Research Article

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Nanoparticles doped with methyl jasmonate: foliar application to Monastrell vines under two watering regimes. An alternative to improve grape volatile composition?

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Abstract

Background: Elicitors induce defense mechanisms, triggering the synthesis of secondary metabolites. Irrigation has implications for a more sustainable viticulture and for grape composition. The aim was to investigate the influence on grape aroma composition during 2019 and 2020 of the foliar application of amorphous calcium phosphate (ACP) nanoparticles and ACP doped with methyl jasmonate (ACP-MeJ), as an elicitor, with rainfed or regulated deficit irrigation (RDI) grapevines.

Results: In both growing seasons, nearly all terpenoids, C_{13} norisoprenoids, benzenoid compounds and alcohols increased with ACP-MeJ under the RDI regimen. In 2019, under the rainfed regime, ACP treatment increased limonene, *p*-cymene, α-terpineol, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), 2-ethyl-1-hexanol, (E,E)-2,4-heptadienal, and MeJ concentration in comparison with control grapes. In 2020, the rainfed regime treated with ACP-MeJ only increased the nonanoic acid content. Grape volatile compounds were most influenced by season and watering status whereas the foliar application mainly affected the terpenoids.

Conclusion: A RDI regime combined with the elicitor ACP-MeJ application could improve the synthesis of certain important volatile compounds, such as *p*-cymene, linalool, α-terpineol, geranyl acetone, β-ionone, 2-phenylethanol, benzyl alcohol, and nonanoic acid in Monastrell grapes.

Keywords: nanoparticles; methyl jasmonate; primary aroma; RDI; elicitor

INTRODUCTION

The foliar application of elicitors and the water regime to which the grapevine is subjected during its biological cycle are two of the agronomic techniques that are becoming more important in viticulture. Elicitors are inducing substances that trigger the activation of a series of defense response mechanisms by the plant.[1,2] The exogenous application of elicitors has begun to be used as an alternative and sustainable tool to phytosanitary products.[3,4] It has also been shown that they can increase resistance to abiotic and biotic stress, as well as increasing the synthesis of phenolic[5] and volatile compounds.[1,6,7] Jasmonic acid is a phytohormone derived from linoleic and linolenic fatty acids that acts as a plant response signal to various types of stress, inducing defense mechanisms including the synthesis of secondary compounds.[1] Methyl jasmonate (MeJ) is the methyl ester of jasmonic acid and, when applied at veraison, has been demonstrated to improve the volatile composition in both grapes and wines in different grape varieties.[1,6-8] Despite the advantages observed with its use, its high

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price and volatility are two drawbacks that limit the use of MeJ in agriculture.[1,9]

On the other hand, nanotechnology offers a unique approach to overcome the shortcomings of many conventional treatments. Among the proposed nanomaterials, calcium phosphate nanoparticles are of particular interest due to their biocompatiblity and biodegradability, releasing plant macronutrients (Ca^{2+} and PO_4^{3-}) upon dissolution. These nanomaterials also have outstanding capacity to incorporate ions in their structure and to adsorb a large amount of molecules on their surface due to the presence of a highly reactive surface hydrated layer, which facilitates the subsequent ionic exchange in aqueous media.[10] Amorphous calcium phosphate (ACP) nanoparticles have been doped successfully with substances of agronomic interest such as urea[11] or MeJ.[5,12,13] Amorphous calcium phosphate nanoparticles provide a gradual release of MeJ and protection against thermal degradation achieving a prolonged action, greater utilization and avoiding leaching losses, which would, presumably, increase their absorbability by the plant.[14]

The current climate change scenario, with more recurrent drought phenomena and heatwaves, will increase evapotranspiration and the water requirements of vines, limiting water resources, as well as phenological progress and causing a change in grape composition (in general, nowadays, the berries contain more sugar, less organic acids, and show a higher pH, with changes in some volatile compounds).[15] In this sense, the controlled use of efficient irrigation has been postulated as a basic and necessary strategy to be implemented, especially for areas with a Mediterranean climate.[16] Thus, the use of irrigation techniques, such as controlled deficit irrigation – regulated deficit irrigation (RDI) – which provide the plant with volumes of water during certain moments of its phenological stage previously stipulated, to supply its basic needs and maintain high productive yields, improving water use efficiency (WUE), and grape and wine quality,[17,18] is becoming increasingly important in viticulture. Authors such as Bouzas-Cid *et al.*[19] and Romero *et al.*[20] have reported that the secondary metabolites content, which confers interesting sensory characteristics to grapes and wines, can be enhanced by the RDI strategy.

On the other hand, the character and quality of grapes and wine are mainly defined/determined by the aroma. Some of the molecules involved in the wine aroma are biosynthesized in grapes (called 'varietal aromas') or formed from the harvest until the beginning of the alcoholic fermentation (called 'pre-fermentative aromas'),[19] and the other molecules result from winemaking (called 'fermentative aromas') synthetized by yeast or lactic bacteria in the alcoholic or malolactic fermentations, respectively[21] and 'ageing aromas', which came from the wine conservation stage.[22] In grapes, the volatile compounds responsible for the called 'varietal aroma', belonging to several chemical groups, such as terpenoids, C_{13} norisoprenoids, esters, benzenoid compounds, thiols, and methoxypyrazines.[1,7,23,24] Terpenoids (also called isoprenoids) represent one of the major groups of secondary metabolites in plants. Depending on cultivar, the glycosylated (bound to a sugar moiety) terpenoids are more abundant than free terpenoids.[25] Some of them, such as linalool, α-terpineol, nerol, geraniol, and citronellol, which emit floral fragrances, are among those with the lowest perception thresholds, so are among the most odoriferous compounds known.[26] C_{13} norisopreinods are also among the most important aroma molecules in grape and wine and provide floral and fruity attributes. Among them, β-damascenone and β-ionone are the two most important compounds, providing rose and violet aromas.[25] However, others C_{13} norisopreinods, such as the 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), which are detected at very low thresholds, provide a very distinctive flavor of kerosene scent. Among the benzenoid derivates, 2-phenylethanol is important because it confers a rose aroma. The alcohol compounds are synthetized at late stages of grape development.[27] *n*-Hexanol and hexanal, related to the herbaceous or grassy aroma descriptors, are the majority C6 compounds in the musts;[24] however, depending on their concentration, they can impact the quality of the wine negatively.[28] The conditions to which the vines are subjected during ripening affect the development of the different components of the grapes, which, as a result of the consequences of the climate change, favor a mismatch between technological and phenolic maturity, and similarly, influence the volatile profile of the wines.[29] Thus, agronomic management can affect both, the concentration and the profile of these (and others) plant secondary metabolites in two different ways – directly, by changing the molecular biosynthesis, and indirectly, due to the grape volume and weight variations, which, consequently, changes the molecules concentration.[30]

The main purpose of this work was to evaluate, in a pioneering way, whether the foliar application of the elicitor MeJ loaded on nanoparticles, influences the volatile compounds of grapes from Monastrell grapevines under two different watering regimes, rainfed and RDI, and in two consecutive growing seasons.

MATERIALS AND METHODS

Samples and grapevine treatments

Red grapes from *Vitis vinifera* L. Monastrell cultivar on 1103P rootstock were used. They were grown in a commercial vineyard located in Albacete (Southeastern Spain, 38° 43′ 43.3″ N, 1° 28′ 12.6″ W, elevation above sea level: 820 m), during two consecutive seasons (2019 and 2020). The climate in the area is defined as typical Mediterranean semiarid. Climate data, obtained from a weather station located near the experimental plot (http://crea.uclm.es/siar/datmeteo/consulta.php), showed similar annual rainfall (mm) and reference evapotranspiration (ET_0) (mm) for both seasons (500 and 1,200 mm, respectively). However, 2019 was rainier (372 mm) than 2020 (166 mm) during the growing season (from April to the beginning of October-harvest time), with similar ET_0 (916 mm, in 2019 *vs* 882 mm in 2020). Likewise, mean annual and growing season temperatures were the same in both years (20.1 and 15.3 °C, respectively), with absolute maximum temperature (27.5 °C in 2019 *vs* 28.1 °C in 2020), and absolute minimum temperature (13 °C *vs* 12.5 °C, respectively), very similar (Fig. 1). Plants were trained to a double Guyot system on a vertical trellis, planted in North–South oriented rows with 3 m × 1.5 m, row and vines spacing. The essay involved the foliar application of three treatments in 2019: control, ACP nanoparticles, and ACP nanoparticles doped with MeJ (ACP-MeJ) at 1 mM concentration.[13] In 2020, due to the coronavirus 2019 (COVID-19) pandemic situation, it was only possible to synthesize the ACP-MeJ nanoparticles, so the control and ACP-MeJ treatments were applied in the vineyard. For the control plants, just a water solution of Tween 80 (Sigma-Aldrich, Madrid, Spain) at 0.1% (v/v) as wetting agent was used. Likewise, Tween 80 (Sigma–Aldrich) was utilized in all the other treatments, to prepare the solutions with nanoparticles. The synthesis and full characterization of naked ACP nanoparticles and ACP-MeJ nanoparticles were described in detail elsewhere.[14,31] Each treatment was sprayed (200 mL/plant) over the leaves of the grapevines twice, at veraison and 1 week later.

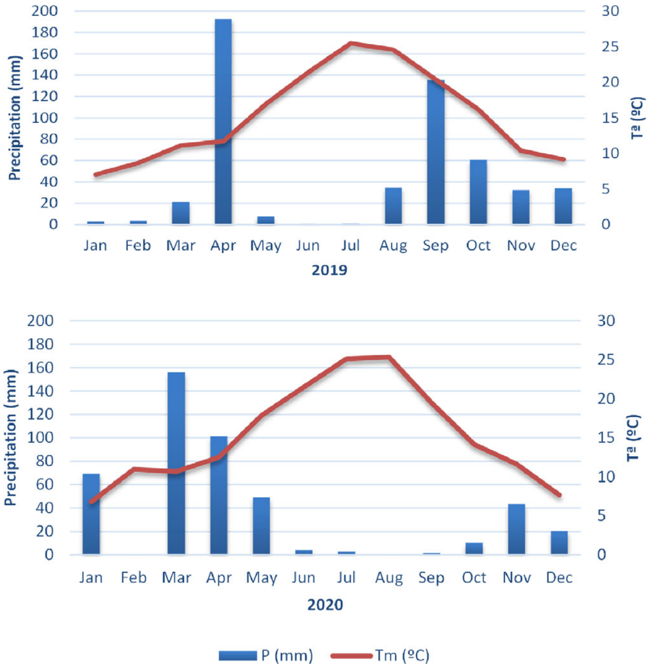

Figure 1. Monthly accumulated precipitation (mm) and mean temperature (°C) during the two seasons (2019 and 2020). Data were collected from the Ontur meteorological station (http://crea.uclm.es/siar/datmeteo/consulta.php).

Two watering strategies: non-irrigated (rainfed) and RDI were performed on the treated grapevines. In the RDI regime, plants received 30% of the estimated crop evapotranspiration (ETc), from prior to veraison (when their stem water potential [Ψs] reached values of −0.8 MPa) to harvest. Each season, a drip irrigation system provided weekly irrigations to the grapevines, with a total irrigation volume of 134 mm (in 2019) and 140 mm (in 2020).

For each watering regime, all foliar treatments were sprayed in four replicates set up in a completely randomized block design with three plants per replicate. Grape samples were harvested at optimum maturity, i.e., when the weight of 100 grapes was constant and the probable alcohol reached around 13 (% v/v). For each treatment and replicate, a random set of 150 berries was destemmed and crushed to obtain the must and to determinate the enological parameters. Another set of 50 berries from each sample were frozen at −20 °C until the analysis of the volatile composition was conducted.

Enological parameters

The must samples were characterized physico-chemically to determine °Brix, probable alcohol, pH, total acidity, color intensity and total polyphenol index (TPI) according to the methodology established by the International Organization of Vine and Wine.[32] An automated enzymatic test with Miura One equipment (TDI, Barcelona, Spain) was used to determine the tartaric and malic acids content as well as the total phenolic compounds (TPC). As the treatments in the vineyard were carried out in quadruplicate, the results of the must enological parameters are shown as the average of the four analyses ($n = 4$).

Analysis of volatile compounds by headspace solid-phase micro-extraction

The volatile composition of grape samples was assessed by headspace solid-phase micro-extraction (HS-SPME) and subsequent analysis by gas chromatography (GC) coupled to mass spectrometry (MS) following the methodology described by Garde-Cerdán *et al.*[1] Briefly, the grape samples were homogenized using Ultra-Turrax T-18 (IKA, Staufen, Germany) equipment at 18 000 rpm for 1 min. Each sample was centrifuged (2060 g, 10 min, 20 °C) in order to use the supernatant. To extract the volatile compounds by headspace, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm) (Supelco, Bellefonte, PA, USA) solid-phase micro-extraction (SPME) fiber was used. First, 9 mL of sample (previously centrifuged) and 2.5 g of NaCl (Sigma-Aldrich, Madrid, Spain) were added to a 20 mL vial and conditioned for 15 min at 60 °C with stirring. Subsequently, extraction with SPME fiber was carried out at 60 °C for 105 min, with stirring.

After extraction, the SPME fiber was introduced automatically into gas chromatography (GC) equipment coupled to a mass spectrometry detector (Agilent, Palo Alto, CA, USA). The desorption process from the SPME fiber was conducted at 250 °C for 15 min. The desorbed compounds were split in a SPB-20 fused silica capillary column (30 m × 0.25 mm Internal Diameter (ID) × 0.25 μm film thickness) (Supelco). A helium flow rate of 1.2 mL min^{-1} was used as carrier gas. The injections were achieved in splitless mode (1 min). The oven temperature was 40 °C for 5 min, after which it was increased at 2 °C min^{-1} to a final temperature of 220 °C, which was held for 20 min. The MS was operated in electron ionization mode at 70 eV. The acquisitions were performed in full scan (35–300 m z^{-1}) and the detector and transfer line temperatures were 150 and 230 °C, respectively. Identification was performed using the data system library National Institute of Standards and Technology of the U.S. Department of Commerce (NIST) comparing the mass spectrum and retention index of standards (Sigma–Aldrich), and data shown in bibliography. Semi-quantification was performed, relating the areas of each compound to the area and known concentration of the internal standard (2-octanol).

As the treatments were performed in quadruplicate, the results of grape volatile compounds are expressed as the average of four analyses ($n = 4$).

Statistical analysis

An ANOVA was used to analyze the results. An SPSS v21.0 statistical package for Windows (SPSS, Chicago, IL, USA) was utilized. The Duncan test (at $P \leq 0.05$) was used in order to know the significant differences between means. Multifactor analysis between treatments (T), watering regimes (W), seasons (S), and their interactions (T × W, T × S, W × S and T × W × S) was carried out (Duncan test at $P \leq 0.05$, 0.01 and 0.001). Discriminant analysis was performed with the volatile compounds of the samples.

RESULTS AND DISCUSSION

Influence of foliar treatments on enological parameters of Monastrell grapes under non-irrigated (rainfed) and RDI strategies

In 2019, neither the treatments nor the watering regimes to which the Monastrell vines were subjected showed any effect on the enological parameters of the grapes (Table 1). In 2020, rainfed vines treated with ACP-MeJ showed higher color intensity than the control. The grapevines treated with ACP-MeJ in the RDI system, increased the total acidity and the malic acid content compared with the control (Table 1). After foliar application of various elicitors, including MeJ, Ruiz-García *et al.*[33] and Garde-

Table 1. Enological parameters in Monastrell grapes for control grapevines and for grapevines treated with amorphous calcium phosphate (ACP) and amorphous calcium phosphate doped with MeJ (ACP-MeJ) nanoparticles, under non-irrigated (rainfed) and regulated deficit irrigation (RDI) conditions, in the 2019 and 2020 seasons

	2019						2020			
	Rainfed			RDI			Rainfed		RDI	
	Control	ACP	ACP-MeJ	Control	ACP	ACP-MeJ	Control	ACP-MeJ	Control	ACP-MeJ
Weight of 100 berries (g)	149.4 ± 15.9a	144.1 ± 8.3a	153.9 ± 23.4a	185.1 ± 18.6a	166.3 ± 7.8a	171.2 ± 6.9a	173.2 ± 17.0a	166.2 ± 24.7a	195.4 ± 11.3a	192.7 ± 29.6a
°Brix	22.1 ± 1.1a	20.9 ± 1.9a	21.2 ± 2.4a	20.3 ± 2.5a	20.3 ± 1.3a	19.2 ± 1.2a	23.4 ± 1.5a	22.6 ± 1.2a	22.8 ± 1.3a	22.2 ± 0.5a
Probable alcohol (%, v/v)	12.9 ± 0.8a	12 ± 1.3a	12.2 ± 1.6a	11.6 ± 1.7a	11.4 ± 1.1a	10.9 ± 0.8a	13.7 ± 1.0a	13.1 ± 0.8a	13.3 ± 0.9a	12.9 ± 0.3a
pH	3.7 ± 0.1a	3.5 ± 0.1a	3.5 ± 0.1a	3.5 ± 0.1a	3.5 ± 0.1a	3.5 ± 0.1a	3.7 ± 0.1a	3.7 ± 0.1a	3.7 ± 0.1a	3.6 ± 0.1a
Total acidity* (g/L)	5.5 ± 0.3a	5.6 ± 0.5a	5.5 ± 0.3a	5.4 ± 0.7a	5.6 ± 0.6a	5.6 ± 0.3a	4.6 ± 0.5a	4.4 ± 0.7a	4.5 ± 0.2a	4.8 ± 0.1b
Tartaric acid (g/L)	2.7 ± 0.2a	3.1 ± 0.5a	3.4 ± 0.4a	2.7 ± 0.7a	3.0 ± 0.3a	3.2 ± 0.2a	5.7 ± 0.6a	6.5 ± 0.7a	5.5 ± 0.0a	5.7 ± 0.2a
Malic acid (g/L)	1.6 ± 0.3a	1.5 ± 0.3a	1.3 ± 0.2a	1.6 ± 0.1a	2.1 ± 0.2a	2.0 ± 0.3a	1.3 ± 0.1a	1.5 ± 0.1a	1.9 ± 0.1a	2.4 ± 0.2b
Color intensity	5.0 ± 0.3a	5.8 ± 0.4a	5.2 ± 0.8a	4.8 ± 0.4a	4.1 ± 0.4a	4.6 ± 0.5a	10.1 ± 1.5a	12.6 ± 1.1b	10.8 ± 0.6a	10.8 ± 0.9a
TPI	20.3 ± 1.1a	21.5 ± 1.0a	19.7 ± 3.3a	18.7 ± 0.5a	16.6 ± 1.5a	16.7 ± 2.7a	27.9 ± 5.6a	31.3 ± 3.3a	25.6 ± 4.4a	24.2 ± 3.6a
TPC (mg/L)	722.9 ± 56.6a	718.3 ± 102.6a	731.1 ± 105.5a	707.1 ± 39.4a	610.2 ± 37.7a	642.6 ± 74.7a	759.6 ± 172.8a	786.8 ± 88.7a	659.7 ± 100.4a	624.5 ± 94.4a

Note: All the parameters are given with their means ± standard deviation (n = 4). For each parameter, water status regime and season, different letters indicate significant differences between treatments ($P \leq 0.05$).
Abbreviations: TPI, total polyphenol index; TPC, total phenolic compounds.
*As g/L of tartaric acid.

Table 2. Mean values ± standard deviation of enological parameters across elicitors treatments (T), water status (W) and seasons (S) factors and their interactions (T × W, T × S, W × S, T × W × S)

	Treatments (T)		Water status (W)		Season (S)		Multifactorial analysis[a]			
	Control	ACP-MeJ	Rainfed	RDI	2019	2020	T × W	T × S	W × S	T × W × S
Weight of 100 berries (g)	175.8 ± 22.7a	171.0 ± 25.0a	160.7 ± 21.0a	186.1 ± 19.3b	164.9 ± 21.3a	181.9 ± 23.4b	ns	ns	ns	ns
°Brix	22.2 ± 1.9a	21.3 ± 1.9a	22.3 ± 1.7a	21.1 ± 2.0a	20.7 ± 2.0a	22.7 ± 1.2b	ns	ns	ns	ns
Probable alcohol (%, v/v)	12.9 ± 1.3a	12.3 ± 1.3a	13.0 ± 1.2a	12.2 ± 1.4a	11.9 ± 1.4a	13.3 ± 0.8b	ns	ns	ns	ns
pH	3.6 ± 0.1b	3.5 ± 0.1a	3.6 ± 0.1a	3.6 ± 0.1a	3.5 ± 0.1a	3.7 ± 0.1b	ns	ns	ns	ns
Total acidity* (g /L)	4.9 ± 0.6a	5.1 ± 0.6a	5.0 ± 0.7a	5.1 ± 0.6a	5.5 ± 0.4a	4.6 ± 0.4b	ns	ns	ns	ns
Tartaric acid (g/L)	4.2 ± 1.5a	4.7 ± 1.5a	4.6 ± 1.7a	4.3 ± 1.4a	3.0 ± 0.6a	5.9 ± 0.6b	ns	ns	ns	ns
Malic acid (g/L)	1.6 ± 0.2a	1.8 ± 0.5a	1.4 ± 0.2a	2.0 ± 0.3b	1.6 ± 0.4a	1.8 ± 0.4a	**	ns	ns	ns
Color intensity	7.7 ± 2.9a	8.3 ± 3.6a	8.2 ± 3.5a	7.7 ± 3.2a	4.9 ± 0.5a	11.1 ± 1.3b	*	*	ns	ns
TPI	22.8 ± 4.8a	22.9 ± 6.4a	24.6 ± 6.1a	21.3 ± 4.8a	18.9 ± 2.4a	27.2 ± 4.7b	ns	ns	ns	ns
TPC (mg/L)	712.3 ± 101.6a	686.7 ± 104.6a	740.6 ± 108.5b	658.5 ± 78.9a	691.4 ± 72.5a	707.7 ± 127.3a	ns	ns	ns	ns

Note: For each parameter, water status regime and season, different letters indicate significant differences between treatments ($P \leq 0.05$).
Abbreviations: TPI, total polyphenol index; TPC, total phenolic compounds.
*As g/L of tartaric acid.
[a] Statistical significance: *$P \leq 0.05$ and **$P \leq 0.01$, respectively and ns, not significant ($P > 0.05$).

Cerdán *et al.*[1,9] also found no important differences in the enological parameters of musts in comparison with the control.

On the other hand, although total the soluble solids concentration of grapes (°Brix) reflects the probable grape alcohol and generally decreases with increasing vineyard water applications, as observed by Intrigliolo *et al.*[34] in their post-veraison irrigation study, in our case that effect was not observed in either of the two seasons that were studied (Table 1). The fact that, at harvest, °Brix was not different in the RDI vines in comparison with the rainfed regime is noteworthy because, currently, consumers demand wines that are balanced and that have a lower alcohol content. Thus, agronomic techniques are being investigated to achieve these objectives. However, as no significant effects of the irrigation treatment were observed for enological parameters, except for malic acid and TPC, it is possible that the influence of the water status of the grapevines could have accentuated the difference in weight of the grapes, which was higher with RDI regime than with rainfed irrigation (Table 2), due to a slight dehydration of the latter berries compared to the RDI ones and not to a direct effect on the berry during ripening.

From the multifactorial analysis of the grape enological data it can be seen that the treatment only influenced the pH, which showed lower values in the ACP-MeJ treated samples than in the control (Table 2). On the other hand, the watering regime modified the 100 grapes' weight. This was higher in grapes from the grapevines under the RDI regime, which also had higher malic acid content and lower TPC in comparison with those under the rainfed regime (Table 2). The factor that showed the greatest influence on these enological parameters was the season, their being content higher in samples from 2020 (the driest year, which during the grape vegetative-productive period received half as much precipitation as 2019, with similar temperature and evapotranspiration – Fig. 1) in comparison with those from 2019, except for malic acid and TPC, the values of which remained unchanged in both years. The interactions between the factors studied were only significant in the case of the treatment by water regime (T × W) for the malic acid and color intensity grapes content, and the latter parameter also when interacting treatment by season (T × S).

Foliar treatments influence on volatile compounds of Monastrell grapes under non-irrigated (rainfed) and RDI strategies

In general, the ACP and ACP-MeJ treatments increased the volatile compounds in grapes with respect to the control samples, except in the grapes from the rainfed treatment in 2020, where there were hardly any differences between the foliar treatments (Table 3). Thus, in 2019, under a rainfed regime, only the content of linalool, methyl salicylate and total other compounds increased in the control grapes in comparison with the treated ones. When MeJ was applied to Sangiovese grapevines, D'Onofrio *et al.*[7] observed no effect on the methyl salicylate content of the grapes. However, they reported the year dependence of the treatment effect and the interaction between MeJ foliar application and season.

Among the terpenoids, in 2019, under the rainfed regime, the limonene and *p*-cymene content in the treated ACP-MeJ samples was higher than that in the control sample, with intermediate values in the ACP treatment samples (Table 3). Both treatments (ACP and ACP-MeJ) increased the α-terpineol and total terpenoids content in the grapes in comparison with the control. In the case of the C_{13} norisoprenoids group, only the TDN content of the ACP and ACP-MeJ-treated samples increased in comparison with the control, with no significant differences between treatments in the rest of the compounds determined or in the total, as occurred in the case of the benzenoid compounds (Table 3). This positive effect of the elicitors on the concentration of certain volatile compounds, mainly terpenoids and C_{13} norisoprenoids, was also observed in Monastrell samples by Gómez-Plaza *et al.*[6] Thus, D'Onofrio *et al.*[7] reported that elicitation activates terpene metabolism by increasing geranylgeranyl diphosphate synthase and terpene synthase, which leads to the increase in these compounds.

On the other hand, and with respect to the benzenoid compounds, one of the precursors of 2-phenylethanol in grapes is phenylethyl-α-D-glucopyranose,[35] but in addition, phenylpropanoid-benzenoid volatiles, along with some phenolic compounds and other secondary metabolites, derived from the phenylalanine activity.[36] However, the content of the amino acid phenylalanine was hardly modified in the Monastrell treated grapes in comparison with the control.[13] Marín-San Román *et al.*[8] did not observe increases in the amount of 2-phenylethanol and 2-phenylethanal after treating Grenache grapes with MeJ. Nevertheless, its content increased when Phe or Phe + MeJ were applied, probably due to the mechanism of phenylalanine transformation, as was also reported by Garde-Cerdán *et al.*[37] in Tempranillo grapes. Therefore, presumably, the metabolic synthesis induction of the benzenoid compounds is also linked to other factors such as the elicitor application dose and timing, grapevine water status and season conditions during grape ripening, exposure of bunches to the sun, and so forth.

Regarding alcohols, in 2019, under the rainfed regime, the concentration of 2-ethyl-1-hexanol and total alcohols increased in the treated samples in comparison with the control (Table 3). However, *n*-nonanol was not influenced by the treatments. Likewise, in the alcohol family, in the carbonyl compounds family, in 2019, under the rainfed regime, the content of one of them, (E,E)-2,4-heptadienal, and the total carbonyl compounds improved in the treated samples in comparison with the control samples but the heptanal content was not influenced by the treatments (Table 3).

In relation to the C6 compounds content in the samples, *n*-hexanol presented higher values in the control grapes than in the ACP-treated samples and the concentration of hexanal increased in the ACP-treated samples in comparison with those from the ACP-MeJ (Table 3). The (E)-2-hexenal content and the total content of the C6 compounds were not affected by the foliar treatments. The fact that the C6 compounds were hardly affected by the treatments is positive for the aroma because these compounds, derived from the fatty acids and responsible for green aromas,[25] at high levels, can produce undesirable aromas.[23] However, after the application of MeJ to Cabernet Sauvignon grapevines, Ju *et al.*[38] reported increases in C6 compounds, mainly related to the increase in the amounts of *n*-hexanol and hexanal.

Regarding the group of 'other compounds', in 2019, under the rainfed regime, the content of MeJ also increased in ACP-treated samples in comparison with ACP-MeJ and the control samples (Table 3). The nonanoic acid concentration was higher in the control samples than in those from the ACP-MeJ treatment, with intermediate values in the ACP-treated grapes.

In relation to the plants under the RDI watering regime, in samples of 2019 (the wettest year but with similar amount of irrigation than 2020, 134 versus 140 mm irrigation, respectively), most of the volatile compound content was generally higher in the treated ACP-MeJ samples than in the control and ACP ones (Table 3). Thus, in the terpenoids group, only the concentrations of limonene and β-myrcene were not affected by the foliar treatments, the total terpenoids content being twofold higher in the ACP-MeJ treated samples than those from the control and the ACP-treated grapes had intermediate values of total terpenoids content. Authors such as Bouzas-Cid *et al.*[19] also reported that the irrigation of the grapevines affected the content of certain terpenoids, increasing the concentration of α-terpineol and decreasing geraniol content in Treixadura wines.

The total C_{13} norisoprenoids content in 2019, under the RDI regime, as well as the (E)-β-damascenone content, was higher in the treated samples than in the control (Table 3). The concentration of β-ionone (which provides violet notes) and TDN (aroma typically described as pretolor kerosene),[39] was higher in the ACP-MeJ grapes than in those from the ACP and control treatments. These results agree with those obtained by Marín-San Román *et al.*[8] after they applied MeJ, Phe, and MeJ + Phe to Grenache vines. They suggested that the high increase of β-ionone in grapes from the MeJ application (80% in comparison with the control samples) and MeJ + Phe (54%) was due to the fact that MeJ accelerates the β-carotene degradation,[40] precursor of β-ionone.[39]

The ACP-MeJ treatment increased the concentration of all the benzenoid compounds and their total content in samples from 2019 season under RDI watering status, in comparison with the control and ACP samples (Table 3). Similarly, *n*-nonanol values increased in the samples treated with the ACP-MeJ and 2-ethyl-1-hexanol, and the total alcohols increased in the treated samples compared with those from the control (Table 3). The control grapes showed higher heptanal content than the treated grapes, and the ACP-MeJ treated samples increased their (E,E)-2,4-heptadienal content and total carbonyl compounds in comparison with the ACP-treated and control grapes.

On the other hand, none of the C6 compounds in samples from 2019 season, under RDI regime, were influenced by the foliar treatments applied in the field, nor was the content of MeJ, methyl salicylate or the total of other compounds (Table 3). The nonanoic acid content in ACP-MeJ-treated grapes increased in comparison with the control and the ACP-treated samples (Table 3).

Regarding the samples from the 2020 season, in the rainfed vines, only the content of geranyl acetone, 2-ethyl-1-hexanol, total alcohols, nonanoic acid, and the total of the other compounds showed differences between the foliar treatments; the content of the latter two compounds was higher in the ACP-MeJ-treated grapes than in the control grapes, and the content of the other mentioned compounds was lower in the ACP-MeJ compared with the control grapes (Table 3). Marín-San Román *et al.*[8] reported, in their Grenache study that, although geranyl acetone was the most abundant terpenoid in control grapes, the foliar application of Phe, MeJ, and the combination of both, decreased the content of this volatile compound. Similarly, after application of Phe and MeJ in Tempranillo grapevines, Garde-Cerdán *et al.*[1,37] also observed geranyl acetone diminution in grapes. As well as in our Monastrell grapes, Marín-San Román *et al.*[8] also found no differences in linalool and α-terpineol content with the foliar application of Phe to Grenache vines. Similarly, during the first year of their assay, Garde-Cerdán *et al.*[1] found that MeJ application in Tempranillo vines (in conventional size doses, so at a dose 10 times higher than our ACP-MeJ) did not affect the total terpenoids content, total C_{13} norisoprenoids content, total benzenoid compounds, total C6

Table 3. Volatile compounds concentration (μg/L) in Monastrell grapes from control grapevines and from grapevines treated with amorphous calcium phosphate (ACP) and amorphous calcium phosphate doped with MeJ (ACP-MeJ) nanoparticles, under non-irrigated (rainfed) and regulated deficit irrigation (RDI) conditions, in 2019 and 2020 seasons

	2019						2020			
	Rainfed			RDI			Rainfed		RDI	
	Control	ACP	ACP-MeJ	Control	ACP	ACP-MeJ	Control	ACP-MeJ	Control	ACP-MeJ
Terpenoids										
Limonene	0.50 ± 0.02a	0.64 ± 0.14ab	0.81 ± 0.16b	0.66 ± 0.03a	0.84 ± 0.43a	1.17 ± 0.31a	0.38 ± 0.06a	0.32 ± 0.05a	0.44 ± 0.15a	0.76 ± 0.01b
p-Cymene	0.44 ± 0.09a	0.60 ± 0.13ab	0.72 ± 0.15b	0.67 ± 0.04a	0.59 ± 0.10a	1.11 ± 0.30b	0.34 ± 0.06a	0.40 ± 0.05a	0.50 ± 0.08a	0.75 ± 0.01b
Linalool	0.36 ± 0.02b	0.53 ± 0.04c	0.27 ± 0.07a	0.46 ± 0.08a	0.89 ± 0.64ab	1.93 ± 0.98b	0.33 ± 0.10a	0.28 ± 0.08a	0.34 ± 0.15a	1.00 ± 0.01b
α-Terpineol	0.35 ± 0.10a	0.63 ± 0.09b	0.87 ± 0.25b	0.54 ± 0.05a	1.55 ± 0.10b	1.47 ± 0.21b	0.36 ± 0.10a	0.45 ± 0.11a	0.42 ± 0.24a	0.99 ± 0.04b
Geranyl acetone	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.00a	0.11 ± 0.03b	0.03 ± 0.00b	0.02 ± 0.00a	0.01 ± 0.00a	0.02 ± 0.00b
β-Myrcene	1.87 ± 0.04a	2.06 ± 0.40a	1.87 ± 0.31a	2.14 ± 0.33a	2.39 ± 1.09a	3.06 ± 1.00a	0.63 ± 0.20a	0.76 ± 0.12a	1.02 ± 0.08a	2.15 ± 0.03b
Total	3.56 ± 0.18a	4.48 ± 0.62b	4.56 ± 0.68b	4.51 ± 0.42a	6.29 ± 2.22ab	8.85 ± 2.67b	2.06 ± 0.41a	2.22 ± 0.27a	2.75 ± 0.66a	5.67 ± 0.00b
C_{13} norisoprenoids										
(E)-*β*-Damascenone	3.90 ± 0.66a	3.96 ± 0.24a	3.39 ± 1.20a	4.33 ± 0.79a	8.53 ± 0.39b	8.77 ± 4.10b	2.22 ± 0.73a	2.95 ± 0.37a	2.68 ± 0.49a	3.57 ± 0.83a
(Z)-*β*-Damascenone	0.25 ± 0.03a	0.30 ± 0.03a	0.26 ± 0.05a	0.39 ± 0.01a	0.60 ± 0.16a	0.59 ± 0.18a	0.16 ± 0.07a	0.24 ± 0.03a	0.21 ± 0.05a	0.27 ± 0.07a
β-Ionone	0.18 ± 0.06a	0.21 ± 0.04a	0.16 ± 0.03a	0.26 ± 0.02a	0.25 ± 0.07a	0.40 ± 0.11b	0.02 ± 0.01a	0.01 ± 0.00a	0.02 ± 0.00a	0.05 ± 0.00b
β-Cyclocitral	0.17 ± 0.06a	0.22 ± 0.01a	0.20 ± 0.03a	0.23 ± 0.02a	0.28 ± 0.10a	0.31 ± 0.06a	0.04 ± 0.01a	0.05 ± 0.00a	0.06 ± 0.03a	0.07 ± 0.00a
TDN	0.27 ± 0.04a	0.75 ± 0.04b	0.64 ± 0.19b	0.54 ± 0.03a	0.83 ± 0.17a	1.43 ± 0.34b	0.25 ± 0.09a	0.29 ± 0.05a	0.25 ± 0.14a	0.41 ± 0.05a
Total	4.76 ± 0.53a	5.45 ± 0.32a	4.65 ± 1.24a	5.76 ± 0.79a	10.48 ± 0.62b	11.50 ± 4.63b	2.69 ± 0.85a	3.53 ± 0.45a	3.22 ± 0.47a	4.37 ± 0.96a
Benzenoid compounds										
2-Phenylethanol	12.85 ± 4.46a	14.70 ± 1.14a	15.29 ± 2.88a	12.78 ± 0.51a	11.58 ± 3.09a	18.04 ± 1.78b	3.18 ± 2.64a	1.55 ± 0.11a	2.30 ± 0.26a	3.41 ± 0.01b
2-Phenylethanal	5.63 ± 2.55a	8.75 ± 1.99a	5.26 ± 0.77a	8.34 ± 1.49a	7.19 ± 0.42a	11.78 ± 2.21b	0.97 ± 0.26a	0.77 ± 0.05a	1.16 ± 0.16a	0.95 ± 0.12a
Benzyl alcohol	7.66 ± 2.26a	6.74 ± 0.37a	6.05 ± 0.97a	6.27 ± 0.97a	6.83 ± 0.31a	12.21 ± 3.98b	0.92 ± 0.25a	0.93 ± 0.08a	1.29 ± 0.19a	2.12 ± 0.22b
Total	26.14 ± 9.13a	30.18 ± 1.84a	26.6 ± 4.33a	27.39 ± 1.39a	25.6 ± 3.25a	42.04 ± 4.72b	5.07 ± 2.79a	3.25 ± 0.09a	4.75 ± 0.54a	6.49 ± 0.27b
Alcohols										
n-Nonanol	0.11 ± 0.04a	0.14 ± 0.02a	0.12 ± 0.02a	0.13 ± 0.02a	0.14 ± 0.03a	0.23 ± 0.02b	0.07 ± 0.04a	0.05 ± 0.00a	0.07 ± 0.01a	0.08 ± 0.00a
2-Ethyl-1-hexanol	1.63 ± 0.10a	2.78 ± 0.39b	2.65 ± 0.38b	2.63 ± 0.17a	3.24 ± 0.54b	3.49 ± 0.30b	1.81 ± 0.29b	1.37 ± 0.12a	1.64 ± 0.39a	1.92 ± 0.03a
Total	1.74 ± 0.10a	2.92 ± 0.40b	2.78 ± 0.39b	2.75 ± 0.15a	3.38 ± 0.55b	3.72 ± 0.32b	1.89 ± 0.31b	1.42 ± 0.11a	1.72 ± 0.40a	2.00 ± 0.03a
Carbonyl compounds										
Heptanal	0.08 ± 0.03a	0.07 ± 0.01a	0.06 ± 0.03a	0.07 ± 0.01b	0.05 ± 0.01a	0.05 ± 0.01a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.02 ± 0.00b
(E,E)-2,4-Heptadienal	2.12 ± 0.45a	3.25 ± 0.46b	2.83 ± 0.30b	2.16 ± 0.03a	2.15 ± 0.11a	3.08 ± 0.77b	0.18 ± 0.04a	0.20 ± 0.06a	0.27 ± 0.10a	0.33 ± 0.00a
Total	2.20 ± 0.47a	3.32 ± 0.46b	2.89 ± 0.32b	2.24 ± 0.03a	2.20 ± 0.11a	3.13 ± 0.77b	0.19 ± 0.04a	0.21 ± 0.06a	0.28 ± 0.10a	0.35 ± 0.00a
C6 compounds										
n-Hexanol	12.39 ± 1.43b	9.70 ± 0.39a	11.32 ± 0.98ab	7.23 ± 1.36a	8.28 ± 1.62a	7.25 ± 0.78a	6.55 ± 0.95a	7.12 ± 1.13a	13.55 ± 5.32a	16.91 ± 3.38a
Hexanal	16.25 ± 2.77ab	19.84 ± 2.64b	14.19 ± 1.67a	11.59 ± 1.81a	11.17 ± 2.47a	11.35 ± 1.40a	6.64 ± 0.19a	5.98 ± 2.11a	10.89 ± 6.30a	26.01 ± 21.91a
(E)-2-Hexenal	2.60 ± 0.39a	4.05 ± 2.13a	2.96 ± 0.56a	1.98 ± 0.37a	2.27 ± 0.08a	1.82 ± 0.12a	7.41 ± 0.74a	9.46 ± 1.66a	13.74 ± 0.49a	33.79 ± 25.12a
Total	31.24 ± 4.10a	33.60 ± 4.11a	28.47 ± 2.26a	20.8 ± 3.29a	21.72 ± 3.97a	20.42 ± 1.51a	20.59 ± 1.41a	22.56 ± 4.79a	38.18 ± 11.21a	76.72 ± 47.40a
Other compounds										
Methyl jasmonate	0.22 ± 0.03a	0.67 ± 0.10b	0.20 ± 0.06a	0.35 ± 0.26a	0.33 ± 0.09a	0.24 ± 0.05a	0.04 ± 0.02a	0.07 ± 0.03a	0.03 ± 0.01a	0.04 ± 0.00a
Methyl salicylate	1.26 ± 0.23b	0.59 ± 0.20a	0.57 ± 0.07a	0.49 ± 0.08a	0.41 ± 0.06a	0.75 ± 0.44a	0.42 ± 0.03a	0.43 ± 0.00a	0.47 ± 0.09a	0.55 ± 0.07a
Nonanoic acid	0.83 ± 0.08b	0.68 ± 0.13ab	0.62 ± 0.08a	0.68 ± 0.13a	0.46 ± 0.27a	1.99 ± 0.58b	0.27 ± 0.03a	0.55 ± 0.06b	0.41 ± 0.10a	0.58 ± 0.06b
Total	4.48 ± 0.32c	3.74 ± 0.26b	2.92 ± 0.36a	2.88 ± 0.27a	3.01 ± 1.59a	5.23 ± 1.88a	1.52 ± 0.16a	2.06 ± 0.12b	1.93 ± 0.18a	2.03 ± 0.57a

Note: All the parameters are given with their means ± standard deviation ($n = 4$). For each parameter, water status regime and season, different letters indicate significant differences between treatments (Duncan test, $P \leq 0.05$).

Table 4. Mean values ± standard deviation of the grape volatile compounds (μg/L) across elicitor treatments (T), water status (W) and seasons (S) factors and their interactions (T × W, T × S, W × S, T × W × S)

	Treatments (T)		Water status (W)		Season (S)		Multifactorial analysis[a]			
	Control	ACP-MeJ	Rainfed	RDI	2019	2020	T × W	T × S	W × S	T × W × S
Terpenoids										
Limonene	0.50 ± 0.13a	0.77 ± 0.35b	0.50 ± 0.21a	0.76 ± 0.32b	0.79 ± 0.30b	0.48 ± 0.19a	**	**	ns	ns
p-Cymene	0.49 ± 0.14a	0.74 ± 0.30b	0.47 ± 0.17a	0.76 ± 0.27b	0.73 ± 0.29b	0.5 ± 0.17a	ns	*	ns	ns
Linalool	0.37 ± 0.10a	0.87 ± 0.83b	0.31 ± 0.08a	0.93 ± 0.78b	0.76 ± 0.83a	0.49 ± 0.32a	***	ns	ns	ns
α-Terpineol	0.42 ± 0.15a	0.94 ± 0.41b	0.50 ± 0.26a	0.86 ± 0.45b	0.80 ± 0.47a	0.56 ± 0.29a	***	***	ns	ns
Geranyl acetone	0.03 ± 0.01a	0.04 ± 0.04a	0.03 ± 0.01a	0.04 ± 0.04a	0.05 ± 0.03b	0.02 ± 0.01a	***	***	***	**
β-Myrcene	1.42 ± 0.66a	1.96 ± 0.97a	1.28 ± 0.63a	2.09 ± 0.88b	2.24 ± 0.70b	1.14 ± 0.63a	**	ns	ns	ns
Total	3.22 ± 1.03a	5.33 ± 2.76b	3.10 ± 1.13a	5.44 ± 2.61b	5.37 ± 2.46b	3.17 ± 1.55a	***	ns	ns	ns
C_{13} norisoprenoids										
(E)-β-Damascenone	3.28 ± 1.08a	4.67 ± 3.14a	3.11 ± 0.96a	4.84 ± 3.08b	5.10 ± 2.96b	2.86 ± 0.76a	*	ns	*	*
(Z)-β-Damascenone	0.25 ± 0.10a	0.34 ± 0.17a	0.23 ± 0.06a	0.37 ± 0.17b	0.37 ± 0.16b	0.22 ± 0.07a	ns	ns	**	ns
β-Ionone	0.12 ± 0.11a	0.15 ± 0.16a	0.09 ± 0.09a	0.18 ± 0.17a	0.25 ± 0.12b	0.03 ± 0.02a	**	ns	***	ns
β-Cyclocitral	0.12 ± 0.09a	0.16 ± 0.12a	0.11 ± 0.08a	0.17 ± 0.12a	0.23 ± 0.07b	0.05 ± 0.02a	ns	ns	**	ns
TDN	0.33 ± 0.15a	0.69 ± 0.49a	0.36 ± 0.20a	0.66 ± 0.50b	0.72 ± 0.48b	0.30 ± 0.11a	**	***	***	ns
Total	4.11 ± 1.40a	6.01 ± 3.96a	3.91 ± 1.15a	6.21 ± 3.93b	6.67 ± 3.65b	3.45 ± 0.9a	*	ns	*	*
Benzenoid compounds										
2-Phenylethanol	7.78 ± 5.71a	9.57 ± 7.58a	8.22 ± 6.69a	9.13 ± 6.82a	14.74 ± 3.36b	2.61 ± 1.41a	ns	**	ns	ns
2-Phenylethanal	4.03 ± 3.48a	4.69 ± 4.74a	3.16 ± 2.66a	5.56 ± 4.96a	7.76 ± 3.18b	0.96 ± 0.21a	ns	ns	***	ns
Benzyl alcohol	4.03 ± 3.27a	5.33 ± 4.90a	3.89 ± 3.31a	5.47 ± 4.83a	8.05 ± 3.34b	1.31 ± 0.53a	**	ns	ns	**
Total	15.84 ± 12.09a	19.59 ± 16.51a	15.26 ± 12.41a	20.17 ± 16.11a	30.54 ± 8.51b	4.89 ± 1.74a	**	**	*	ns
Alcohols										
n-Nonanol	0.09 ± 0.04a	0.12 ± 0.07a	0.09 ± 0.04a	0.13 ± 0.07b	0.15 ± 0.06b	0.07 ± 0.02a	***	***	**	ns
2-Ethyl-1-hexanol	1.93 ± 0.48a	2.36 ± 0.85a	1.87 ± 0.55a	2.42 ± 0.77b	2.60 ± 0.72b	1.69 ± 0.31a	ns	***	**	*
Total	2.02 ± 0.50a	2.48 ± 0.92a	1.95 ± 0.57a	2.55 ± 0.84b	2.75 ± 0.76b	1.76 ± 0.33a	ns	***	***	*
Carbonyl compounds										
Heptanal	0.04 ± 0.04a	0.03 ± 0.03a	0.04 ± 0.04a	0.04 ± 0.03a	0.07 ± 0.02b	0.01 ± 0.00a	ns	*	ns	ns
(E,E)-2,4-Heptadienal	1.18 ± 1.01a	1.61 ± 1.44a	1.33 ± 1.23a	1.46 ± 1.29a	2.55 ± 0.60b	0.24 ± 0.08a	ns	**	ns	ns
Total	1.23 ± 1.05a	1.65 ± 1.47a	1.37 ± 1.27a	1.50 ± 1.32a	2.62 ± 0.60b	0.25 ± 0.09a	ns	**	ns	ns
C6 compounds										
n-Hexanol	9.93 ± 4.09a	10.65 ± 4.45a	9.34 ± 2.82a	11.23 ± 5.19a	9.55 ± 2.63a	11.03 ± 5.36a	ns	ns	***	ns
Hexanal	11.35 ± 4.74a	14.39 ± 12.46a	10.77 ± 4.98a	14.96 ± 12.18a	13.35 ± 2.73a	12.38 ± 13.22a	ns	ns	**	ns
(E)-2-Hexenal	6.43 ± 4.89a	12.01 ± 17.45a	5.60 ± 3.14a	12.83 ± 17.53a	2.34 ± 0.59a	16.10 ± 15.61b	ns	ns	*	ns
Total	27.7 ± 9.48a	37.04 ± 32.00a	25.72 ± 5.40a	39.03 ± 32.20a	25.23 ± 5.55a	39.51 ± 31.95a	ns	ns	***	ns
Other compounds										
Methyl jasmonate	0.16 ± 0.18a	0.14 ± 0.09a	0.13 ± 0.09a	0.16 ± 0.18a	0.25 ± 0.13b	0.05 ± 0.02a	ns	ns	ns	ns

Table 4. Continued

	Treatments (T)		Water status (W)		Season (S)		Multifactorial analysis[a]			
	Control	ACP-MeJ	Rainfed	RDI	2019	2020	T × W	T × S	W × S	T × W × S
Methyl salicylate	0.66 ± 0.38a	0.57 ± 0.23a	0.67 ± 0.37a	0.56 ± 0.24a	0.77 ± 0.38b	0.47 ± 0.07a	***	ns	***	**
Nonanoic acid	0.55 ± 0.24a	0.93 ± 0.68b	0.56 ± 0.22a	0.92 ± 0.70a	1.03 ± 0.64b	0.45 ± 0.14a	***	*	**	***
Total	2.70 ± 1.20a	3.06 ± 1.62a	2.74 ± 1.18a	3.02 ± 1.64a	3.88 ± 1.36b	1.88 ± 0.36a	**	ns	ns	***

Note: For each parameter, treatment, water status regime and season, different lower case letters indicate significant differences between treatments ($P \leq 0.05$).
[a] Statistical significance: *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$, respectively; ns, not significant ($P > 0.05$).

compounds, or MeJ content in comparison with the content of those substances in control samples.

In 2020, regarding to the RDI watering status, the ACP-MeJ treatment increased the content of all the terpenoids. Similarly, this ACP-treatment increased two-fold the total of the terpenoids respect to the control samples, as well as was observed in 2019 data. Likewise, the β-ionone, 2-phenylethanol, and benzyl alcohol content, the total benzenoid compounds content, the heptanal content and the nonanoic acid content increased with the ACP-MeJ treatment, in comparison with the control samples (Table 3). These results interesting because terpenoids are one of the main varietal volatile compounds in grapes, because their very low sensory thresholds, are responsible for the citrus and floral aromas of grapes and wines.[27] Thus, although in Monastrell wines these compounds were probably not found above their threshold of perception, they could contribute to the overall aroma of the wine, as possible synergies between aromatic compounds are unknown to date. D'Onofrio *et al.*[7] suggested that the application of MeJ activates the expression of the terpenoid biosynthesis genes. Their results and those from other authors, such as Garde-Cerdán *et al.*[1] and Gómez-Plaza *et al.*[6] also supported this increment of terpenoids content in grapes after MeJ application (it is pointed out that they applied MeJ in a conventional way, at a dose 10 times higher than our ACP-MeJ treatment). According to Bouzas-Cid *et al.*,[19] grapevine water availability is a factor that affects certain terpenoids content. They observed that those wines with greater α-terpineol content and lower geraniol concentration came from the irrigated grapevines.

Multifactorial analysis

Table 4 shows multifactorial analysis applied to the data of volatile compounds for foliar treated samples, for two different watering regimes, and for two consecutive seasons (Table 4). The compounds belonging to the terpenoid family were the most influenced by the foliar treatment, the content of limonene, *p*-cymene, linalool, α-terpineol, and total terpenoids being higher in the ACP-MeJ treated samples than in the controls (Table 4). These results were consistent with those obtained by other authors such as Gómez-Plaza *et al.*,[6] D'Onofrio *et al.*,[7] and Marín-San Román *et al.*,[8] where after MeJ was applied to the vines, the sum of terpenoids increased in Monastrell, Sangiovese, and Grenache varieties, respectively. As Black *et al.*[39] suggested, this could be linked to the fact that foliar treatments were applied during veraison, when the free terpenoids begin to be synthesized in grapes. The nonanoic acid content also increased in the ACP-MeJ samples in comparison with the control. However, the other grape volatile compounds were not affected by the treatment (Table 4).

The multifactorial analysis also showed clearly that the RDI watering regime increased the content of most of the terpenoids, C_{13} norisoprenoids, and alcohols in the grape samples with respect to the rainfed regime (Table 4). Authors such as Qian *et al.*[41] and Ou *et al.*[42] reported that the application of different RDI strategies to grapevines wase linked to increased concentrations of fruity norisoprenoids in the resulting wines. Likewise, Savoi *et al.*[43] revealed that water deficit strategy modulates the terpenoid pathway, with an accumulation of terpenes in grapes. Deluc *et al.*[44] reported an increase in carotenoid cleavage dioxygenases (CCDs), enzymes related to norisoprenoid synthesis.

Most of the compounds were influenced by season, with higher volatile compounds content in 2019 – a year in which the precipitation during the vegetative cycle (372 mm from April to harvest) doubled in comparison with 2020 (166 mm), although the annual precipitation was more similar (526 vs 459, respectively). However, the (E)-2-hexenal content was higher in 2020 than in 2019, and the concentration in grapes of linalool, α-terpineol, *n*-hexanol, hexanal and total C6 compounds, was not affected by the season (Table 4). This strong influence of year in the most of the volatile compounds was also reported by Garde-Cerdán *et al.*,[1] after applying MeJ on Tempranillo grapevines, and by Bouzas-Cid *et al.*[19] in wines from the Treixadura cultivar under rainfed and irrigation conditions. Authors such as Ju *et al.*[38] reported different variation patterns of C6 and C9 compounds in two vintages, suggesting the strong influence of season in the grape volatile compounds (in 2005, ethyl acetate and dodecanoic acid methyl ester were detected and the Cabernet Sauvignon grapes from the 80% ETc RDI treatment increased the content of dodecanoic acid methyl ester, unlike in 2016). These authors also reported that RDI treatments might regulate the biosynthesis of C6 compounds, enhancing the aroma characteristics of the grape varieties.

The interactions between the three factors, treatment (T), water status (W) and season (S), were significant in most of the volatile compounds (Table 4). T × W interaction influenced a large number of volatile compounds: all terpenoids except *p*-cymene, and C_{13} norisoprenoids except (Z)-β-damascenone and β-cyclocitral increasing their content in the treated ACP-MeJ samples in comparison with the control samples and those in which RDI was applied compared to rainfed ones (Table 4). Similarly, the content of benzyl alcohol, total benzenoid compounds, and *n*-nonanol was affected by the interaction of these two factors (T × W). On the other hand, neither carbonyl compounds nor C6 compounds nor the MeJ content were significant in this interaction (Table 4). Other compounds, such as methyl salicylate and nonanoic acid, showed differences regarding the T × W interaction. Also, the interation T × S showed differences for the terpenoids limonene, *p*-cymene, α-terpineol, and geranyl acetone values. In the case of the C_{13} norisoprenoids, only TDN was significant, as well as 2-phenylethanol and total benzenoid compounds (Table 4).

On the other hand, this interaction T × S affected all the alcohols and carbonyl compounds, as well as nonanoic acid content. The W × S interaction was significant in the case of the content of the C_{13} norisoprenoids, the total benzenoid compounds, the

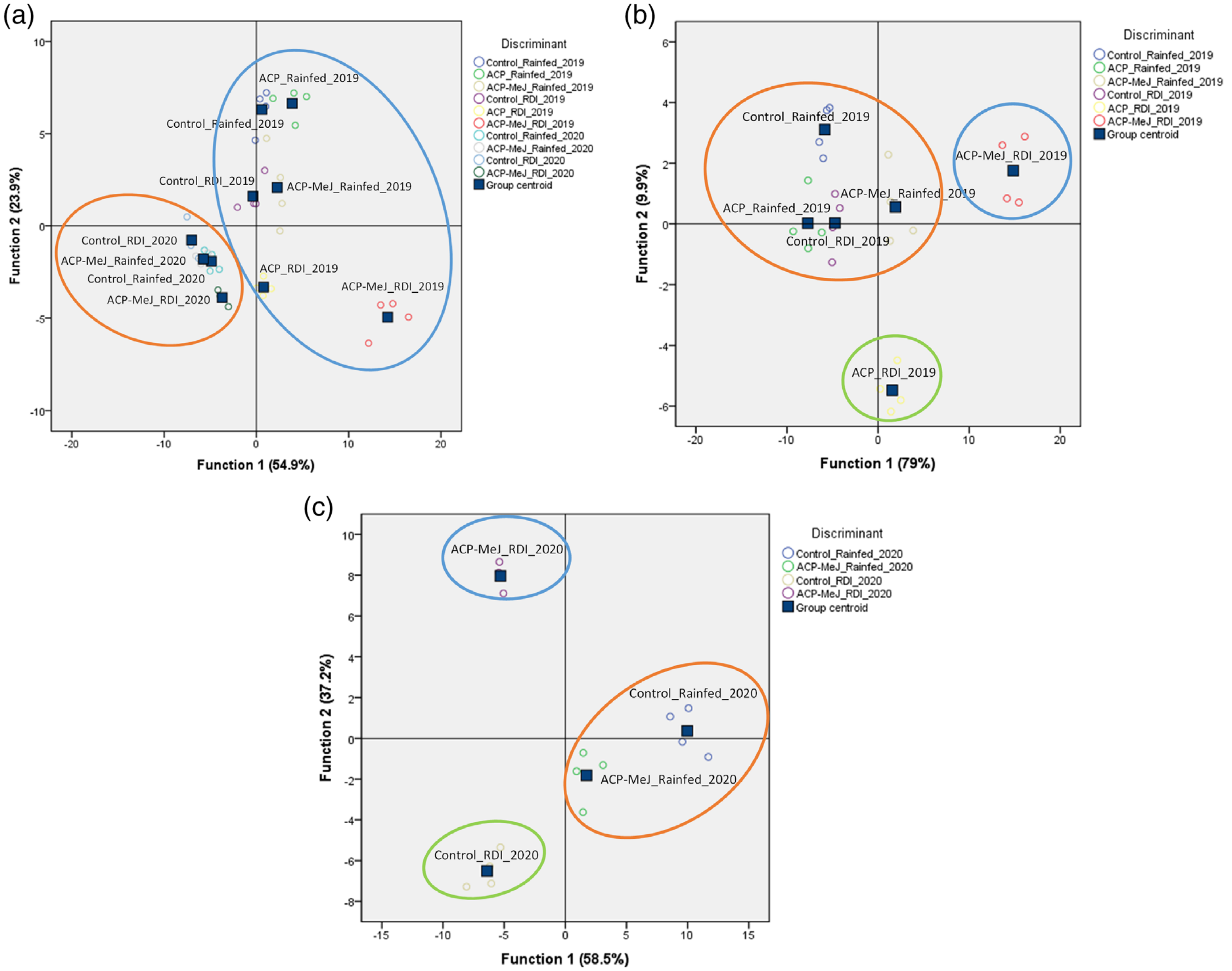

Figure 2. Discriminant analysis performed on data expressed as concentration of the total terpenoids, C_{13} norisoprenoids, benzenoid compounds, alcohols, carbonyl compounds, C6 compounds and other volatile compounds from the Monastrell grapes from control grapevines and from grapevines treated with amorphous calcium phosphate (ACP) and amorphous calcium phosphate with MeJ (ACP-MeJ) nanoparticles, under non-irrigated (rainfed) and regulated deficit irrigation (RDI) conditions, in both seasons (a), in 2019 (b) and in 2020 (c).

alcohols and the C6 compounds (Table 4). The triple interaction of the factors (T × W × S) was significant in the case of geranyl acetone, (E)-β-damascenone, total C_{13} noirisoprenoids, benzyl alcohol, 2-ethyl-1-hexanol, total alcohols, methyl salicylate, nonanoic acid, and the total of other compounds determined in the Monastrell grapes (Table 4). These interactions indicate that the factors analyzed were independent and, thus, the content of these volatile compounds in Monastrell grapes was affected, simultaneously, by the three factors.

Discriminant analysis of volatile compounds

In order to obtain any differentiation, discriminant analysis was performed with the data of the grape volatile compounds studied. In Fig. 2(a), the discriminant analysis was performed with the content data for the terpenoids, C_{13} norisoprenoids, benzenoid compounds, alcohols, carbonyl compounds, C6 compounds, and other volatile compounds from the control Monastrell grapes and those treated with ACP and ACP-MeJ, under the rainfed and RDI regimes, during 2019 and 2020 seasons. Function 1 explained 54.9% of the variance and Function 2 explained 23.9%, reaching a total of 78.8%. In the case of the Function 1, the variables that contributed most to the discriminant model were geranyl acetone, *p*-cymene, methyl jasmonate, methyl salicylate, and (E,E)-2,4-heptadienal, positively correlated, and, β-ionone and 1-hexanol, negatively correlated. For the Function 2, (E,E)-2,4-heptadienal and methyl salicylate were positively correlated, and, α-terpineol and *p*-cymene were negatively correlated. Both discriminant functions made it possible to differentiate the samples from each of the two seasons; thus, in 2020, the volatile compounds values between the treatments were more similar than the data form the different treatments in 2019 (year with less precipitation during the cycle) (Figs 1 and 2(a)).

In order to examine, within each season, the influence of agronomic practices, foliar treatments and irrigation systems on the volatile compounds of the samples, separate discriminants were carried out with data from 2019 (Fig. 2(b)) and 2020 (Fig. 2(c)). With the volatile data of 2019 (year where rainfall was higher and, therefore, the vines presumably had more soil water available than in 2020), Function 1 of the discriminant explained 79% of the variance and Function 2 explained 9.9%, reaching the 89% of the total variance. Geranyl acetone, *p*-cymene and 1-hexanol (Function 1) and geranyl acetone and *p*-cymene (Function 2) were the variables that positively contributed the most to the discriminant model, as well as β-ionone and hexanal (Function 1) and α-terpineol and β-ionone (Function 2), which contributed negatively to the discriminant. In this case, the discriminant allowed the samples treated under the RDI water regime to be differentiated from those of the rest of the treatments, which, except for the rainfed control, were more similar to each other (Fig. 2(b)). The discriminant perfomed with data from 2020, driest year and with the highest absolute maximum temperature (28.1 *vs*. 27.5 °C in 2019) during the growing season) also allowed to differentiate the samples under the rainfed regime from those under the RDI (Fig. 2(c)). Within each watering regime, the effect of the foliar treatments applied was also observed, with the difference between the control and treated samples being more pronounced in the case of the RDI regime. Regarding this discriminant, Function 1 explained 58.5% of the variance and Function 2 explained 37.2%, reaching 95.7% of the total variance. Geranyl acetone and methyl salicylate were the variables that, positively, most contributed to this explantation in both functions of the discriminant. Meanwhile, β-myrcene and 2-phenylethanal (Function 1) and 2-phenylethanal (Function 2), were the variables that, negatively, most contributed to the discriminant (Fig. 2(c)).

CONCLUSIONS

The influence on grape volatile compounds of the foliar application of ACP and ACP-MeJ to Monastrell vines under rainfed and RDI watering strategies was studied. The RDI treatment increased the grape weight and malic acid content and reduced the phenols content in comparison with the rainfed grapes, possibly due to the dilution effect of the grapes with the supplied water. Meanwhile, the foliar treatments hardly changed the enological parameters of the grapes but increased some volatile compounds in the berries in comparison with the untreated samples. The ACP-MeJ treatment applied to the vines under RDI water regime influenced positively the concentration of most of the terpenoids, C_{13} norisoprenoids, benzenoids, and alcohols in Monastrell grapes. Likewise, the content of these volatile compounds was higher in grapes under the influence of the RDI system and those grapes from 2019 (the wetter season) than the rainfed samples and grapes from 2020 season, respectively. This could indicate that the synthesis of these volatile compounds appreciated in the grapes is favored by a high water profile throughout the vine ripening cycle, interacting positively with the treatment of the nanoparticles doped with MeJ. Thus, the results obtained in this study are of great interest. They show that the application of MeJ as ACP-MeJ allowing a longer release of the elicitor in time positively affects the grape volatile composition, which could possibly be reflected in an improvement in the wine quality. The positive interaction of the elicitor treatment on plants whose water status was modulated according to their needs presents this watering system as an interesting tool for managing such a scarce and valuable resource as water, enhancing the influence of the foliar treatment on the volatile composition and quality of the grapes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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