applied sciences

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Foliar Applications of Calcium, Silicon and Their Combination: A Tool to Improve Grape Composition and Quality

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Abstract: Foliar nutrient sprays are broadly used in commercial agriculture. To date, the application of Ca and Si has been little explored in vineyard production but may lead to positive responses at various levels. Therefore, the aim of this work was to evaluate the effect of supplying single or combined calcium (Ca, 120 mM) and silicon (Si, 120 mM) sprays in Tempranillo grape composition. Foliar treatment with Ca + Si foliar enhanced all families of aromatic compounds, whereas single Ca and Si sprays induced lower effects. Regarding phenolic compounds, all foliar treatments led to minor effects. However, all three foliar Ca and Si treatments increased the total grape amino acid content. Consequently, the application of combined Ca and Si sprays to a vineyard is recommended as a tool for improving grape quality.

Keywords: volatile composition; aroma; phenolic compounds; nitrogen composition; Tempranillo; foliar application; must; grape

Citation: Garde-Cerdán, T.; González-Lázaro, M.; Alonso-Ortiz de Urbina, D.; Sáenz de Urturi, I.; Marín-San Román, S.; Murillo-Peña, R.; Torres-Díaz, L.L.; Pérez-Álvarez, E.P.; Fernández, V. Foliar Applications of Calcium, Silicon and Their Combination: A Tool to Improve Grape Composition and Quality. *Appl. Sci.* **2023**, *13*, 7217. https://doi.org/10.3390/app13127217

Academic Editors: Joanna Kapusta-Duch, Teresa Leszczyńska and Ewa Piątkowska

Received: 30 May 2023
Revised: 9 June 2023
Accepted: 12 June 2023
Published: 16 June 2023

1. Introduction

Grapevine is a major agricultural crop that is often exposed to variable stress conditions during its lifespan and growing cycle [1,2]. Among other husbandry techniques, fertilization has been shown to play a key role in grapevine cultivation, since it may affect berry and vine quality [3,4], in addition to improving disease resistance [5,6] and tolerance to other stress factors [7].

Calcium is an essential nutrient for plants that plays key signaling and structural roles [8]. Roots absorb this element from the soil solution, and Ca is subsequently transported to the shoots in the xylem [8]. Calcium mobility in plants is generally low, this element being chiefly accumulated in mature organs despite the high Ca requirements of actively growing plant parts such as expanding leaves, flowers or fruits [9,10].

The delivery of Ca to developing fruits depends on xylem transport and transpiration [11,12], but a contribution of pedicel phloem transport has also been reported [9]. The high stomatal densities of leaves compared to fruits lead to higher transpiration and Ca accumulation rates in foliar tissues compared to fruiting organs [12].

Although Ca is normally available in soils in sufficient amounts, the occurrence of localized Ca deficiencies (i.e., Ca-related disorders) in fruits and vegetables is a common agricultural production problem, which can lead to major economic losses at harvest and postharvest [13]. Calcium-related disorders may involve an array of alterations including the deterioration of cell walls and membranes [13], with many physiological and anatomical

symptoms in plant organs as recently described for grapevine [14]. Given the limited Ca translocation from leaves to fruits during fruit growth and development [10,15], the supply of Ca as sprays onto fruit surfaces is recommended [16,17], as also evaluated in some studies performed with grapevine [3].

On the other hand, Si is a nonessential element that has been found to be beneficial for improving crop yield and quality and also plant tolerance to various abiotic and biotic stress factors [18]. The effect of a Si supply on grapevine has been assessed in a few investigations [19–21], which reported positive changes in grape quality and must composition. Silicon is often provided as foliar sprays that have been shown to induce positive plant responses in terms of improved quality, yield and pest and disease tolerance [22,23].

Foliar nutrient sprays are nowadays widely used in plant production, chiefly as a complementary strategy to root treatments [24,25]. Foliar-applied nutrient solutions may be absorbed by plant surfaces via stomata, the cuticle and cuticular irregularities, trichomes, veins and other modified epidermal structures, such as lenticels [26]. Research in the last 50 years largely focused on the mechanisms of cuticular penetration [24], with limited focus on the permeability of other epidermal structures such as stomata or trichomes, which may have a major contribution to the foliar absorption process [26,27]. Recent studies are providing evidence for their major physical and chemical heterogeneity of plant surfaces, which are generally covered with a cuticle [26]. Indeed, the occurrence of cell wall polysaccharides in [28–30] and even at the very surface of the cuticle [31] has been shown for few species and plant organs, and this may have major implications for understanding the mechanisms of agrochemical spray foliar permeability or pest and pathogen attack, among others [26].

Aware of the positive results reported after the application of Ca and Si sprays to crop plants in the few studies available as reported above, the aim of this investigation was to evaluate the effect of Ca and Si supplied as either single elements or in combination, in terms of Tempranillo berry yield, quality and subsequent must chemical composition under the prevailing growing conditions in La Rioja (Spain).

2. Materials and Methods

2.1. Vineyard, Treatments and Grape Samples

Tempranillo grape (*Vitis vinifera* L.) variety plants grown in Finca La Grajera (Spain) were used for trial development. The vine was planted in 1995 (2.80 m × 1.20 m) and the experiment was performed during the 2020 season. Foliar sprays included Ca nitrate ($CaNO_3$ $4H_2O \geq 99.0\%$, Sigma-Aldrich-Merck, Darmstadt, Germany) or/and 120 mM silicic acid potassium solution (Tradecorp, Madrid, Spain), which were applied separately or in combination. The commercial products were used to prepare solutions with concentrations of 120 mM Ca, 120 mM Si and 120 mM Ca + 120 mM Si. Foliar sprays contained 0.1% (v/v) Genapol X-80 surfactant (Sigma-Aldrich-Merck). Control plants were foliar treated with 0.1% Genapol solutions. All treatments were applied twice, at veraison and one week later, and approximately delivered 200 mL of foliar spray solution per plant. Foliar applications were carried out in triplicate and were performed following a completely randomized block design, with 3 vines for each treatment and repetition.

For harvesting, grapes from all treatments were picked at the optimal technological maturity, i.e., when the weight of 100 berries remained constant and the probable alcohol reached 13 (% v/v). A random set of 150 grape berries was picked, per treatment and replicate, and frozen at −20 °C until the determination of volatile (50 berries), phenolic (50 berries) and nitrogen (50 berries) composition. Another set of 100 berries was used to obtain the average berry weight. Then, the berries were crushed, and the physico-chemical parameters were determined in the must.

2.2. Must Physico-Chemical Parameters

Must general parameters were analyzed by the OIV [32] official methods by measuring total acidity, pH, °Brix and probable alcohol. Glucose, glucose + fructose (calculating fructose concentration by the difference), tartaric and malic acids, total phenols, ammonium and amino nitrogen (N) (yeast assimilable N (YAN) was the sum of the two N fractions) determined using Miura One equipment (TDI, Barcelona, Spain).

As treatments included 3 repetitions, the results of enological parameters also presented as the mean of 3 determinations (n = 3).

2.3. Volatile Compound Determination

The volatile composition was determined in the musts by headspace solid-phase microextraction (HS-SPME) and subsequent analysis by GC-MS (Agilent, Palo Alto, CA, USA), according to the method described by Garde-Cerdán et al. [33]. Briefly, aliquots of 9 mL must plus 2.5 g of NaCl and 10 µL of 2-octanol (internal standard) were put in 20 mL vials. Solutions were then stirred, and the vials were closed for subsequent GC-MS analysis. After completion of the extraction process, the fiber was inserted into the GC injection port (250 °C) for 15 min. The capillary column used was SPB™-20 (30 m × 0.25 mm I.D. × 0.25 µm of film thickness) (Supelco, Bellenfonte, PA, USA). Identification of volatile compounds was carried out using the NIST library, by comparing with the mass spectra and retention time of the chromatographic standards. Since foliar applications involved 3 repetitions, the results of grape volatile compounds are expressed as an average of 3 replicates (n = 3).

2.4. Analysis of Phenolic Compounds

2.4.1. Grape Phenolic Compound Extraction

Grape berry phenolic compounds were extracted according to Garde-Cerdán et al. [34]. Briefly, 50 g grapes were added to a mixture of methanol/water/formic acid (50:48.5:1.5, $v/v/v$) (50 mL). Then, this mixture was homogenized (18,000 rpm, during 1 min). After that, samples were macerated (ultrasonic bath) for 10 min and, then, were centrifuged (3640× g, 10 °C, 10 min). The supernatant was separated, and the resulting pellet was extracted once again. Then, the supernatants collected were mixed, and samples were stored at −20 °C for further analysis.

2.4.2. Extract of Non-Anthocyanin Phenolic Compounds

The extraction of non-anthocyanin phenolic compounds was performed following the procedures described by Garde-Cerdán et al. [34], by using PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Santa Clara, CA, USA). Cartridges were put in the extraction system (Visiprep™ SPE Vacuum Manifold, Sigma-Aldrich-Merck). Then, 3 mL of grape phenolic extracts were diluted with 0.1 N HCl (9 mL). The cartridges were conditioned with methanol (5 mL) and water (5 mL). After that, the diluted samples were passed through the cartridges and washing was undertaken (5 mL of 0.1 N HCl + 5 mL of water); 2 × 3 mL of ethanol were used in order to elute the non-anthocyanin phenolic compounds fraction. Then, this fraction was dried in an evaporator (35 °C) and redissolved in a methanol (1.5 mL) aqueous solution (20% v/v). The anthocyanin-free fraction was employed in order to determine flavonols, hydroxycinnamic and hydroxybenzoic acids, stilbenes and flavanols.

2.4.3. Phenolic Compound Determination

Phenolic compounds were analyzed as described by Garde-Cerdán et al. [34] using an Agilent 1260 Infinity II chromatograph equipped with a diode array detector (DAD). Samples were filtered and injected into a Lichrospher® 100 RP-18 column (Agilent) with pre-column Lichrospher® 100 RP-18 (Agilent) (40 °C). For the analysis of anthocyanins, grape extracts were injected (10 µL). For the analysis of non-anthocyanin phenolic compounds (flavonols, hydroxycinnamic and hydroxybenzoic acids, stilbenes and flavanols), 20 µL was injected. For quantification, DAD chromatograms were extracted: anthocyanins (520 nm),

flavonols (360 nm), hydroxycinnamic acids and stilbenes (320 nm) and gallic acid and flavanols (280 nm).

Since field treatments were performed in triplicate, the results for phenolic compounds are the average of the analyses of three samples (n = 3).

2.5. Nitrogen Compound Determination

The analyses of amino acids were carried out by HPLC using an Agilent 1260 Infinity Series coupled to a DAD and a fluorescence detector (FLD) according to Garde-Cerdán et al. [35]; 100 µL of sarcosine and 100 µL de norvaline (internal standards) were added to 5 mL of must. The mixture was submitted to a derivatization with o-phthaldialdehyde (Agilent) and with 9-fluorenylmethylchloroformate (Agilent). The nitrogen compound separations were undertaken on a Hypersil ODS (250 × 4.0 mm, I.D. 5 µm, Agilent) column, the injected volume being 10 µL. The identification of the nitrogen compounds was carried out according to the retention times of the standards (Sigma-Aldrich, St. Louis, MO, USA). Their quantification was undertaken using the calibration graphs ($R^2 > 0.96$).

Since the foliar applications were carried out in triplicate, the results of amino acids are expressed as the average of the 3 replicates (n = 3).

2.6. Statistical Analyses

The statistical elaboration of the data was conducted using SPSS Version 21.0 (SPSS, Chicago, IL, USA). Physico-chemical parameters and volatile, phenolic and nitrogen compounds data were managed using the variance analysis (ANOVA) ($p \leq 0.05$).

3. Results and Discussion

In this study, we assessed the potential of Ca and Si sprays supplied two times during the growing season for improving grape quality by measuring several physico-chemical parameters. Positive results were obtained, especially when treating vines with combined Ca plus Si sprays as described in the paragraphs below for the different variables measured.

3.1. Must Enological Parameters

Table 1 shows general grape parameters for control and foliar sprayed vines, which were supplied Ca, Si and Ca + Si. Foliar Ca application decreased the weight of 100 berries, as previously observed by Martins et al. [36]. This effect may be associated with a delay in berry maturation. However, °Brix was unaffected by the three foliar treatments (Table 1). On the other hand, Ca spraying increased total phenols, amino N and YAN contents in must compared to the control must; except for total phenols, the effect of Ca application was significant in comparison with Si and Ca + Si foliar treatments (Table 1). Foliar Si sprays increased the fructose must content in comparison to the values recorded for control, Ca and Ca + Si musts. Furthermore, Ca + Si foliar application led to musts with a higher ammonium N content than control and Si musts (Table 1). Therefore, the overall effect of foliar treatments on general must parameters was slight. Gomes et al. [37] described an increase in some acids in the grapes after spraying vines with Si and Ca, in contrast with the general parameters shown in Table 1. However, Losada et al. [21] observed an absence of effect on the °Brix, pH and total acidity of must after foliar application of monosilicic acid to grapevines, which is in agreement with our results (Table 1).

Table 1. General parameters in musts from control and Ca, Si and Ca + Si foliar treatments.

	Control	Ca	Si	Ca + Si
Weight of 100 berries (g)	235.30 ± 25.45 b	183.03 ± 33.68 a	222.00 ± 19.29 ab	239.63 ± 0.60 b
°Brix	22.80 ± 1.73 a	22.53 ± 1.10 a	24.83 ± 1.76 a	24.00 ± 1.11 a
Probable alcohol (% *v*/*v*)	13.33 ± 1.18 a	13.13 ± 0.76 a	14.72 ± 1.22 a	14.14 ± 0.77 a
Glucose + Fructose (g/L)	231.95 ± 19.45 a	232.52 ± 11.04 a	265.52 ± 23.85 a	249.23 ± 19.62 a
Glucose (g/L)	116.27 ± 10.39 a	115.63 ± 5.28 a	130.83 ± 11.32 a	124.31 ± 1048 a
Fructose (g/L)	115.68 ± 9.09 a	111.68 ± 9.22 a	133.02 ± 31.74 b	118.19 ± 16.90 a
pH	3.53 ± 0.14 a	3.73 ± 0.14 a	3.61 ± 0.02 a	3.68 ± 0.24 a
Total acidity (g/L) *	5.23 ± 0.89 a	4.72 ± 0.16 a	4.95 ± 0.10 a	5.40 ± 0.89 a
Tartaric acid (g/L)	5.02 ± 0.37 a	5.03 ± 0.59 a	4.42 ± 0.13 a	4.48 ± 0.86 a
Malic acid (g/L)	2.64 ± 0.05 a	2.21 ± 0.26 a	2.23 ± 0.18 a	2.63 ± 0.46 a
Total phenols (mg/L)	472.47 ± 42.30 a	722 ± 172.75 b	625.57 ± 6.58 ab	592.33 ± 50.60 ab
Ammonium nitrogen (mg N/L)	91.78 ± 15.13 a	123.50 ± 5.07 ab	98.02 ± 13.29 a	138.84 ± 27.30 b
Amino nitrogen (mg N/L)	108.24 ± 7.92 a	205.92 ± 30.80 b	119.97 ± 17.16 a	97.97 ± 9.24 a
YAN (mg N/L) **	200.02 ± 22.12 a	329.42 ± 35.87 b	217.99 ± 26.04 a	236.81 ± 23.80 a

* As g/L of tartaric acid. ** YAN: yeast assimilable nitrogen. All parameters are listed with the standard deviation (n = 3). Different letters indicate significant differences between the treatments ($p \leq 0.05$).

3.2. Effect of the Ca, Si and Ca + Si Foliar Treatments on Must Volatile Composition

The must volatile compounds content of control and Ca, Si and Ca + Si foliar treatments is shown in Figures 1 and 2 and Table 2.

Terpenoids are one of the most important families of varietal compounds for aromatic typicity, since these compounds contribute to floral and citrus aroma [38]. The application of Ca + Si sprays to vines strongly increased the total must terpenoids content (Figure 1l) because of the increase in the concentration of limonene, p-cymene, linalool, α-terpineol, nerol, geraniol and neral (Figure 1a–d,f,g,k), although the content of geranyl acetone showed a decrease (Figure 1j).

Foliar Ca application also showed positive effects in terms of varietal aromatic quality, as it increased the synthesis of p-cymene, geraniol and neral (Figure 1b,g,k), consequently raising the total terpene concentration (Figure 1l). However, foliar Si treatment did not affect the total concentration of this family of aromatic compounds (Figure 1l), despite it increased p-cymene, linalool and nerol concentration (Figure 1b,c,f), whereas it decreased the content of isogeraniol and geranic acid (Figure 1h,i).

Regarding C_{13} norisoprenoids, which together with terpenoids is the other major family of varietal compounds [39–41], the only treatment that significantly favored their synthesis was the foliar application of Ca + Si (Figure 2), with the single exception of β-ionone, whose concentration decreased in the musts after this application (Figure 2c). Calcium spray application to grapevines had no effect on the amount of these aromatic compounds (Figure 2), while the application of Si only affected the concentration of β-ionone in the musts (Figure 2c), decreasing it, so that the concentration of total C_{13} norisoprenoids also decreased (Figure 2g).

As for benzenoid compounds, all spray treatments had an influence on the concentration of these compounds, which generally increased, especially in the Ca + Si combined treatment (Table 2). The application of Ca and Si favored the synthesis of 2-phenylethanol and 2-phenylethanal, while the Ca + Si treatment also increased the concentration of eugenol in the musts (Table 2). Benzenoid compounds also belong to the varietal aroma, conferring pleasant, fruity aromas, and 2-phenyethanol is characterized by the aroma of rose [42]. These compounds come from phenolic acids or from aromatic amino acids [41,43].

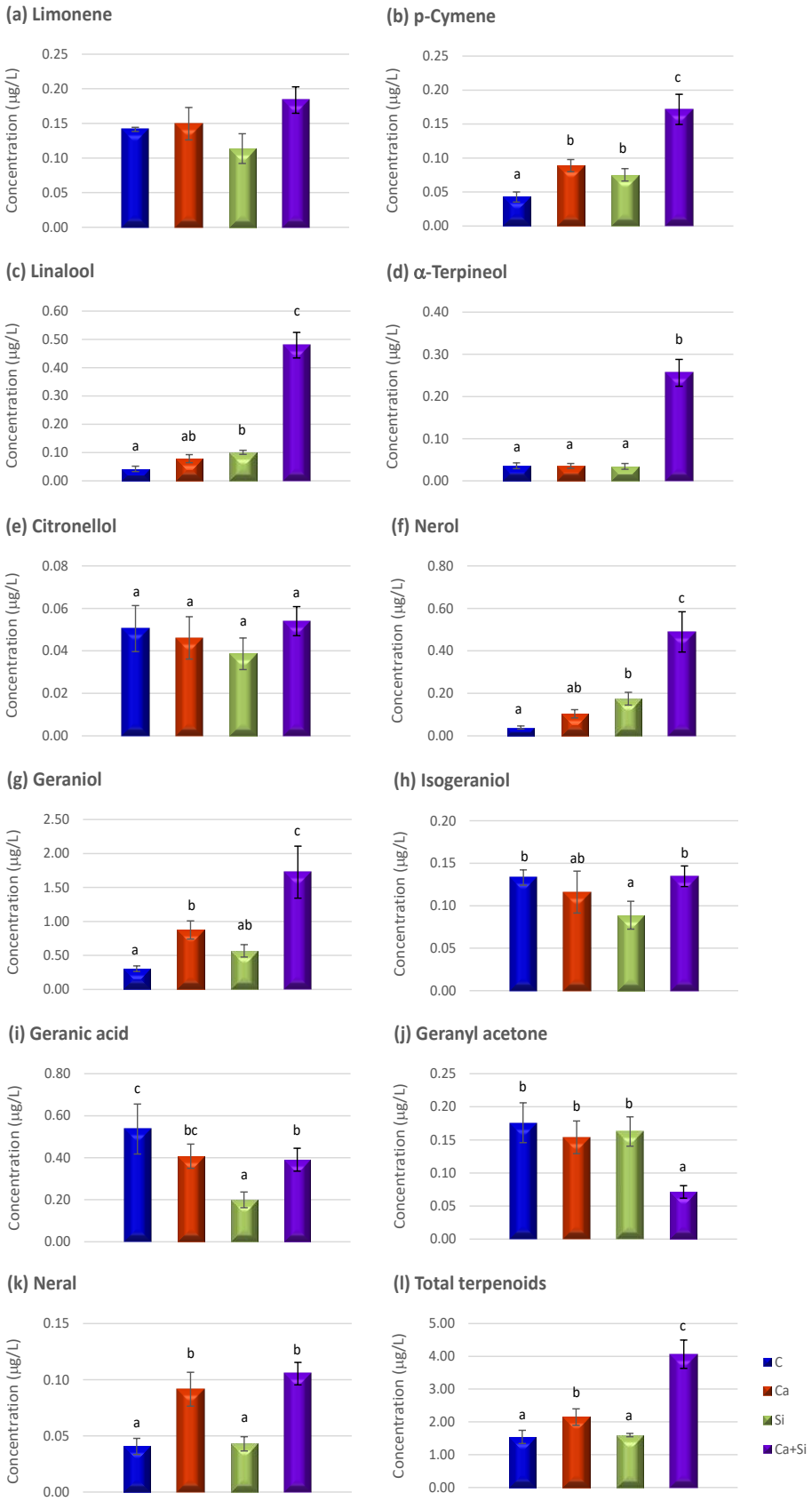

Figure 1. Terpenoids concentration (µg/L) in musts of control (C) and Ca, Si and Ca + Si foliar treatments. The mean volatile compound contents are shown the standard deviations SD (n = 3). Different letters indicate significant differences between foliar applications ($p \leq 0.05$).

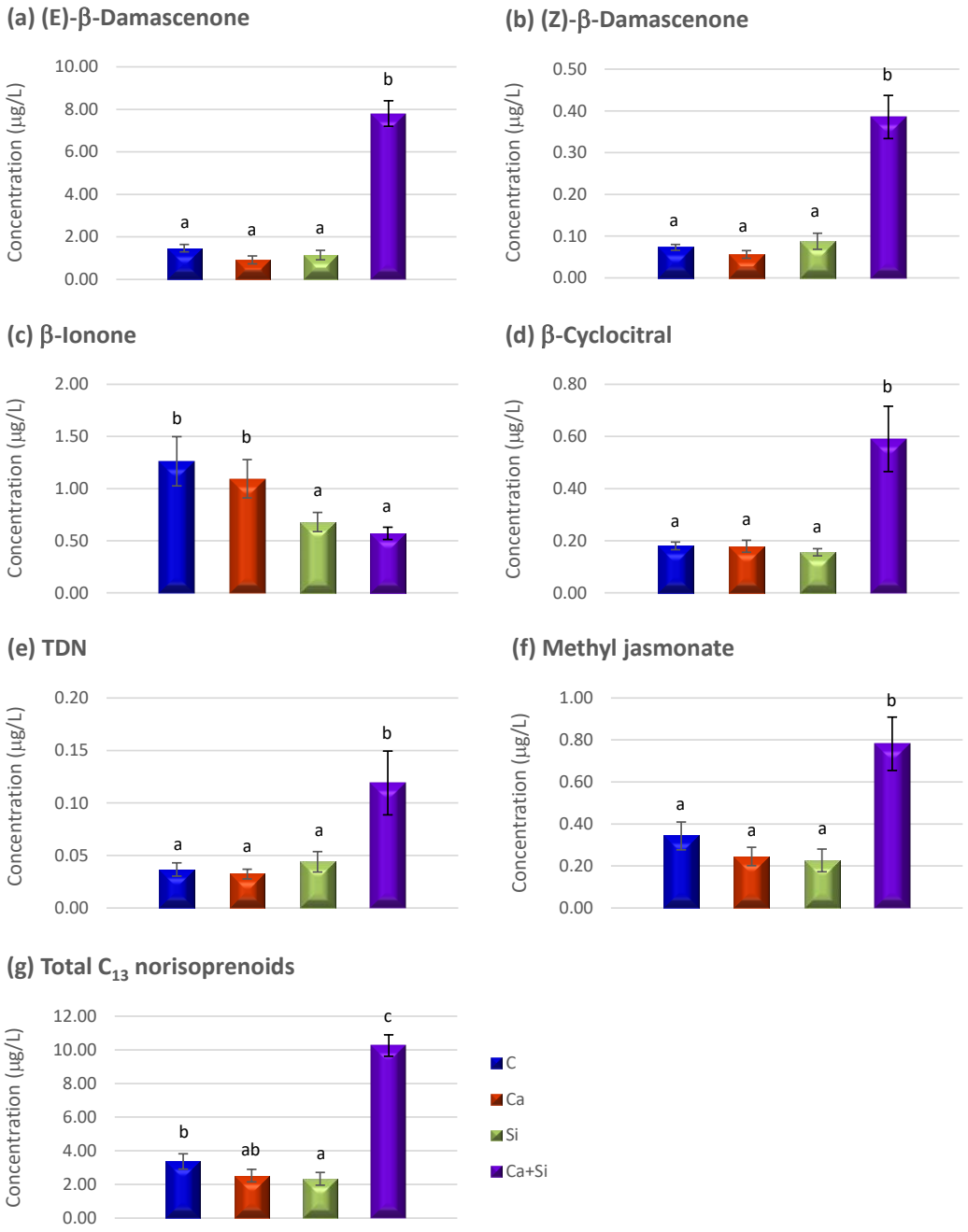

Figure 2. C_{13} norisoprenoids concentration (µg/L) in musts from control (C) and Ca, Si and Ca + Si foliar treatments. The volatile compound concentrations are means ± SD (n = 3). Different letters indicate significant differences between foliar applications ($p \leq 0.05$).

Of the two esters found in the musts, only the concentration of methyl salicylate was influenced by foliar applications, decreasing in the Si treatment and increasing in association with Ca + Si spray application, the total content following a similar trend (Table 2). Nonetheless, esters are present in grapes in small quantities. These compounds are related to fruity aromas and, hence, play an important role in wine aroma because they are formed in high amounts during the alcoholic fermentation [44].

Table 2. Benzenoid compounds, esters, alcohols, carbonyl compounds and C6 compounds concentrations (μg/L) in musts from control and Ca, Si and Ca + Si foliar treatments.

	Control	Ca	Si	Ca + Si
Benzenoid compounds				
2-Phenylethanol	6.68 ± 1.23 a	11.32 ± 1.07 b	14.86 ± 2.54 b	14.84 ± 3.53 b
2-Phenylethanal	8.25 ± 1.35 a	18.58 ± 4.08 b	16.69 ± 2.10 b	26.84 ± 5.05 c
Benzyl alcohol	2.40 ± 0.48 ab	1.85 ± 0.47 a	2.46 ± 0.49 ab	2.78 ± 0.12 b
Eugenol	0.02 ± 0.00 a	0.03 ± 0.01 a	0.04 ± 0.01 a	0.06 ± 0.01 b
Total	*17.35 ± 3.05 a*	*31.78 ± 2.62 b*	*34.04 ± 3.53 b*	*44.52 ± 8.47 c*
Esters				
Hexyl acetate	0.07 ± 0.01 ab	0.06 ± 0.01 a	0.06 ± 0.01 a	0.09 ± 0.02 b
Methyl salicylate	0.12 ± 0.01 b	0.11± 0.02 b	0.06 ± 0.01 a	0.33 ± 0.02 c
Total	*0.20 ± 0.02 b*	*0.18 ± 0.01 b*	*0.12 ± 0.01 a*	*0.42 ± 0.01 c*
Alcohols				
1-Heptanol	0.05 ± 0.01 a	0.13 ± 0.02 b	0.18 ± 0.02 b	0.30 ± 0.06 c
1-Octanol	0.48 ± 0.08 a	0.76 ± 0.12 a	0.70 ± 0.10 a	1.83 ± 0.34 b
1-Nonanol	0.24 ± 0.04 a	0.27 ± 0.05 a	0.51 ± 0.09 b	0.96 ± 0.14 c
1-Octen-3-ol	0.80 ± 0.08 a	0.70 ± 0.14 a	0.67 ± 0.03 a	1.19 ± 0.12 b
2-Ethyl-1-hexanol	0.89 ± 0.15 b	0.57 ± 0.12 a	0.56 ± 0.12 a	2.38 ± 0.11 c
Total	*2.46 ± 0.34 a*	*2.44 ± 0.24 a*	*2.62 ± 0.12 a*	*6.66 ± 0.42 b*
Carbonyl compounds				
Heptanal	0.06 ± 0.00 a	0.08 ± 0.02 a	0.05 ± 0.01 a	0.11 ± 0.02 b
Octanal	0.07 ± 0.01 a	0.08 ± 0.01 a	0.07 ± 0.01 a	0.06 ± 0.01 a
(E)-2-Octenal	0.15 ± 0.03 a	0.16 ± 0.03 a	0.14 ± 0.01 a	0.17 ± 0.03 a
Nonanal	0.94 ± 0.08 a	1.37 ± 0.32 b	0.79 ± 0.14 a	1.63 ± 0.19 b
(E)-2-Nonenal	0.14 ± 0.03 a	0.23 ± 0.05 b	0.12 ± 0.02 a	0.21 ± 0.02 b
Decanal	0.22 ± 0.04 c	0.15 ± 0.02 b	0.12 ± 0.02 ab	0.09 ± 0.02 a
(E,E)-2,4-Hexadienal	2.83 ± 0.17 b	1.79 ± 0.41 a	1.12 ± 0.20 a	2.86 ± 0.55 b
(E,E)-2,4-Heptadienal	2.29 ± 0.42 c	1.16 ± 0.20 b	0.56 ± 0.12 a	2.57 ± 0.34 c
(E,E)-2,4-Nonadienal	0.16 ± 0.02 b	0.15 ± 0.02 ab	0.11 ± 0.02 a	0.26 ± 0.03 c
(E,E)-2,4-Decadienal	0.04 ± 0.01 a	0.04 ± 0.01 a	0.03 ± 0.01 a	0.06 ± 0.01 b
γ-Decalactone	0.31 ± 0.06 a	0.42 ± 0.07 ab	0.43 ± 0.10 ab	0.63 ± 0.17 b
6-Methyl-3,5-heptadien-2-one	0.05 ± 0.01 b	0.02 ± 0.00 a	0.03 ± 0.00 a	0.07 ± 0.01 c
Total	*4.95 ± 0.19 b*	*4.49 ± 0.13 b*	*2.99 ± 0.36 a*	*6.15 ± 0.77 c*
C6 compounds				
Hexanal	80.75 ± 18.07 a	100.09 ± 17.52 a	86.51 ± 13.31 a	111.05 ± 20.55 a
n-Hexanol	46.85 ± 5.20 a	45.33 ± 10.17 a	66.65 ± 2.15 b	65.55 ± 11.66 b
(E)-2-Hexenal	36.31 ± 7.23 a	43.23 ± 4.70 a	35.66 ± 6.12 a	46.02 ± 9.12 a
(Z)-3-Hexen-1-ol+(E)-2-Hexen-1-ol	2.95 ± 0.57 ab	2.09 ± 0.39 ab	1.95 ± 0.40 a	3.01 ± 0.69 b
Total	*166.86 ± 22.47 a*	*190.74 ± 22.85 ab*	*190.78 ± 19.23 ab*	*225.63 ± 41.71 b*

The volatile compounds contents are listed with the standard deviation (n = 3). Different letters indicate significant differences between foliar applications ($p \leq 0.05$).

Regarding alcohols, only the Ca + Si foliar treatment increased their total concentration (Table 2). The application of Ca had an inverse effect on the concentration of two alcohols, 1-heptanol and 2-ethyl-1-hexanol, the first one increasing and the latter decreasing (Table 2). The same result was observed for Si, and, moreover, after this foliar application, 1-nonanol concentration increased in the musts. These compounds are related to the green and herbaceous aromas of grapes and wines [45].

The largest family of volatiles found in the musts was carbonyl compounds, whose total content increased when Ca + Si was applied to the vines and decreased when Si was applied, with no effect associated with Ca sprays (Table 2). Calcium treatments actually favored the synthesis of nonanal, (E)-2-nonenal, decanal and (E,E)-2,4-heptadienal, whereas it decreased the concentration in the musts of (E,E)-2,4-hexadienal and 6-methyl-3,5-heptadien-2-one. Foliar application of Si decreased the must concentrations of decanal, (E,E)-2,4-hexadienal, (E,E)-2,4-heptadienal and 6-methyl-3,5-heptadien-2-one. However,

the combined Ca + Si application favored the synthesis of most of these compounds, i.e., heptanal, nonanal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, γ-decalactone and 6-methyl-3,5-heptadien-2-one, and only decreased the content of decanal (Table 2). Carbonyl compounds are presented in low quantities in grapes. These compounds mostly are formed during alcoholic fermentation and present one or more aldehyde and ketone functions [46].

Finally, the C6 compounds, which are pre-fermentative aromas related to green and herbaceous notes [45], were hardly modified by the foliar treatments supplied to the vineyard, since only the concentration of n-hexanol was affected, increasing with the applications of Si and Ca + Si (Table 2) and only showing a total concentration increase with Ca + Si spray.

We failed to find any study in which the effect of Ca and Si application to the vineyard was evaluated in terms of the aromatic composition of the grapes, as assessed in our investigation.

3.3. Influence of Foliar Ca, Si and Ca + Si Treatments on Grape Phenolic Compounds

Table 3 shows the results of grape phenolic composition in control and foliar-treated grapevines.

Table 3. Phenolic compound concentration (mg/kg) in grapes from control and Ca, Si and Ca + Si foliar treatments.

	Control	**Ca**	**Si**	**Ca + Si**
Anthocyanins				
Delphinidin-3-glc	78.63 ± 7.06 a	73.38 ± 16.52 a	117.93 ± 13.51 b	93.43 ± 8.72 ab
Cyanidin-3-glc	13.36 ± 0.27 a	11.78 ± 3.35 a	25.50 ± 7.91 b	16.47 ± 4.16 ab
Petunidin-3-glc	61.52 ± 3.98 a	59.88 ± 11.44 a	50.19 ± 56.05 a	72.35 ± 3.09 a
Peonidin-3-glc	26.46 ± 1.77 a	27.53 ± 7.04 a	44.99 ± 13.29 a	35.04 ± 0.83 a
Malvidin-3-glc	188.29 ± 8.85 a	217.85 ± 27.95 a	216.19 ± 4.13 a	225.16 ± 47.53 a
Delphinidin-3-acglc	7.59 ± 0.56 a	7.56 ± 0.80 a	8.55 ± 0.29 a	8.04 ± 0.55 a
Cyanidin-3-acglc	3.86 ± 0.04 a	3.83 ± 0.14 a	3.79 ± 0.17 a	3.84 ± 0.04 a
Petunidin-3-acglc	5.89 ± 0.34 a	5.82 ± 0.44 a	6.05 ± 0.08 a	6.05 ± 0.36 a
Peonidin-3-acglc	16.72 ± 0.57 a	16.07 ± 1.86 a	18.33 ± 1.12 a	17.62 ± 1.24 a
Malvidin-3-acglc	3.68 ± 0.11 a	3.64 ± 0.08 a	3.54 ± 0.11 a	3.61 ± 0.03 a
Delphinidin-3-cmglc	11.23 ± 1.01 a	12.89 ± 0.81 a	10.92 ± 1.15 a	12.33 ± 1.02 a
Cyanidin-3-cmglc	5.30 ± 0.13 ab	4.82 ± 0.38 a	6.22 ± 0.63 b	5.61 ± 0.81 ab
Petunidin-3-cmglc	4.14 ± 0.09 a	4.33 ± 0.22 a	3.71 ± 0.06 a	4.23 ± 0.48 a
Peonidin-3-cmglc	13.96 ± 0.17 a	14.21 ± 2.08 a	14.57 ± 1.19 a	14.56 ± 0.53 a
Malvidin-3-*cis*-cmglc	4.92 ± 0.31 ab	5.18 ± 0.16 b	4.38 ± 0.17 a	4.98 ± 0.48 ab
Malvidin-3-*trans*-cmglc	9.03 ± 0.42 a	9.14 ± 1.01 a	10.44 ± 0.73 a	10.41 ± 0.24 a
Malvidin-3-cfglc	55.04 ± 3.91 a	62.37 ± 8.52 a	49.38 ± 11.05 a	58.57 ± 11.17 a
Total	*509.62 ± 15.49 a*	*540.28 ± 68.71 a*	*594.69 ± 82.25 a*	*592.32 ± 40.16 a*
Flavonols				
Myricetin-3-glcU+3-gal	23.61 ± 1.16 a	32.53 ± 7.83 a	29.08 ± 1.15 a	31.82 ± 3.84 a
Myricetin-3-glc	78.09 ± 2.09 a	111.54 ± 32.97 a	110.22 ± 8.88 a	121.79 ± 16.72 a
Quercetin-3-glcU	27.17 ± 2.55 a	42.39 ± 17.18 a	36.57 ± 4.26 a	35.06 ± 3.43 a
Quercetin-3-glc	31.39 ± 5.88 a	50.16 ± 23.86 a	49.84 ± 4.98 a	61.51 ± 12.84 a
Laricitrin-3-glc	13.31 ± 0.59 a	21.15 ± 5.21 ab	20.06 ± 1.54 ab	23.91 ± 4.28 b
Kaempferol-3-gal	8.30 ± 0.02 a	8.64 ± 0.17 a	8.99 ± 0.55 a	9.30 ± 0.74 a
Kaempferol-3-glcU+3-glc	1.92 ± 0.20 a	3.74 ± 2.55 a	2.70 ± 0.25 a	4.13 ± 1.65 a
Isorhamnetin-3-glc	3.05 ± 0.92 a	4.91 ± 2.12 a	9.29 ± 0.56 b	11.78 ± 1.66 b
Syringetin-3-glc	7.71 ± 0.35 a	12.75 ± 3.74 a	12.11 ± 1.81 a	15.74 ± 4.55 a
Total	*195.55 ± 9.54 a*	*287.80 ± 94.12 a*	*278.85 ± 17.29 a*	*315.04 ± 48.23 a*

Table 3. *Cont.*

	Control	Ca	Si	Ca + Si
Flavanols				
Catechin	29.28 ± 2.27 a	35.94 ± 4.41 a	33.92 ± 18.41 a	24.07 ± 4.65 a
Epicatechin	34.33 ± 3.82 b	50.48 ± 6.35 c	17.37 ± 3.02 a	22.28 ± 3.30 a
Epicatechin-3-gallate	6.45 ± 0.75 a	7.16 ± 1.41 a	4.18 ± 2.29 a	3.83 ± 1.62 a
Epigallocatechin	3.35 ± 0.12 a	5.35 ± 1.28 a	4.47 ± 0.43 a	4.98 ± 0.62 a
Procyanidin B1	8.89 ± 0.15 b	10.05 ± 0.84 b	2.37 ± 0.01 a	2.98 ± 0.20 a
Procyanidin B2	13.93 ± 2.75 ab	20.54 ± 2.34 c	17.50 ± 2.09 bc	11.09 ± 1.01 a
Total	*96.23 ± 8.13 ab*	*129.53 ± 16.02 b*	*79.80 ± 25.37 a*	*69.22 ± 3.56 a*
Hydroxybenzoic acids				
Gallic acid	3.73 ± 0.33 a	5.06 ± 0.27 a	4.41 ± 0.64 a	5.76 ± 1.62 a
Hydroxycinnamic acids				
trans-Caftaric acid	4.85 ± 0.75 bc	5.84 ± 0.24 c	4.34 ± 0.65 b	3.05 ± 0.42 a
trans+*cis*-Coutaric acids	3.46 ± 1.30 ab	4.07 ± 0.23 b	3.23 ± 0.49 ab	2.17 ± 0.02 a
trans-Fertaric acid	0.91 ± 0.04 a	1.18 ± 0.16 a	1.00 ± 0.03 a	1.14 ± 0.21 a
Caffeic acid	0.15 ± 0.01 ab	0.22 ± 0.06 b	0.06 ± 0.02 a	0.09 ± 0.02 a
p-Coumaric acid	0.06 ± 0.01 a	0.07 ± 0.01 a	0.12 ± 0.01 a	0.29 ± 0.20 a
Ferulic acid	0.80 ± 0.03 a	1.09 ± 0.46 a	1.43 ± 0.12 a	1.35 ± 0.01 a
Total	*10.23 ± 2.06 ab*	*12.45 ± 1.09 b*	*10.18 ± 1.08 ab*	*8.08 ± 0.05 a*
Stilbenes				
trans-Piceid	0.53 ± 0.01 a	0.73 ± 0.24 a	0.85 ± 0.07 a	0.74 ± 0.23 a
cis-Piceid	0.88 ± 0.06 a	1.38 ± 0.37 a	1.13 ± 0.00 a	1.41 ± 0.17 a
trans-Resveratrol	0.16 ± 0.02 a	0.30 ± 0.13 a	0.22 ± 0.06 a	0.28 ± 0.03 a
cis-Resveratrol	0.16 ± 0.01 a	0.32 ± 0.09 a	0.40 ± 0.06 a	0.37 ± 0.16 a
Total	*1.73 ± 0.06 a*	*2.73 ± 0.67 a*	*2.61 ± 0.07 a*	*2.80 ± 0.12 a*

Abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-*p*-coumaroylglucoside; cfglc, caffeoylglucoside; glcU, glucuronide; gal, galactoside. The phenolic compounds contents are listed with the standard deviation (n = 3). Different letters indicate significant differences between foliar applications ($p \leq 0.05$).

Phenolic compounds are related to wine quality since these compounds affect color, mouthfeel, and wine aging potential. Regarding anthocyanins, Ca sprays only increased the malvidin-3-cis-cmglc concentration of grapes in comparison with the Si treatment, whereas differences with control and Ca + Si grapes concentrations were not found (Table 3). Foliar application of Si increased the concentration of delphinidin-3-glc and cyanidin-3-glc when compared with control and Ca grapes. In addition, the concentration of cyanidin-3-cmglc increased, although this increment was only significant for Ca foliar application because differences with control and Ca + Si foliar treatment in these compounds were not observed (Table 3). Variations on total anthocyanin contents among control and foliar-treated samples were not observed (Table 3). Therefore, foliar application of Ca, Si and Ca + Si did not improve the anthocyanin content of grapes, in contrast with some previous studies that described an increase in anthocyanins content in grapes after Ca or Si foliar treatments [47–49]. Anthocyanins are the compounds responsible for the red color in red grapes and wines, so the improvement of their content in grapes is a goal for winemakers. Sut et al. [48] evaluated the effect of Si foliar treatment on four grape varieties and described a significant increase in the anthocyanin content of the Oseleta cultivar. However, the foliar application of Si stimulated specific metabolic changes in each particular cultivar analyzed. Yu et al. [49] showed that foliar application of Ca increased anthocyanin, sugar and Ca contents in the grape skin, probably because of Ca effects for increasing the expression of genes related to anthocyanin biosynthesis, modification and transport. Unfortunately, in our study, this effect was not clearly observed as described above.

Flavonols are compounds that can act as co-pigments and, thus, contribute to wine color stabilization [50]. For this family of compounds, the foliar application of Ca did not produce a significant effect in comparison with control grapes (Table 3). However, Martins et al. [36] described an increase in some specific flavonols in grapes after a Ca spray supply. Silicon foliar treatment led to grapes with a higher isorhamnetin-3-glc concentration than

control grapes, whereas the foliar application of combined Ca plus Si (Ca + Si) increased the laricitrin-3-glc and isorhamnetin-3-glc concentration in comparison with control grapes (Table 3). Therefore, for flavonols, the Ca + Si foliar treatment showed a higher effect than the single Ca and Si foliar sprays. It is possible that there was a synergistic effect between Ca and Si in the biosynthesis of this family of phenolic compounds. However, the total flavonol concentration of grapes did not vary between control and foliar treatments (Table 3). A minor effect or a decrease in some flavonols was reported for musts and wines of Sauvignon blanc after spraying inorganic Ca and Si salts [37], which is in agreement with the results we obtained for the application of single Ca and Si sprays.

Flavanols are related to astringency and bitterness of wines [51], which are mainly due to the occurrence of catechins and tannins. Calcium foliar treatment increased the epicatechin and procyanidin B2 concentration of grapes, in comparison with control and Ca + Si samples (Table 3). In addition, the total flavanol concentrations of Ca-sprayed grapes was the highest, although this increase was not significant compared to control ones. Grapes from grapevines sprayed with Si had a lower concentration of epicatechin and procyanidin B1 than control grapes, whereas the Ca + Si treatment induced a decrease in epicatechin, procyanidin B1 and procyanidin B2 values compared to control grapes (Table 3). These results do not agree with those reported by Gomes et al. [37]. These authors observed an increase in epicatechin and catechin content because of foliar Si application. On the other hand, they also showed higher concentrations of epicatechin in relation to Ca spraying.

Differences in gallic acid concentration between control and foliar treatments were not observed (Table 3). A similar lack of effect on this acid was observed after foliar Ca treatment by Martins et al. [36].

Hydroxycinnamic acids contribute to the astringent properties of grapes and wines [52]. The grapes from grapevines treated with Ca sprays presented had the highest concentrations of *trans*-caftaric acid, *trans*+*cis*-coutaric acids, caffeic acid and total hydroxycinnamic acids (Table 3). However, these increases were not significant in comparison with control grapes content. Silicon foliar treatment did not induce changes in any hydroxycinnamic acids when compared to control grapes, whereas Ca + Si sprays decreased *trans*-caftaric acid concentrations (Table 3). Previously, Gomes et al. [37] described a higher concentration of *trans*-caftaric acid in the musts of vines sprayed with Ca and Si salts. Martins et al. [36] also showed an increase in grape phenolic acids after Ca treatment. However, the results from the present work did not support the hypothesis that Ca and Si foliar treatments influence the phenolic composition of musts [37].

Finally, the concentration of stilbenes was not affected by any of the foliar treatments evaluated (Table 3). These compounds are of interest because of their healthy properties for humans. However, previous studies showed an increase in the content of some stilbenes after Ca and Si treatments. For example, Martins et al. [36] observed an increase in cis-piceid content after Ca treatment, while the remaining stilbenes were not affected. Gomes et al. [37] reported an increase in trans-resveratrol content with Ca and Si treatments compared to the content of control grapes.

3.4. Influence of the Ca, Si and Ca + Si Foliar Treatments on Must Nitrogen Compounds

Table 4 shows the must amino acid concentrations of control and foliar-treated grapevines. In general, the concentration of all amino acids was affected by the foliar sprays with respect to the control samples, excepting arginine (Arg), γ-aminobutyric acid (Gaba) and ornithine (Orn), which were not influenced by the foliar treatments. In addition, tyrosine (Tyr) and cysteine (Cys) were not detected in any of the samples (Table 4). The application of Ca + Si sprays increased aspartic acid (Asp), asparagine (Asn) and citrulline (Cit) concentrations with respect to the other treatments. Likewise, this combined Si and Ca treatment led to the highest values of histidine (His), valine (Val), isoleucine (Ile) and leucine (Leu) but without difference with the Ca sprayed samples (Table 4). In the case of the single application of Ca and Si, only the concentration of lysine (Lys) was higher in Ca-

sprayed samples compared to the control and Ca + Si treatment, with no differences with Si grapes. Likewise, the Ca treatment increased the content of total amino acids without proline (Pro) with respect to the control samples, with no differences regarding the other two spray treatments (Table 4). Foliar applications increased the concentration of glutamic acid (Glu), serine (Ser), glycine (Gly), alanine (Ala), tryptophan (Trp), phenylalanine (Phe), proline (Pro) and total amino acids with respect to the control samples (Table 4).

Table 4. Amino acid concentration (mg/L) in musts from control and Ca, Si and Ca + Si foliar treatments.

	Control	Ca	Si	Ca + Si
Aspartic acid (Asp)	12.49 ± 0.45 a	14.59 ± 1.41 a	15.57 ± 2.31 a	19.37 ± 1.10 b
Glutamic acid (Glu)	21.47 ± 3.69 a	32.89 ± 6.33 b	29.34 ± 2.71 ab	35.82 ± 6.34 b
Asparagine (Asn)	1.85 ± 0.12 a	2.72 ± 0.04 b	1.82 ± 0.26 a	3.86 ± 0.16 c
Serine (Ser)	27.13 ± 2.48 a	38.85 ± 7.02 b	30.79 ± 2.74 ab	31.36 ± 3.54 ab
Histidine (His)	13.69 ± 2.79 a	31.33 ± 12.12 ab	13.93 ± 1.00 a	37.87 ± 18.12 b
Glycine (Gly)	2.02 ± 1.71 a	5.40 ± 0.16 b	5.20 ± 0.60 b	5.18 ± 0.12 b
Threonine (Thr)	34.10 ± 2.02 a	54.43 ± 4.71 c	40.60 ± 7.09 ab	47.28 ± 8.83 bc
Citrulline (Cit)	4.86 ± 0.47 a	8.83 ± 1.55 a	8.27 ± 2.94 a	19.47 ± 7.20 b
Arginine (Arg)	559.42 ± 40.36 a	745.72 ± 178.86 a	708.59 ± 81.93 a	587.11 ± 156.69 a
Alanine (Ala)	58.21 ± 18.68 a	93.29 ± 3.97 b	84.24 ± 6.84 b	88.15 ± 10.66 b
γ-Aminobutyric acid (Gaba)	137.71 ± 19.97 a	152.83 ± 16.78 a	160.01 ± 19.39 a	169.63 ± 5.92 a
Tyrosine (Tyr)	n.d.	n.d.	n.d.	n.d.
Cysteine (Cys)	n.d.	n.d.	n.d.	n.d.
Valine (Val)	4.43 ± 0.59 a	23.00 ± 3.55 c	11.50 ± 0.07 b	22.08 ± 0.11 c
Methionine (Met)	3.42 ± 1.01 a	9.35 ± 1.78 c	5.23 ± 0.37 ab	7.28 ± 1.63 bc
Tryptophan (Trp)	21.05 ± 2.06 a	29.00 ± 3.24 b	22.73 ± 2.94 ab	24.84 ± 2.90 ab
Phenylalanine (Phe)	4.88 ± 0.57 a	9.42 ± 0.72 b	9.55 ± 1.35 b	9.75 ± 0.60 b
Isoleucine (Ile)	2.94 ± 0.46 a	13.65 ± 2.09 c	5.58 ± 0.55 b	13.09 ± 0.29 c
Ornithine (Orn)	2.12 ± 0.89 a	3.58 ± 0.70 a	2.89 ± 0.28 a	2.50 ± 0.65 a
Leucine (Leu)	5.71 ± 0.70 a	22.51 ± 4.77 b	9.52 ± 1.85 a	18.74 ± 1.90 b
Lysine (Lys)	2.07 ± 0.03 a	3.20 ± 0.58 b	2.63 ± 0.43 ab	1.73 ± 0.41 a
Proline (Pro)	295.23 ± 41.76 a	373.10 ± 28.58 b	357.07 ± 16.24 b	358.02 ± 28.96 b
Total amino acids	*1214.78 ± 42.53 a*	*1666.82 ± 232.04 b*	*1525.07 ± 72.80 b*	*1504.02 ± 101.89 b*
Total amino acids without Pro	*919.55 ± 32.39 a*	*1293.71 ± 220.99 b*	*1168.00 ± 66.85 ab*	*1145.13 ± 121.64 ab*

Abbreviation: n.d., not detected. The nitrogen compounds contents are listed with the standard deviation (n = 3). Different letters indicate significant differences between foliar applications ($p \leq 0.05$).

According to the results obtained by Sut et al. [48] with four different grape varieties, Si sprays stimulated cultivar-specific metabolic changes in grapes. Thus, for instance, the total amino acids and Pro content increased in Chardonnay and Garganega white cultivars; as well, the Arg content decreased in Garganega berries after foliar Si application. Stines et al. [53] reported that Pro accumulation is generally improved in the late phases of grape maturation, matching an increase in sugar content. This fact is consistent with the increase in fructose (Table 1), Pro and total amino acids observed in our Tempranillo grapes in association with the foliar application of Si compared to the control (Table 4). To the best of our knowledge, the effect of foliar Ca applications on grapevines has not been previously assessed in terms of grape N composition, and these are the first results showing the influence of this fertilization treatment on such a grape quality parameter.

4. Conclusions

In this study, the effect of Ca and Si foliar treatments applied to the vineyard either as single elements or in combination was assessed on the Tempranillo grape quality and chemical composition. A slight effect was observed with respect to general must parameters. Regarding aroma composition, the foliar application of Ca + Si increased the total concentration of all the aromatic compounds families evaluated. Foliar Ca sprays enhanced the total terpenoid and total benzenoid concentrations, while Si foliar application only

increased the total benzenoid concentration. Further studies on the foliar application of Ca and Si in vineyards should be carried out to clarify their effect on the grape phenolic composition. The effect of foliar treatments may vary depending on grape variety, as derived from the limited enhancement of grape phenolic content recorded in this investigation, compared with previous studies performed with other cultivars. In addition, no clear synergistic effect was observed between Ca and Si treatments to improve grape phenolic compound concentrations. In relation to grape amino acid concentrations, all foliar treatments enhanced some individual amino acids and total amino acids, so that Ca and Si sprays can be suitable tools for raising N compound concentrations in grapes.

Author Contributions: Conceptualization, T.G.-C. and V.F.; methodology, T.G.-C., M.G.-L., E.P.P.-Á. and V.F.; formal analysis, D.A.-O.d.U., I.S.d.U. and S.M.-S.R.; data curation, R.M.-P., L.L.T.-D. and M.G.-L.; writing—original draft preparation, T.G.-C., M.G.-L., E.P.P.-Á. and V.F.; writing—review and editing, D.A.-O.d.U., I.S.d.U., S.M.-S.R., R.M.-P. and L.L.T.-D. All authors have read and agreed to the published version of the manuscript.

Funding: M.G.-L. thanks the Universidad de La Rioja for her Margarita Salas 2022 contract funding by the Ministerio de Universidades and the European Union (Financed by the European Union-Next GenerationEU). S.M.-S.R. and R.M.-P. thank Gobierno de La Rioja and INIA-Gobierno de La Rioja, respectively, for their predoctoral contracts (E-26-2018-0134063; PRE2019-088633). E.P.P.-Á. thanks the Ministerio de Ciencia, Innovación y Universidades for her postdoctoral financial support received from the "Juan de la Cierva-Incorporación" Spanish Program (IJC2019-040502-I). Thanks to the Tradecorp company for supplying us with the silicon product.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in the published paper.

Conflicts of Interest: The authors declare no conflict of interest.

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