraniol was obtained, in contrast to ACP-Ur, where a higher content of most of the compounds of this family, and therefore the total terpenoids, was obtained (Table 4). Significant differences were found between the treatments MeJA+Ur and ACP-MeJA+Ur, where, with the application of MeJA+Ur, a higher content of linalool, α-terpineol and geraniol was obtained, whereas γ-geraniol, nerol, neral and geranyl acetone increased in the musts with the application of ACP-MeJA+Ur, compared to MeJA+Ur (Table 4). It should be noted that the nano-treatments were applied at much lower urea and methyl jasmonate concentrations, at 0.4 kg N ha^{-1} and 1 mM, respectively, than the conventional applications, at 6 kg N ha^{-1} and 10 mM, respectively.

C_{13} norisoprenoids

Comparing the conventional and nano forms (Table 4), the foliar application of MeJA resulted in a higher methyl jasmonate content in the grape musts compared to ACP-MeJA. Between the conventional and nano form of Ur, a higher content of most compounds within this chemical family was found when Ur was applied, whereas ACP-Ur exhibited higher levels of β-cyclocitral and β-ionone (Table 4). Similarly, higher values of β-cyclocitral, β-ionone and total C_{13} norisoprenoids were found in the samples treated with ACP-MeJA+Ur compared to those treated conventionally.

Benzenoid compounds

Based on Table 4, the content of 2-phenylethanal only showed significant differences with the application of Ur, with a higher concentration observed with ACP-Ur. On the other hand, for 2-phenylethanol, there were significant differences between the conventional treatments and the nano-treatments, obtaining higher contents with the MeJA, ACP-Ur and ACP-MeJA+Ur applications; furthermore, significant effects were observed between the treatments individually and jointly, obtaining a higher concentration of 2-phenylethanol in the musts with the application of MeJA+Ur and ACP-MeJA (Table 4).

Total benzenoids content only showed significant differences between the conventional treatments and the nano-treatments with the application of Ur, with a higher concentration observed with ACP-Ur (Table 4). However, in the combined or individual form of the biostimulants, a significant effect was observed when they were applied conventionally, quantifying a higher content of total benzenoids in the musts with applications of MeJA and MeJA+Ur (Table 4).

Alcohols

Differences between MeJA and ACP-MeJA samples only were found with respect to 1-octen-3-ol content, obtaining a higher content with MeJA (Table 4). On the other hand, a statistically significant increase in the concentration of 1-octen-3-ol, 1-dodecanol and total alcohols was observed when Ur was applied in nano form. Finally, the foliar application of MeJA+Ur resulted in higher levels of 1-nonanol and 1-decanol, with respect to its nano form, whereas, with the application of ACP-MeJA+Ur, a significant increase of 1-octen-3-ol and 1-dodecanol was obtained, compared to its conventional form (Table 4).

Carbonyl compounds

All the compounds identified in this family decreased their content in the musts with the application of ACP-MeJA compared to MeJA, presenting statistically significant differences in (*E*)-2-nonenal, decanal, 6-methyl-3,5-heptadien-2-one and (*E*,*E*)-2,4-nonadienal (Table 4). The opposite effect was observed with ACP-Ur and Ur applications, where lower contents of most compounds were obtained when Ur was applied. A similar result was evident with the application of MeJA+Ur, concerning ACP-MeJA+Ur (Table 4).

Within this family, nonanal was the main major compound, increasing significantly after treatment with urea nanoparticles. Similarly, Cheng *et al.*[38] reported a high nonanal content after foliar application of urea in Cabernet Sauvignon wine.

Esters

According to Table 4, a statistically significant effect was obtained when MeJA and MeJA+Ur were applied, obtaining a higher content of ethyl 2-hexenoate and hexyl acetate in the musts, respectively, compared to the nano form. However, the content of total esters was significantly higher with the application of ACP-MeJA+Ur compared to the conventional form (Table 4).

C6 compounds

Foliar treatments with nanoparticles significantly decreased C6 compounds in the musts with respect to the conventional form of treatments (Table 4); this effect of amorphous calcium phosphate nanoparticles could be positive for aroma because high levels of C6 compounds can contribute undesirable herbaceous flavours to the wine.[4]

One-factor statistical analysis: influence of individual versus *combined treatments*

Overall, with the combination of MeJA and Ur treatments in conventional and/or nano form, concentrations were intermediate or similar to those observed when some of the biostimulants were applied individually (Table 4). Thus, one-factor statistical analysis indicated that there were statistically significant differences in TDN, β-damascenone, methyl jasmonate, total C_{13} norisoprenoids, 6-methyl-3,5-heptadien-2-one, (*E*,*E*)-2,4-decadienal and (*Z*)-3-hexen-1-ol content, which decreased in the musts with the combination of MeJA+Ur (Table 4). By contrast, 2-phenylethanol, 1-nonanol, hexyl acetate, ethyl 2-hexenoate and total esters increased in concentration when the same treatment (MeJA+Ur) was applied. Comparable outcomes were observed upon the application of nano-treatments, revealing substantial impacts on the majority of the identified compounds in this work, both individually and in combination (Table 4). Notably, the concentration of most compounds showed significant differences when comparing individual nano-treatments with the combined application of ACP-MeJA and ACP-Ur. In cases where distinctions did arise, as with 1-dodecanol and (*Z*)-3-hexen-1-ol, the application of ACP-MeJA+Ur resulted in statistically significant variations relative to their individual forms, with an increase in 1-dodecanol and a decrease in (*Z*)-3-hexen-1-ol (Table 4).

Discriminant analysis of volatile compounds

Figure 3 shows the results obtained after performing the discriminant analysis of the volatile compounds content in the musts (control, MeJA, ACP-MeJA, Ur, ACP-Ur, MeJA+Ur and ACP-MeJA+Ur). The analysis was performed with the concentrations of the identified compounds, taking each variable as independent values. As can be seen, samples were grouped as follows: ACP-MeJA; control and MeJA; Ur and ACP-Ur, and finally MeJA+Ur and ACP-MeJA+Ur (Fig. 3). Function 1 explained 65.6% of the

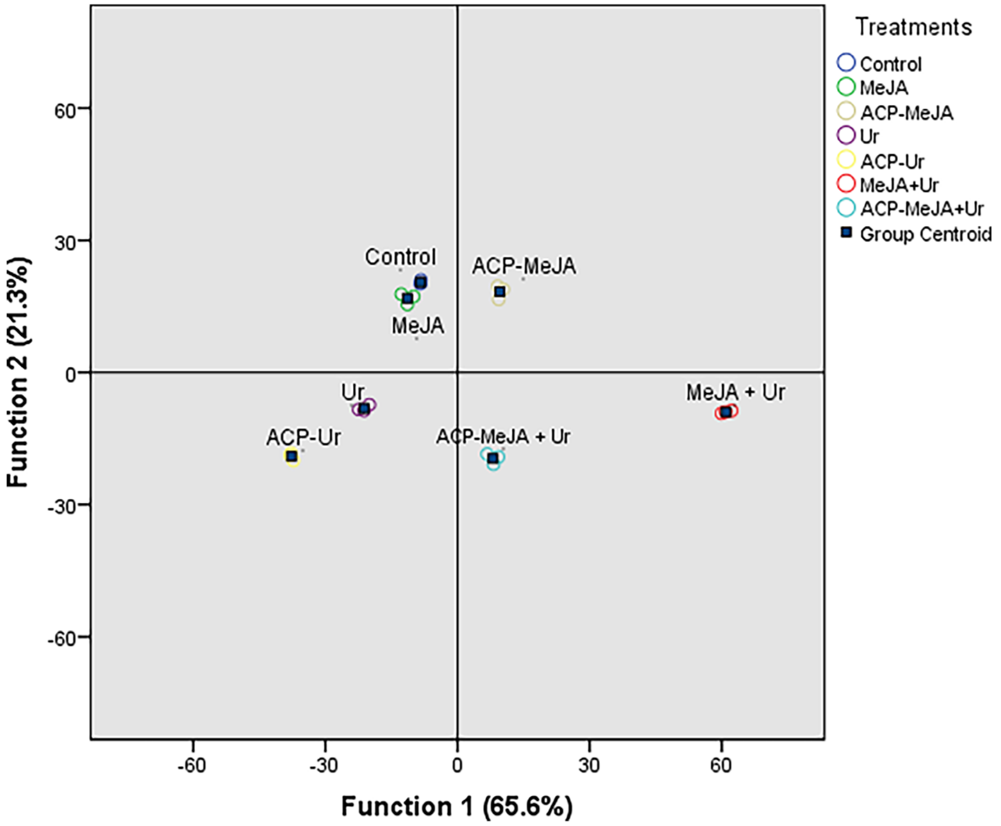

Figure 3. Discriminant analysis of the volatile compounds belonging to the must samples obtained in grapes from control, methyl jasmonate (MeJA), methyl jasmonate on nanoparticles (ACP-MeJA), urea (Ur), urea on nanoparticles (ACP-Ur), methyl jasmonate + urea (MeJA+Ur) and methyl jasmonate + urea on nanoparticles (ACP-MeJA+Ur) foliar treatments.

variance and function 2 explained 21.3% of the variance, accounting for 86.9%. Considering the coefficient of the canonical functions, the variables that contributed most to the discriminant model in function 1 according to their weight were: (E,E)-2,4-decadienal, hexyl acetate, (*E*)-2-heptenal, (*E*)-2-hexenal, 2-phenylethanol, geranyl acetone, (*Z*)-3-hexen-1-ol, ethyl 2-hexenoate and methyl jasmonate. Moreover, the variables most favoured the discriminant model in Function 2 were: methyl jasmonate, (*Z*)-3-hexen-1-ol, (*E*,*E*)-2,4-decadienal, ethyl 2-hexenoate, (*E*)-2-heptenal, (*E*)-2-hexenal, geranyl acetone, hexyl acetate and 2-phenylethanol.

When comparing the data obtained in the discriminant analysis with the results of the ANOVA, a higher content of 2-phenylethanol and hexyl acetate was obtained in the musts with the foliar application of MeJA+Ur, which are results that agree with the ANOVA (Fig. 3 and Table 3) and also explain the value obtained in the coefficients of the canonical discriminant function in function 1. According to the discriminant analysis of function 2, the variable with the highest weight was methyl jasmonate identified in the control samples, results that agree with those obtained in Fig. 2(f). In the case of (*Z*)-3-hexen-1-ol, the results obtained in Table 3 match with the data of the discriminant analysis for function 2 because, in the musts of the ACP-MeJA treatment, a higher concentration of the compound in question was achieved compared to the other foliar applications. Similarly, (*E*,*E*)-2,4-decadienal showed similarity in functions 1 and 2 of the discriminant analysis with the ACP-Ur treatment.

CONCLUSIONS

The present study reveals that conventional and nanoparticulate foliar treatments have a variable effect on the overall parameters of grape must. Foliar treatments, such as MeJA and MeJA+Ur, significantly affected the content of malic acid and total phenols in grape must, wheras other treatments, such as Ur and ACP-Ur, did not show noticeable changes. In addition, MeJA and MeJA+Ur significantly increased amino nitrogen and YAN content compared to the control, as well as ACP-MeJA and ACP-MeJA+Ur. These findings underline the need for further research to fully understand the impact of nanoformulated treatments on the overall parameters of grape must.

The results showed that most of the volatile compounds and their total content did not change with the application of MeJA compared to the control sample and ACP-MeJA. However, the application of ACP-MeJA decreased the concentration of 14 aromatic compounds in the musts compared to the control sample and MeJA; the decrease was mainly in carbonyl compounds and C6 compounds (and their totals), which may be positive as a result of their undesirable herbaceous notes. The ACP-MeJA treatment increased the content of 2-phenylethanal, 1-nonanol and (*Z*)-3-hexen-1-ol, with respect to the control and MeJA; except for 2-phenylethanol content, where no significant differences were found in the one-factor analysis, standing out because it contributes to the rose aromas.

Ur treatment resulted in an increase in the concentration of six volatile compounds compared to control and ACP-Ur treated samples, suggesting that Ur may increase the presence of certain volatile compounds. Some undesirable compounds decreased, as well as other compounds with positive aroma, in the Ur-treated samples, and this similar behaviour was observed in the ACP-Ur-treated samples. Foliar application of MeJA+Ur increased the concentration of several compounds, including 2-phenylethanal, heptanol, 1-nonanol, γ-decalactone, esters (and their total) and 1-hexanol, compared to the control and ACP-MeJA+Ur. However, except for 2-phenylethanal and ethyl 2-hexenoate, the one-factor analysis indicated that no significant differences in their concentrations were observed when MeJA+Ur and ACP-MeJA+Ur were applied, which is important in terms of aroma. Finally, ACP-MeJA+Ur treatment reduced the concentration of 10 compounds in the musts, with some belonging to carbonyl compounds and C6 compounds. This reduction occurred with respect to the control and MeJA+Ur treated samples. The impact of individual and combined foliar applications exhibited a variable effect on the volatile composition.

In general, nanoparticle treatments showed effects similar to conventional treatments. It was evident that nanoparticle treatments reduced total C6 compounds and some carbonyl compounds. This is of novel interest because their presence at high levels in the must is undesirable and detrimental to wine quality. In addition, some positive aroma compounds, such as nerol, neral, geranyl acetone, β-cyclocitral, β-ionone and 2-phenylethanol, increased with a lower dose of Ur (0.4 kg N ha^{-1} *versus* 6 kg N ha^{-1}) and 2-phenylethanal increased significantly with lower doses of MeJA (1 mM *vs*. 10 mM). Therefore, the application of nanoparticles is promising in the development of new strategies for the administration of biostimulants in vineyards when aiming to generate a more sustainable and modern viticulture. The results of the present study provide information on the efficiency of nanoparticles to improve the aromatic quality of musts. However, more studies are needed to corroborate their effect.

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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.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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